rpth research & practice in thrombosis & haemostasis

ARTERIAL THROMBOEMBOLISM

ACUTE CORONARY SYNDROMES

PB0001 | Clopidogrel Resistance and P2y12 Receptor Gene Polymorphisms in Patients with Non-ST Elevated Acute Coronary Syndrome

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Background: The prevalence of antiplatelet drug resistance during antiplatelet therapy varies from 20% to 45% for clopidogrel usage. The reasons and risk factors of this state are not completely known. As P2Y12 receptor plays key role in platelet activation and data on its role for clopidogrel resistance is inconsistent the study of this gene polymorphisms is actual and perspective.
Aims: The aim of this study was to investigate the association of P2Y12 polymorphisms and clopidogrel resistance due to the platelet aggregation at patients with non-ST elevated acute coronary syndrome.
Methods: 200 patients with non-ST elevated acute coronary syndrome from Bukovinian region (Ukraine) were genotyped in order to the H1(744T) and H2 (744C) haplotypes of the P2Y12 receptor gene. Aggregation studies with J.Born method. Decreasing of ADP-induced platelet aggregation < 10%, 10-29%, and ≥30% compared to the basal level considered as "clopidogrel resistance", "partial clopidogrel resistance" or "clopidogrel sensitiveness".
Results: In addition to specific genotype presentation (H1/H1; H1/H2; H2/H2) we have divided patients into the groups: homozygotes with H1/H1 (68 persons, 34.0%), homozygotes with H2/H2 mutation (18 persons, 9.0%) and heterozygotes H1/H2 (114 persons, 57%).
The spontaneous and ADP-induced aggregation of platelets after 7 days of dual antiplatelet therapy was significantly higher in H1/H2 and H2/H2 genotypes in compare to the H1/H1 genotype (p,p1< 0.05). The time of aggregation was shortest in mutant H2/H2 genotype (p< 0.05). The higher incidence of "clopidogrel resistance" and "partial clopidogrel resistance" patients was registered in H2/H2 genotype (odds ratio - 9.72), and, also, in H1/H2 genotype (odds ratio - 3.55), Mantel-Haenszel χ^2 - 20.62, p< 0.001. Generalized odds ratio (Agresti's alpha) was 3,746 [CI 95% 2,122-6,613].
Conclusions: This study supports the association of P2RY12 gene polymorphisms with platelet aggregation level, platelet dysfunction and higher incidence of "clopidogrel resistance" in genotypes H2/H2 and H1/H2 at patients with non-ST elevated acute coronary syndrome.

PB0002 | Improved Antithrombotic Activity and Diminished Bleeding Side Effect of a PEGylated αIIbβ3 Antagonist, Disintegrin

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Background: The applicability of protein drugs is confined by protein degradation and rapid elimination. PEGylation of polypeptides improves protein stability by sterically obstructing the degradation by serum proteases and reduces renal clearance by the increased mass.
Aims: To produce a pharmaceutically modified disintegrin derivative with improved physicochemical, pharmacokinetic, and antithrombotic properties.
Methods: We utilized NH_2-terminal-specific PEGylation technique to modify the disintegrin derivative KGDRR. We compared the antithrombotic activities of intact KGDRR (RR) and PEGylated KGDRR (P-RR) both *in vitro* and *in vivo* systems. In addition, the functional half-life in inhibiting platelet aggregation and the tendency in causing bleeding side effect were investigated.
Results: P-RR exhibited optimal potency in inhibiting platelet aggregation of human and mouse platelet-rich plasma induced by collagen or ADP *in vitro* with a lower IC_{50} than the intact derivative RR. In illumination-induced mesenteric venous thrombosis model, RR and P-RR efficaciously prevented occlusive thrombosis in dose-dependent manner. In rotational thromboelastometry assay, P-RR did not induce hypocoagulation in human whole blood even given at a higher concentration (30 μg/mL), while RR slightly prolonged clotting time. However, at equally efficacious antithrombotic dosages, both RR and

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P-RR caused neither severe thrombocytopenia nor bleeding in FcγRIIa-transgenic mice. The functional half-life assay revealed that PEGylation prolonged the *in vivo* half-life of RR (RR: 15.65 hrs vs. P-RR: 20.45 hrs).
Conclusions: P-RR exhibits higher antithrombotic potency and longer half-life *in vivo* as compared with native RR on a molar basis. In addition, P-RR exhibits a better safety profile at an efficacious antithrombotic dose *in vivo*. Therefore, PEGylation may be one of the ideal options in modifying disintegrin derivatives as the safe therapeutic agents for integrin-related diseases.

PB0003 | Correlation of D-Dimer Levels with Troponin I in Patients with Acute Myocardial Infarction

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Background: D-Dimer levels are elevated in patients with acute myocardial infarction and signify disease severity. However, the association of structural markers of myocardial injury with D-Dimer levels is not well studied.
Aims: Aim of this study was to assess the correlation between elevated D- Dimer levels and cardiac enzymes (Troponin I) in patients with acute myocardial infarction.
Methods: A total of 301 patients with Acute Myocardial Infarction were included in the study during a period of one year, from August 2018 to August 2019. Our patient population consisted of 230 males and 71 females. Average age of our patients was 43.2 years (range 26.7-87.5 years). Serial standard 12-lead electrocardiography (ECG) was performed in each patient. D-dimer, and troponin I testing was done for all the patients. D- Dimer levels were performed on Cobas-6000 chemistry analyzer and Troponin I levels were performed by electrochemiluminescence method.
Results: Of the 301 patients studied, all patients had elevated D-Dimer with an average D-dimer levels of 817 ng/ml. A total of 257 patients had elevated Troponin I levels with an average of 159 ng/ml (Range 27-631 ng/ml). A strong correlation between D Dimer levels and Troponin I was noted in these patients.(p value=0.003).
Conclusions: Based on the results of this study, it is evident that the measurement of D-dimer level correlates with Troponin-T in patients with acute myocardial infarction.

PB0004 | How Much Evidence Is Needed to Conclude against the Use of Oxygen Therapy in Acute Myocardial Infarction?

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Background: Oxygen therapy (OT) has often been used for the treatment of patients with acute myocardial infarction (AMI). However, the evidence from randomized control trials (RCTs) on the use of OT in AMI is conflicting and many recent systematic reviews have explored to summarize the evidence.

TABLE 1 RCTs included in systematic reviews

Systematic review	Included RCTs								
	Kirkeeide 1976	Ohlmann 2005	Wilson 1997	Hanchard 2012	Chen 2012	Jacob 2013	Khashnood 2016	Hofmann 2017	Hesdorf 2017
Khan 2016	Yes	Yes	No	Yes	No	Yes	No	NA	NA
Li 2016	Yes	Yes	No	Yes	Yes	No	No	NA	NA
Cabello 2018	Yes	Yes	Yes	Yes	No	Yes	No	NA	NA
Fu 2017	Yes	Yes	Yes	Yes	Yes	No	No	No	No
Li 2018	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No
Loembe 2018	Yes	Yes	Yes	Yes	No	Yes	No	No	No
Abuzaid 2018	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No
Jagtap-Jaswal 2018	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes

Yes: RCT included in SR; No: RCT not included in SR; NA: RCT published after the publication of SR
AMI: acute myocardial infarction; SR: systematic literature review

Aims: To synthesize the evidence from systematic reviews assessing the benefit and harm associated with OT for the treatment of AMI.
Methods: We searched the PubMed, Embase, Cochrane, and Epistemonikos databases (for the last five years) for systematic reviews assessing OT for AMI patients. Reviews including ACS population were excluded; reviews specifically focusing on the AMI population were included. Two researchers independently performed the assessment for inclusion, quality assessment (AMSTAR), and data extraction.
Results: This overview included eight systematic reviews (7 non-Cochrane, 1 Cochrane) that qualified for inclusion.
The individual reviews constituted 4-8 RCTs, with total of nine unique RCTs included across the reviews, with a patient population ranged 871-7998. Included RCTs were published from 1976 to 2017. The majority of the reviews (5) were of medium to high quality. Across reviews, OT did not show improvement in key outcomes of all-cause mortality, recurrent ischemia/MI, and pain. In contrast, OT was associated with an increased rate of recurrent MI, longer average hospital length of stay, and a trend in increased mortality.
Conclusions: Evidence from the recent systematic reviews, and constituting primary studies, unanimously confirms the uncertainty around the perceived beneficial effect of OT while its harmful effect cannot be ruled out for the patients with AMI. The finding support retiring the use of OT in AMI population, particularly in normoxaemic AMI population, until a well-conducted, high-quality RCTs suggests otherwise.

PB0005 | White Clot is More Permeable and Stronger than the Red Clot

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Background: For a heart attack, an occlusive thrombus must resist a high arterial shear rate and blood pressure. White clots formed under high shear in arteries may have different permeability and ultimate strength compared to red clots formed in veins. We quantify the permeability and ultimate strength as critical properties for vWF versus fibrin clots.
Aims: To quantify the permeability and ultimate strength of white clots and compare these to red clots.

Methods: To create white clots, heparinized (3.5 IU/ml) porcine whole blood was perfused through two stenotic flow systems coated with type 1 fibrillar collagen having high shear >3,500 s^{-1}. Red clots were generated by re-calcifying a citrated porcine blood (3.2%) under stagnant conditions. The blood flow was driven by a constant pressure head or a constant flow rate by a syringe pump. The upstream pressure and mass flow rate were measured and applied to Darcy's law to quantify the bulk permeability. The upstream blow-out pressure was used to calculate the ultimate strength of the clots.

Results: The platelet dominant white clots were significantly more permeable (Figure 2A, 0.1 to 17 μm^2). The red blood cell and fibrin-rich clots had much smaller permeabilities of < 0.001 μm^2 ($p < 0.05$). The white clots demonstrated an ultimate strength of 4.6±1.3 kPa that was 7x stronger than; whereas, the red clots yielded a weaker 0.63±0.3 kPa (Figure 2B, $p < 0.001$).

Conclusions: White clots are 2-4 orders of magnitude more permeable than the red clots. White clots also have seven times greater ultimate strength. Both attributes allow the white clots to occlude a stenotic artery where a red clot would be too weak. The two types of clots are quite different in structure and mechanics to create thrombosis and hemostasis to stop arterial flow.

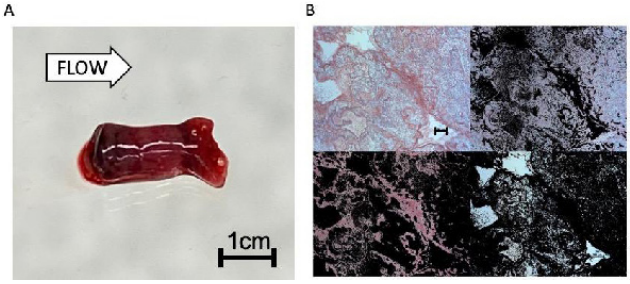

FIGURE 1 A, A white clot B, Histology images. Platelets (upper right), Fibrin (bottom left), and void space (bottom right)

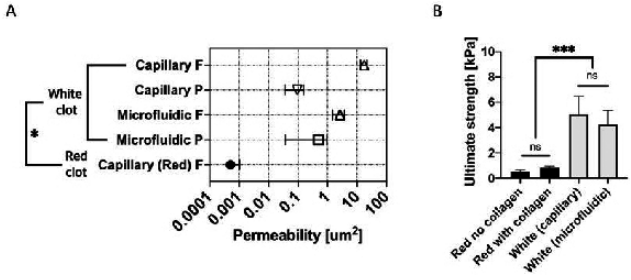

FIGURE 2 A, Permeability with 2 stenotic chambers and 2 flow conditions (F: constant flow, P: constant pressure head). B, Clot ultimate strengths

PB0006 | Thrombophilia and Myocardial Infarction with Non-Obstructive Coronary Arteries

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Background: Myocardial infarction with nonobstructive coronary arteries (MINOCA) is rare condition and occurs in 5% to 10% of all patients with myocardial infarction (MI). The underlying pathophysiological mechanisms are poorly understood, although several different mechanisms have been proposed, including plaque disruption, spasm, thromboembolism, dissection, other reasons. The presence of inherited thrombotic disorders could influence the risk of MINOCA, according to studies about 14% thrombophilia is found in patients with MINOCA.

Aims: The aim of this study is to investigate the impact of thrombophilia on the risk of MINOCA.

Methods: MI was diagnosed by significant elevation of a cardiac biomarker and new ST/T changes, qualitative coronary angiography findings to allow determination of the presence/absence of obstructive coronary artery disease. MINOCA was defined as the presence of MI criteria in the absence of obstructive coronary artery disease (ie, no epicardial vessel with a stenosis ≥50% on angiography). The study involved 26 patients with MINOCA (6 females and 20 males, mean age 46.69 years±12.99) and 35 patients with MI with obstructive coronary arteries (25 males and 10 females, 64.00 years±11.97). All subjects were recruited to the study during their stay in the hospital. For all patients genetic testing for inherited thrombophilia - Factor V Leiden G1691A, Prothrombin G20210A, MTHFR C677T polymorphism, PAI-1(SERPIN1) 4G/5G polymorphism - was performed by real-time PCR technique.

Results: Study have shown that MINOCA was higher in patient younger than 40 years ($p = 0.002$). Factor V Leiden G1691A increase the risk of MINOCA in 2.5 (CI - 1.8-3.3), $p = 0.029$. Prothrombin G20210A, MTHFR C677T polymorphism, PAI-1(SERPIN1) 4G/5G polymorphism also increase the risk of MINOCA, but it was no significant.

Conclusions: Previous results of our work indicate influence of Factor V Leiden G1691A on the risk of MINOCA, especially in young person.

PB0007 | Contribution of Fibrin and von Willebrand Factor to Platelet Adhesion on Ruptured Atherosclerotic Plaque in Acute Myocardial Infarction

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Background: Arterial thrombus formation is considered to be initiated by platelet adhesion on subendothelial matrix. However, the ruptured sites of atherosclerotic plaques are characterized by less matrix proteins. We recently reported presence of intraplaque erythrocytes and/or fibrin in coronary high-risk plaques.

Aims: We tried to identify factors providing platelet scaffolds at the ruptured sites of coronary plaques, and also studied intraplaque hemorrhage-related findings in ruptured plaques.

Methods: We histologically examined 73 coronary aspirated thrombi with plaque components obtained within 24 hours of the onset of acute myocardial infarction. In addition, we investigated thrombus formation using tissue factor-coated flow chambers under 1,000/s with perfusion of anti-coagulated rabbit blood.

Results: In the aspirated specimens, necrotic debris (95%), macrophages (95%), and cholesterin clefts (81%) were highly frequently observed at the interface areas of plaque components and thrombi. On the other hand, fibrous matrix (47%) and calcification (23%) were found partly as small foci. Tissue factor localized in the necrotic core and macrophages. Fibrin and von Willebrand factor deposited within the plaques and beneath platelet adhesion/aggregation in the all specimens. Extracellular DNA was observed in 15% of the interface area, and citrullinated histone H3 immunopositive area accounted for only 0.5% of the plaque area. We also found bilirubin deposition, intracellular iron deposition in about one-fifth of the plaques. Biliverdin reductase and ferritin are predominantly expressed in yellowish or grayish macrophages. Iron deposition was also found in degenerative fibrous matrix. In a flow chamber system, the treatment by thrombin inhibitor and monoclonal antibody for von Willebrand factor suppressed platelet adhesion/aggregation and fibrin deposition on tissue factor coated glass under high shear rate.

Conclusions: Fibrin and/or von Willebrand factor rather than matrix proteins and neutrophils extracellular traps may contribute to platelet adhesion at the ruptured areas of coronary plaques.

PB0008 | A Case Report of a Patient with Hereditary Angioedema and Samter's Syndrome Treated with Anticoagulant of Direct Action Anti Xa (Rivaroxaban), Secondary to Angioplasty due to Ischemic Heart Disease

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Background: Hereditary angioedema (HAE) is a genetic pathology caused by a deficit of the complement inhibitor (C1-INH). The incidence of HAE combined with Samter's syndrome (SSx) is low, making diagnosis, treatment and disease monitoring to be difficult.

Aims: To prevent intra stent thrombosis in a patient with HAE, SSx and ischemic heart disease after coronary angioplasty.

Methods: A clinical case of a 67-year-old female patient with HAE due to deficiency of the complement C1-INH inhibitor and SSx who develops aspirin allergy is presented. In 2006, because she manifests complications, receives treatment with nandroparin 2850 IU / 24 hours. With 3-year ischemic heart disease, she underwent catheterization with the presence of a right coronary lesion with stent placement. Upon receiving treatment with clopidrogel 75 mg, the patient begins with adverse reactions of angioedema. Subsequently with ticagregol 90 mg / 24 hours evolves with dyspnea on exertion and allergies. Under these circumstances it is decided to prescribe rivaroxaban 2.5mg / 24 hours.

Results: The patient after 6 months of rivaroxaban treatment, is asymptomatic, has no precordial pain, has no electrocardiographic changes of ischemia or cardiovascular manifestations. Laboratory and imaging tests without alarm data.

The aforementioned patient is required dual therapy with Aspirin and Clopidrogel to avoid intra stent thrombosis, however this management by HAE and SSx is not possible, so it is decided to prescribe rivaroxaban, based on the results of the COMPASSS and ATLAS ACS 2-TIMI 51 studies. Rivaroxaban is indicated in the prevention of atherothrombotic events in adult patients after an acute coronary syndrome.

Conclusions: The administration of rivaroxaban can be a safe treatment alternative for patients with HAE and SSx who develop heart disease, reducing the risk of intra stent thrombosis. Our patient with rivaroxaban has evolved favorably during the 6 months of follow-up.

PB0009 | Differential Effects of Clopidogrel with or without Aspirin on Platelet Reactivity and Coagulation Activation: A Randomized Placebo-Controlled Trial in Healthy Volunteers

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Background: Dual antiplatelet therapy (DAPT) is standard in acute coronary syndrome because of efficacy in reducing vascular outcomes albeit at an increased bleeding risk.

Aims: To compare effects of clopidogrel-monotherapy with clopidogrel-based DAPT on platelet reactivity and coagulation activation.

Methods: In a randomized, parallel-group, double-blind trial 44 volunteers received a loading dose of clopidogrel (600mg) plus aspirin (100mg) or placebo followed by 6 days of clopidogrel (150mg) plus aspirin (100mg) or placebo. Multiple electrode aggregometry (MEA), the vasodilator-stimulated phosphoprotein (VASP), prothrombin fragment 1.2 (f1.2), d-Dimer, and thromboxane B_2(TxB_2) were measured in venous blood at different timepoints before first study drug intake and over the course of treatment. Data are given as mean differences (Δmean [95% confidence interval])or geometric mean ratios (GMR [95% confidence interval]) to describe changes in time and differences between groups.

Results: Clopidogrel-based DAPT and clopidogrel-monotherapy comparably decreased adenosine diphosphate-triggered platelet aggregation (MEA-ADP) (Δmean: −37.1 (−44.4; −29.9), p< 0.0001 and −32.2 (−39.8; −24.6), p< 0.0001) and VASP (Δmean: −36.1 (−46.9; −25.3), p< 0.0001 and −43.1 (−54.4; −31.8), p< 0.0001) at 2h. Arachidonic acid-triggered aggregation (MEA-AA) was reduced by clopidogrel-based DAPT and clopidogrel-monotherapy (Δmean: −63.8 (−73.9; −53.8), p< 0.0001 and −24.3 (−34.9; −13.7), p< 0.0001) at 2h. Over 8 days, clopidogrel-based DAPT and clopidogrel-monotherapy comparably affected MEA-ADP and VASP, whereas MEA-AA was significantly stronger influenced by clopidogrel-based DAPT (Δmean: −70.1 (−79.5; −60.7), p< 0.0001 and −21.2 (−31.0; −11.4), p< 0.0001).

Clopidogrel-based DAPT almost entirely eliminated TxB_2-generation at 8 days (GMR: 0.03 (0.02; 0.05), p< 0.0001), but clopidogrel monotherapy also reduced TxB_2-generationat 8 days (GMR: 0.52 (0.32; 0.86), p=0.01).

Both treatments did not affect f1.2 at 2 hours and 8 days. d-Dimer was slightly reduced by clopidogrel-based DAPT (0.94 (0.89; 1.00), p=0.04) at 2h but not after 8 days. Clopidogrel-monotherapy did not decrease d-Dimer.

Conclusions: Clopidogrel-based DAPT and clopidogrel-monotherapy comparably affect platelet and coagulation activation.

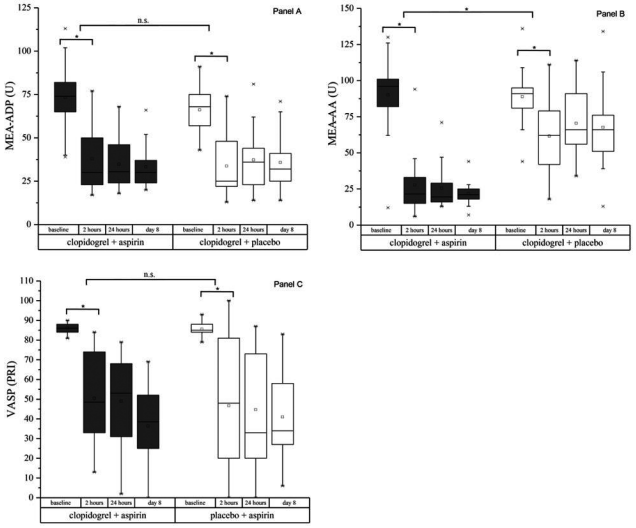

FIGURE 1 Boxplots demonstrating the effects of clopidogrel-based DAPT and clopidogrel-monotherapy on platelet aggregation (MEA-ADP, MEA-AA) and VASP

PB0010 | Hydrodynamic Activation of von Willebrand Factor under Flow Conditions May Contribute to Thrombotic Complications in Coronary Heart Disease

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Background: von Willebrand Factor (vWF) molecules are able to change their conformation from globular to fibrillar under flow conditions, making available domains that bind to the glycoprotein platelet membrane receptors. The critical blood flow shear rate threshold for hydrodynamic activation of vWF is estimated to be approximately 5000 s^{-1}. If the threshold for activation of vWF is lower than this value, conformational changes in vWF can lead to an increase in the risk of thrombosis in arteries, including coronary arteries, where the maximum shear rates are 1200-1300 s^{-1}.

Aims: To investigate the vWF-dependent platelet adhesion to the protein coated surface of patients with coronary heart disease (CHD) under shear rates below critical (1200-1300s^{-1}).

Methods: Using a microfluidic system, that mimics blood flow in vessels, we measured platelet adhesion to a fibrinogen-coated surface under shear rates of 1200-1300 s^{-1}. Platelet adhesion kinetics was measured in blood samples from 22 patients with CHD aged 45 to 60 years. The control group consisted of 8 healthy volunteers aged from 30 to 55 years.

Results: The inhibition of GPIb platelet-receptors by specific monoclonal antibodies decreased platelet adhesion to fibrinogen surface under shear rates of 1200-1300s^{-1} by 15.4±10% in healthy volunteers and by 72.3±18% in persons with CHD (p< 0.01). Inhibition

of GPIIb/IIIa receptor decreased platelet adhesion by 75.3±6.2% in healthy volunteers and by 80.2±6.6% in patients with CHD (p>0.05).
Conclusions: At low shear rates platelet adhesion in healthy volunteers is mainly dependent on platelet interaction with fibrinogen. In persons with CHD, the interaction of platelets with both fibrinogen and vWF is equally important. Abnormal hydrodynamic activation of vWF at low shear rates may contribute to atherothrombotic complications in CHD patients.
This work was supported by the grant of the Russian Science Foundation (project #16-15-10098).

PB0011 | Antiphospholipid Antibodies in Acute Myocardial Infarction

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Background: Antiphospholipid antibody syndrome (APS), an important cause of acquired thrombophilia with persistently positive antiphospholipid antibodies (aPL). There are limited big data regarding the acute myocardial infarction (AMI) patients with aPL -positive.
Aims: Accordingly, we aimed to assess the association of aPL -positive with the clinical phenotype of AMI.
Methods: The study population consisted of 778 patients with AMI. Clinical characteristics and data were determined according to the medical history, present clinical symptoms and laboratory results. Data were compared between aPL -positive and aPL -negative patients. From July 2018 to July 2019.

TABLE 1 Clinical characteristics of overall AMI, aPL-negative, and aPL-positive groups

Characteristic	Overall AMI (n=778)	aPL-negative AMI (n=732)	aPL-positive AMI (n=46)	p
Age (y,mean±SD)	60.31±12.46	61.01±12.22	49.33±11.24	<0.001
Sex (male), n (%)	592 (76.69)	552 (75.41)	40 (86.96)	0.075
Prior MI, n (%)	135 (17.35)	121 (16.55)	14 (30.43)	0.016
Prior stroke, n (%)	95 (12.21)	89 (12.16)	6 (13.04)	0.859
Prior VTE, n (%)	17 (2.19)	13 (1.78)	4 (8.70)	0.002
Any arterial or venous thrombosis, n (%)	222 (28.53)	200 (27.32)	22 (47.83)	0.003
Hypertension, n (%)	495 (63.62)	482 (65.85)	13 (28.26)	<0.001
Hyperlipidemia, n (%)	703 (90.36)	693 (94.67)	10 (21.74)	<0.001
Diabetes mellitus, n (%)	286 (36.76)	272 (37.16)	14 (30.43)	0.359

Results: aPL was measured in 778 AMI patients. Of those, forty-six (5.91%) were positive for laboratory tests exploring aPL antibodies. Among them, 7 patients (15.2%) had a triple positive aPL profile. When compared with the aPL -negative group, aPL -positive patients were significantly younger (49.33±11.24 vs. 61.01±12.22 years, p < 0.001), had more frequently a history of any arterial or venous thrombosis (47.83% vs. 27.32%, p= 0.003), and had less cardiology risk factor such as hypertension (28.26% vs. 65.85%, p < 0.001) and hyperlipidemia (21.74% vs. 94.67%, p < 0.001). On multivariate logistic regression analysis, after adjustment for age, diabetes, hyperlipidemia and hypertension showed that aPL -positive (odds ratio:4.72; p < 0.001) contribute independent risks for arteriovenous thrombosis events. Patients younger than 55years old with aCL, LA or β2 GPI any positive had a high risk of arteriovenous thrombosis events (odds ratio:4.98; p < 0.001). Among them, the odds ratio of arteriovenous thrombosis events associated with LA, aCL and β2 GPI in patients younger than 55 years old was 5.11 (p < 0.001), 4.45 (p =0.013) and 4.30 (p =0.044), respectively.
Conclusions: In conclusion, aPL -positive patient is an important subgroup of AMI with unique clinical phenotype. The presence of aPL should be suspected in patients with recurrent arteriovenous thrombosis or in patients with younger AMI.

PB0012 | BDNFVal66Met Polymorphism Affects Cardiac Remodeling and Modulates Macrophage Polarization After Myocardial Infarction

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Background: Brain-derived neurotrophic factor (BDNF) is a member of neurotrophin family, known for its role in homeostasis of cardiovascular system. Low circulating BDNF levels were associated with future coronary events, mortality and adverse left ventricular cardiac remodeling. Recently the BDNFVal66Met polymorphism was related to increased propensity for arterial thrombosis related to acute myocardial infarction.
Aims: This study investigated the effect of BDNFVal66Met polymorphism on cardiac remodeling after myocardial infarction in mice focusing on macrophage role. To sustain the relevance of data obtained in mice, the phenotype of macrophages derived from $BDNF^{Val/Val}$ or mutant $BDNF^{Met/Met}$ human patients was analyzed.
Methods: Wild-type ($BDNF^{Val/Val}$) and homozygous BDNFVal66Met mice ($BDNF^{Met/Met}$) underwent left anterior descending (LAD) coronary artery ligation, left ventricular parameters were obtained

by cardiac magnetic resonance imaging (cMRI) and infarct size assessed by histology. Cardiac or peritoneal macrophages were isolated after LAD and thioglycollate elicitation. Human macrophages were obtained by differentiating circulating monocytes isoalted from $BDNF^{Val/Val}$ and $BDNF^{Met/Met}$ coronary heart disease patients (13/group). Mouse and human macrophages were charcatherized in terms of M1 and M2 markers by flow cytometry, qRT-PCR, and migration ability by agarose spot assay. The study complies Helsinki declaration and was approved by Centro Cardiologico Monzino Ethical Committee. All partecipants provided written informed consent.

Results: After LAD-ligation $BDNF^{Met/Met}$ mice displayed lower ejection fraction, higher infarct size and greater contralateral cardiomyocyte dimensions than $BDNF^{Val/Val}$. Cardiac $BDNF^{Met/Met}$ macrophages showed an enhanced polarization towards M1 phenotype. Similarly, mutant peritoneal macrophages showed a greater expression of M1 markers, associated whit a higher round-to-spindle ratio. In addition, $BDNF^{Met/Met}$ macrophages exhibited increased migratory ability and expression of inflammatory genes (IL-6, TNF-α and COX-2). Human $BDNF^{Met/Met}$ macrophages displayed a similar phenotype to the one observed in mutant mice.

Conclusions: Overall these data suggest that BDNFVal66Met polymorphism, influencing macrophage polarization and activation, can contribute to affect cardiac remodeling after myocardial infarction.

PB0013 | Plasma Exosomes in Patients with ST-Elevation Acute Myocardial Infarction (STEMI) and in Patients with Stable Coronary Artery Disease (CAD)

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Background: Exosomes (EXOs), a subgroup of extracellular vesicles, are important mediators of inter-cellular communication. They reflect the physio-pathological state of the parental cell, suggesting their potential as diagnostic and prognostic tool in pathological conditions. The signature and the implication of EXOs in ST-elevation acute myocardial infarction (STEMI) are still partially unknown.

Aims: This study is aimed at identifying the potential differences in EXOs profile between STEMI and stable coronary artery disease (CAD).

Methods: EXOs were isolated from plasma of STEMI (at hospital admission; n=18) and CAD (absence of acute coronary symptoms from at least 12 months; n=31) male patients using commercial kits. EXOs were validated by analyzing the morphology (transmission electron microscopy) and the expression of specific exosome markers (western blot, WB). Concentration and size distribution were determined by Nanoparticle Tracking Analysis and protein cargo screened by proteomic approach and confirmed by WB. The study complies with Declaration of Helsinki and was approved by the Centro Cardiologico Monzino Ethical Committee. All patients provided written informed consent.

Results: EXOs from STEMI patients displayed a higher concentration (p=0.0012) and mode (p=0.0005) compared to CAD patients. The greater expression of the platelet marker CD41 (p=0.0007) in STEMI patients suggests an important platelet contribution to circulating EXOs in these patients. By proteomic analysis, we identified about 20 proteins differentially expressed in STEMI- and CAD-EXOs which are mainly involved in inflammation, integrin signaling pathway and blood coagulation. Some of these proteins have been previously recognized as independent predictor of major adverse cardiac events in patients with acute myocardial infarction (AMI) and associated with increased long-term mortality after AMI.

Conclusions: The pronounced differences emerged between plasma EXOs of STEMI and CAD patients provide evidence that EXO signature reflects the clinical manifestation, suggesting their potential application in clinical practice. Larger studies are required to confirm these data.

PB0014 | Interventions Needed in Antithrombotic Therapy in a Real Life Hospitalized Population; Evaluation of Interventions Initiated by a Multidisciplinary Antithrombotic Team Regarding Antithrombotic Agent Combinations in In-Patient Care

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Background: Patient population on antithrombotic therapy is growing and the antithrombotic landscape is complex. Combining oral anticoagulants (OAC, e.g. DOAC and VKA) with other antithrombotic agents (platelet aggregation inhibitors, PAI) is associated with high risk of bleeding complications and should be monitored closely to prevent unintentional longer use than indicated. Our hospital has implemented a multidisciplinary antithrombotic stewardship (ASP) to proactively monitor the in-patients safety and efficacy of antithrombotic therapy.

Aims: To determine the number of interventions needed by ASP while monitoring hospitalized patients on combinations of OAC with other antithrombotic agents.

Methods: This prospective, single-center, observational cohort study was conducted in a large teaching hospital between March 2019 and June 2019. Patients using a combination of OAC with PAI (double therapy) or with two PAI's (triple therapy) during hospitalization were included to be monitored by ASP on indication and length of use. Primary outcome was the number of interventions regarding the antithrombotic therapy initiated by ASP.

Results: The study comprised 200 consecutive patients (table 1), of which 123 patients (61.5%) needed an intervention (58% of double and 78% of triple therapy patients). Interventions most initiated were 'define maximum duration of use' (62%) and 'discontinue PAI' (24%). For patients started the antithrombotic combination during hospitalization most needed intervention was to 'define maximum duration of use of the combined antithrombotic therapy' before discharge (70%, n=80), while the intervention needed for those who entered the hospital using a combination of antithrombotic agents was to stop one or two PAI's as the combination was no longer indicated (46%, n=43) (table 2).

Conclusions: Hospitalized patients using an OAC combined with other antithrombotic agents often need an intervention to prevent unnecessary longer use than indicated. Implementation of APS leads to interventions in 61.5% of these hospitalized patients and therefore improves safety and efficacy of antithrombotic medication.

TABLE 1 Baseline characteristics of patients using double and triple antithrombotic therapy and reviewed by ASP

Characteristic	Double antithrombotic therapy (n=160)	Triple antithrombotic therapy (n=40)
Male gender n, (%)	100 (62.5)	100 (62.5)
Age, year [range]	73 [36-95]	71 [38-87]
Length of hospitalization, days [range]	12 [1-77]	15 [2-82]
Cardiac ward type n, (%)	94 (58.8)	37 (92.5)
Combination newly started during hospitalization, n (%)	102 (63.8)	32 (80.0)
VKA combined with PAI n, (%)	73 (45.6)	19 (40.0)
DOAC combined with PAI n, (%)	77 (48.1)	19 (40.0)
LMWH combined with PAI n, (%)	10 (6.3)	2 (5.0)
Death during hospitalization, n (%)	5 (3.1)	1 (2.5)

TABLE 2 Comparison of interventions of patients with double or triple therapy started during current hospitalization vs started not within current

Type of intervention	Number of patients using double or triple antithrombotic therapy started during hospitalization (n = 80) (%)	Number of patients using double or triple antithrombotic therapy not started during hospitalization (n = 43) (%)
Define maximum date of use	56 (70)	20 (46.5)
Discontinue PAI	9 (11.3)	20 (46.5)
Discontinue OAC	3 (3.8)	1 (2.3)
Add PAI	1 (1.3)	1 (2.3)
Add LMWH	1 (1.3)	0 (0)
Other	10 (0)	1 (2.3)

PB0015 | Changes of Soluble Fibrin-Monomer Complex Concentration in Different Types of Coronary Artery Disease

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Background: Soluble fibrin-monomer complex (SFMC) as a feature of thrombosis is shown to be raised in patients with acute coronary syndrome (ACS). Most studies exist regarding myocardial infarction (MI). Limited investigations include patients with unstable angina (UA). Almost no data is found concerning types of UA, namely, new-onset (NUA) and crescendo (CUA) angina.

Aims: To compare the concentration of SFMC in patients with different types of CAD (MI, NUA, CUA, stable angina (SA)).

Methods: In this cross-sectional study, we collected blood samples in 94 patients with CAD - 10 patients with MI, 57 patients with UA (38 patients with PUA and 19 patients with NUA), 27 patients with SA. Concentration of SFMC was determined by colorimetric ortho-phenantroline method. There were no significant difference between groups with respect of age (63.66+/–7.79 y.o.), gender (females:males=33.7:66.3) and body mass index (28.91+/-4.53 kg/m^2). Written consent was obtained from each participating subject. The data was analysed with SPSS v.22.

Results: We have identified increased SFMC concentration in ACS patients compared with SA (22.00 (12.00-26.00) mcg/ml vs 15.00 (9.75-21.50) mcg/ml, p=0.03). SFMC concentration in UA (22.00 (11.00-28.00) mcg/ml) was higher than in SA (p=0.016), and did not differ from MI patients' concentration (22.00 (15.00-26.00) mcg/ml, p>0.05). SFMC concentration in NUA was higher in comparison with CUA (24.00 (22.00-28.00) mcg/ml vs 19.00 (7.50-25.75) mcg/ml, p=0.013). There were no difference between SFMC concentration in SA vs CUA (p>0.05) and MI vs NUA (p>0.05). The association between types of CAD and SFMC concentration was established (Chi^2_8=30.691, p=0.0001, Cramer's V=0.375).

Conclusions: We have found dissimilarity of SFMC concentration depending on CAD type (including the one between NUA and CUA). The statement about increased thrombotic activity in patients with ACS need more profound analysis.

TABLE 1 Dissimilarity of SFMC concentration depending on CAD type

	SFMC ≤10 mcg/ml, %	10<SFMC <20 mcg/ml, %	SFMC ≥20 mcg/ml, %	
Stable angina	23.1	50.0	26.9	100%
Crescendo angina	29.4	20.6	50.0	100%
New onset angina	5.3	0	94.7	100%
Myocardial infarction	0	30.0%	70.0	100%

PB0016 | In-Vivo Biomarkers of Platelet Function in Women with Ischemic Heart Disease: What We Learned from the EVA Study

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Background: Ischemic heart diseases (IHD) is not synonymous with obstructive flow-limiting coronary artery disease (CAD), especially in women. Platelets play a pivotal role in the pathogenesis of myocardial ischemia. However, there is limited data on the of sex differences among biomarkers of platelet function *in-vivo*.

Aims: To identify sex differences in biomarkers of platelet function in patients with IHD.

Methods: We performed platelet function studies in a subgroup of patients from the "Endocrine Vascular disease Approach" (EVA) study, an ongoing observational study recruiting IHD adults undergoing urgent or elective angiography. No obstructive disease was defined for a coronary stenosis < 50% for acute myocardial infarction (MINOCA) or for chronic stable angina (INOCA).

Results: 199 patients were included (mean age 67±11; 37% women). Women experienced more frequently acute coronary syndrome compared to men (54.8 vs 41.3%, p=0.001) with a higher proportion of no obstructive disease (27% vs 4%; p< 0.05). Among adults with stable angina, men were more frequently presenting with obstructive disease compared to women (77% vs 62%, p< 0.05). Overall, the biomarkers of platelet function were similar between men and women. However, when we stratified their level according to the clinical presentation (ACS vs Stable Angina) and the type of CAD (obstructive vs no obstructive) we found statistically significant differences: women with MINOCA had higher levels of thromboxane B_2 compared to women with obstructive disease or INOCA (187±68 vs 125±51 pg/ml, p=0.003); women with MINOCA presented also the highest level of soluble P-selectin (38±18 vs 26±10ng/ml, p< 0.05), while no differences were observed in men. Of note, soluble CD40L level was higher in women with INOCA compared to men with INOCA (3.18±2.13 vs 1.65±0.5, p=0.03).

Conclusions: High levels of platelet activation biomarkers identify women with no obstructive disease in acute and chronic IHD. Identification of sex-specific biomarkers may improve the personalized management of IHD.

PB0018 | Clot Formation, Ultrastructure and Lysis in Patients with Acute Phase Myocardial Infarction

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Background: Patients with established coronary artery disease have impaired clot structure and lysis. The role of clot structure in the acute phase of the disease is unknown.

Aims: Investigate clot structure in the acute phase of myocardial infarction (AMI) compared to healthy controls.

Methods: Plasma clots from AMI patients (within 12 hours of symptoms onset) or healthy controls were analyzed by turbidity and lysis. Clot pore size was determined by permeation. Clot ultrastructure was determined by confocal and scanning electron microscopy. Average fiber radius and protofibril packing were investigated by turbidimetry.

Results: Analysis on clot formation, using turbidity, showed increased lag time (24%, p< 0.05), suggesting changes in protofibril packing and increased fiber size for AMI patients compared to controls. Additionally, AMI clots formed more quickly as evidenced by average rate of clotting (2.9-fold increase, p< 0.001) and time to maximum absorbance (38% decrease, p< 0.0001). Decreased plasminogen in AMI patients (23%, p< 0.05) had strong effects on clot lysis, including increased time to half lysis (1.72-fold, p< 0.0001) and increased time to maximum lysis rate (7.96-fold, p< 0.0001). Taken together, these results suggest AMI patients form less porous clots made from densely packed fibers with decreased numbers of protofibrils, which was confirmed using permeation (1.05-fold decrease, p< 0.0001), confocal and scanning electron microscopy (46% increase, p< 0.0001), and turbidimetry (16% decrease, p< 0.05) respectively.

Conclusions: AMI plasma form denser and less permeable clots that lyse more slowly than healthy controls. These data show marked abnormalities in clot structure that contribute to the high risk of thrombosis during the acute phase of myocardial infarction.

PB0019 | Myocardial Infarction as a First Symptom of Activated Protein C Resistance Associated to Hyperhomocyteinemia

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Background: Coagulopathy in myocardial infarction with normal coronary arteries can reach 36% of cases.
Aims: We aimed to report a new case of a combined coagulopathy in a patient with myocardial infarction.
Methods: Case report.
Results: We report the case of a 62 year-old-male patient who was admitted for acute coronary syndrome without ST elevation and with high Troponin level. He had no history of diabetes, hypertension or tobacco. Coronary angiography revealed completely normal coronary arteries without narrowing. 1 week later he presented a second episode of myocardial infarction spontaneously reperfused. Coronary artery thrombosis was suspected and an extensive work-up for thrombophilia revealed hyperhomocysteinemia at 47 µmol/l associated to activated protein C resistance. The patient was treated with oral anticoagulation and susbtitutional treatment of folic acid with a good outcome 1 year after discharge. Controlled homocysteinemia was at 18 µmol/l.
Conclusions: Thrombophilia should be considered in patients with myocardial infarction with normal coronary arteries without apparent cardiovascular risk factors. Combined thrombophilia may expose to an increased risk for thrombosis.

PB0020 | Postoperative Dual Antiplatelet Therapy Is Influenced by Platelet Regeneration

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Background: Current guidelines recommend (re)start of dual antiplatelet therapy as soon as possible after coronary artery bypass grafting (CABG) in patients at risk. However the choice of ADP receptor antagonist and dosing remain unclear. Competing thrombotic and haemorrhagic risks as well as increased platelet turnover interfering with antiplatelet therapy are challenges to the antithrombotic management. Platelet function testing has been proposed for guidance of DAPT in the perioperative setting.
Aims: We assessed platelet function together with platelet turnover in patients with DAPT in the immediate postoperative period.
Methods: Nonbleeding patients (n=193) with recent acute coronary syndrome or coronary stenting were given Clopidogrel 75mg/d from day +1 after CABG. Aspirin therapy was continued without interruption perioperatively. The platelet reactivity index (PRI) according to Vasodilator Stimulated Phosphoprotein (VASP) testing was assessed once or repeatedly during the first 10 days of DAPT-initiation. The PRI range from 20-60% was considered target range; PRI< 20% and >60% were interpreted as low and high on treatment platelet reactivity (LTPR and HTPR), respectively. Platelet regeneration was assessed by the immature platelet fraction (IPF; Sysmex® Hematology Analyzer). PRI results were compared between IPF-low and -high groups.
Results: The mean PRI value dropped into the target range after two intakes of 75mg Clopidogrel (mean PRI=55%) and reached a steady state after four intakes. Interindividual variability was profound. Accordingly, beyond 4 days of Clopidogrel therapy only 45.0% of PRI values were within the target range, whereas HTPR and LTPR was found in 29.2% and 25.8%, respectively. The PRI was significantly lower in the IPF-low as compared to the IPF-high (34.3% vs. 44.1%, $p< 0.05$) with more HTPR in the IPF-high group (33.3% vs 18.3%).
Conclusions: Gradual initiation of ADP receptor inhibiton postoperatively is feasible with clopidogrel without loading. However, the interindividual pharmacodynamic variability is strong, which may be explained by the kinetics of platelet regeneration.

PB0021 | New Rat Model of Ferric Chloride-induced Acute Coronary Artery Thrombosis for Pharmaceutical Treatment of Myocardial Infarction

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Background: Clinically relevant animal models are required to better investigate pharmaceutical and surgical treatment of arterial thrombosis. Myocardial infarction (MI) caused by coronary arterial thrombosis, is frequent in patients with a rupture or injury of an atherosclerotic coronary arterial plaque. This occurs following a percutaneous coronary intervention or is the result of having acute coronary syndrome.
Aims: Our goal was to validate a model of acute MI induced by topical application of ferric chloride (FeCl3) solution on the left anterior descending (LAD) coronary of anesthetized rats.
Methods: Acute MI in male adult rats was induced by topical application of a stripe of filter paper saturated with 50% FeCl3 for a period of 10 min. Occlusion of the LAD resulted from the appearance of an adherent mixed thrombus within 30 minutes. On the ECG, elevated ST segments were observed following occlusion, characteristic of MI.
Results: Upon harvesting the heart, dual staining with Evans blue and triphenyltetrazolium chloride (TTC) revealed an infarct covering 33% of the left ventricular area provoked by the thrombotic occlusion. Animals treated with 30 mg/kg of acetyl salicylic acid (ASA) 30 min prior to FeCl3 application failed to generate the elevated ST-segment and no infarct area was observed. Cardiac troponin I, measured 24h post MI, increased from 0.4 to 343.4 ng/mL. Cardiac function evaluated 24h post MI using echocardiography calculated a 12% reduction of the fractional shortening, 8% of the ejection fraction and 8.7% of the stroke volume while we observed a 9.3% increase in heart rate and a 24.8% increase in end systolic volume.
Conclusions: In conclusion, we present that this rat model of acute MI provoked by application of FeCl3 is a more clinically relevant

model to be used in screening for new antithrombic or thrombolytic pharmaceutical treatments.

PB0022 | Smoking Status as Risk Factor and Risk Factors in Young and Elderly Patients Suffered from Myocardial Infarction

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Background: The role of smoking in accelerated atherothrombosis is well known but the role of smoking in the pathogenesis of myocardial infarction in young is not well understood.

Aims: To determine smoking status and coleration with haemostatic markers in patients with myocardial infarction younger than 45 years and the group of patients with myocardial infarction older than 65 years.

Methods: In a prospective study, 143 patients were examined, i.e. 80 patients with myocardial infarction younger than 45 years and 63 patients with myocardial infarction older than 65 years. The values of hemostatic and biochemical markers were analyzed using standard coagulometric and biochemical methods.

Results: In the group of patients young than 45-year-old there was significantly higher than in the group of older patients (64.1% vs 15.1%; p< 0.001). In males with myocardial infarction the smoking status was correlated with level of total cholesterol, LDL cholesterol, triglycerides and fibrinogen. There was not significant correlation between these two groups with the level of FVII, F VIII, vWF, F X, F XII and CRP. In the female group correlation between smoking status and stated parameters was not found.

Conclusions: This result suggests the importance of possible specific pathogenic mechanism of induction of myocardial infarction in young.

ATHEROSCLEROSIS

PB0023 | High-Dose Atorvastatin - Loaded Microbubbles-Mediated Sonoporation Therapy using B - Mode Ultrasound - Guided Focused Electrohydraulic Shock Waves Decreases Inflammation and Restenosis after Laser Angioplasty of Advanced Atherosclerotic Severe Stenosis

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Background: The management of advanced atherosclerotic stenosis reduces the risk of myocardial infarction and its related deaths. The laser atherectomy methods that are currently in use, cause to inflammation and subsequent restenosis.

Aims: The aim of this study was to evaluate the effect of high- dose atorvastatin - loaded microbubbles - mediated sonoporation therapy using B- mode ultrasound- guided focused electrohydraulic shock waves on inflammation and intimal hyperplasia reduction after laser angioplasty in the hamster model of abdominal aorta atherosclerotic severe stenosis, wherein diagnostic B- mode ultrasound is combined with sonoporation therapy system, with a goal of increased safety.

Methods: Briefly, Golden Syrian hamsters were submitted to advanced atherosclerotic severe stenosis by primary intravascular radiofrequency (F= 23.7 MHz, P= 10 W) thermal balloon angioplasty- mediated endothelial injury at the abdominal aorta (approximately 0.5 cm superior to the iliac bifurcation) followed by a 1.5% cholesterol- rich diet injury for 12 weeks. Histopathology results showed the formation of advanced atherosclerosis with lipid and neovessel- rich plaque that resulted to severe stenosis (> 70%) in all of the hamsters′ arteries. Then treatment group underwent ArF excimer laser (193 nm) angioplasty followed by shock wave (V= 15 Kv, F= 2 Hz, Impulses= 50)- mediated sonoporation therapy in combination with simultaneously intravenous high- dose atorvastatin (5 mg/Kg/day)- loaded PESDA (Perfluorocarbon- Exposed Sonicated Dextrose Albumin) microbubbles (100ml/kg, 2-5 ×105 bubbles/ml) administration.

Results: Results from histopathology showed a significant reduction in the mean value for macrophages and smooth muscle cells density in the sonoporation therapy group compared with the other groups (p< 0.05).

Conclusions: Enhanced sonoporation therapy effect of focused shock waves, induced by inertial cavitation effect of microbubbles accompanied by anti- inflammatory, lipophilic and pleiotropic effects of high- dose atorvastatin therapy, can cause to reduce inflammation and intimal hyperplasia after laser angioplasty of atherosclerotic severe stenosis.

PB0024 | Synthetic Antisense Oligodeoxynucleotides - Loaded Microbubbles - Mediated Transfection Therapy using B-Mode Ultrasound - Guided Focused Shock Waves Decreases Neointimal Hyperplasia Restenosis after Laser Angioplasty of Advanced Atherosclerotic Severe Stenosis

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Background: intimal cell hyperplasia due to smooth muscle cell proliferation is the dominant cellular event in the restenosis process.

Aims: The aim of this study was to evaluate the effect of Synthetic Antisense Oligodeoxynucleotides - loaded microbubbles- mediated

transfection therapy using focused shock waves on inflammation and intimal hyperplasia reduction after laser angioplasty in the hamster abdominal aorta model of atherosclerotic severe stenosis, wherein diagnostic B- mode ultrasound is combined with transfection therapy system, with a goal of increased safety.

Methods: Briefly, Golden Syrian hamsters were submitted to advanced atherosclerotic severe stenosis by primary intravascular radiofrequency (F= 23.7 MHz, P= 10 W) thermal balloon angioplasty- mediated endothelial injury at the abdominal aorta (approximately 0.5 cm superior to the iliac bifurcation) followed by a 1.5% cholesterol- rich diet injury for 12 weeks. Histopathology results showed the formation of advanced atherosclerosis with lipid and neovessel- rich plaque that resulted to severe stenosis (> 70%) in all of the hamsters′ arteries. Then treatment group underwent ArF excimer laser (193 nm) angioplasty followed by shock wave (V= 15 Kv, F= 2 Hz, Impulses= 50)- mediated gene transfection therapy in combination with simultaneously intravenous Synthetic Antisense Oligodeoxynucleotides - loaded PESDA (Perfluorocarbon- Exposed Sonicated Dextrose Albumin) microbubbles (100ml/kg, 2-5 ×105 bubbles/ml) administration.

Results: Results from histopathology showed a significant reduction in the mean value for macrophages and smooth muscle cells density in the transfection therapy group compared with the other groups ($p< 0.05$).

Conclusions: Focused shock wave -mediated transfection therapy with Synthetic Antisense Oligodeoxynucleotides- loaded PESDA microbubbles can cause to reduce the intimal smooth muscle cells and significantly dilate the luminal cross- sectional area. Focused shock wave - mediated transfection therapy is a feasible and efficient method for the vascular neointimal hyperplasia reduction.

PB0025 | Effect of Phosphomimetic Endothelial Nitric Oxide Synthase - Loaded Microbubbles-Mediated Transfection Therapy using B-Mode Ultrasound-Guided Focused - Low Level Confocal Dual-Pulse Electrohydraulic Shock Waves on Early Stage Atherosclerosis

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Background: In atherosclerosis, local inflammation and associated macrophage activity can lead to foam cell- rich plaque formation, which results to plaque rupture and thrombosis, making inflammation an important therapeutic target in stroke and cardiovascular disease. Foam cells are generated by uncontrolled uptake of modified LDL, especially oxidized LDL (oxLDL).

Aims: In this study, we developed an experimental transfection therapy protocol using focused- low level confocal dual -pulse electrohydraulic shock waves and investigated its effectiveness on macrophage foam cells density reduction, wherein diagnostic B- mode ultrasound is combined with shock wave therapy system, with a goal of increased safety.

Methods: Briefly, Golden Syrian hamsters underwent primary balloon dilatation injury at the abdominal aorta followed by a 1.5% cholesterol-rich diet injury for three weeks. Histopathology results showed the formation of early stage atherosclerosis in all of the hamsters′ arteries. Then animals' arteries in the treatment group at the lesion region, treated using Phosphomimetic Endothelial Nitric Oxide Synthase - loaded microbubbles- mediated transfection therapy using

B-mode ultrasound- guided focused- Low level confocal dual- pulse electrohydraulic shock waves (V= 15 Kv, F= 2Hz, Impulses= 50 and V= 10 Kv, F= 0.5Hz, Impulses= 150). Foam cells density were evaluated in the treatment group compared with the control group using B-mode ultrasonography and histology.

Results: Results from B-mode ultrasonography and histopathology showed a significant reduction in the mean value for foam cells density within the early atherosclerotic lesion in the treatment group compared with the control group ($p< 0.05$).

Conclusions: Enhanced sonoporation therapy effect of shock waves, induced by inertial cavitation effect of microbubbles accompanied by Phosphomimetic Endothelial Nitric Oxide Synthase - loaded microbubbles administration can cause to reduce the foam cells density within the lesion and significantly enhance of protein expression and nitric oxide synthesiss. This protocol is a feasible and efficient method for the vascular early stage atherosclerosis regression.

PB0026 | Intravenous Lipid-Based Encapsulated Paclitaxel Nanoparticles Administration Enhances 5-Aminolevulinic Acid-Mediated B- Mode Ultrasound- Guided Focused- Electrohydraulic Shock Wave Sonodynamic Therapy of Intermediated Stage Atherosclerosis

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Background: Intermediate stage atherosclerosis is highlighted by infiltration of oxidized low-density lipoprotein (LDL) and foam cell formation, resulting in the thickening of arterial walls and decreased arterial lumen space.

Aims: In this study, we aimed to investigate the effect of 5-aminolevulinic acid- mediated sonodynamic therapy using focused- electrohydraulic shock waves accompanied by Intravenous lipid- based encapsulated paclitaxel nanoparticles administration on intermediate stage atherosclerosis regression, wherein diagnostic ultrasound imaging system is combined with the therapy system, with a goal of increased safety.

Methods: Briefly, Golden Syrian hamsters underwent endothelial denaturation using ballooning at the abdominal aorta, before being fed a 1.5% cholesterol-rich diet. After four weeks, the histopathology

results showed macrophages foam cells-rich plaque formation, resulting in vessel wall thickening and intermediate stage atherosclerosis formation in all of the hamsters' arteries. Then treatment group underwent 5-aminolevulinic acid- mediated sonodynamic therapy using extracorporeal low level focused- electrohydraulic shock wave (V= 15 Kv, F= 2Hz, Impulses= 50) accompanied by simultaneously intravenous lipid- based encapsulated paclitaxel (10mg/Kg) nanoparticles administration.

Results: Results from ultrasonography and histopathology showed a significant reduction in the mean value for foam cells density, lumen wall mean thickness and percentage of luminal cross- sectional area of stenosis, in the treatment group compared with the other groups ($p< 0.05$).

Conclusions: Enhanced toxicity effect of paclitaxel and 5-aminolevulinic acid, induced by sonodynamic therapy effect of low level focused- electrohydraulic shock waves accompanied by enhanced sonoporation therapy effect of shock waves, induced by inertial cavitation effect of capsules and microbubbles, can cause to intermediate stage atherosclerosis regression and significantly dilate the luminal cross- sectional area of stenosis.

PB0027 | Immuno-Radio-Metric TSH in TRH Test of Optimal Substitutive Thyroid Hormones

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Background: Polymorphism of deiodinase is genetically determined but mean optimal dosage of substitutive treatment by thyroid hormones attemps to determine preventive iodine request against arteriosclerosis.

Aims: 92 trh tests for optimal iodine request.

Methods: Prospective study of levothyroxine treatment in 52 primary hypothyroidisms.

immuno-radio-metric tsh after trh test in the duration of an internship-residentship-assistantship of 30 months in college de medecine des hopitaux de paris..

setting hopital lariboisiere in paris :2 rue ambroise pare 75475 cedex 10 france.

Results: An early stage of weak michaelis constant of deiodinase turn over supervenes as stigmate of iodine deficient request in grades of bio-clinic classification of primary hypothyroidism.

Conclusions: Definition of incipient myxedema as obeso genesis grade

PB0028 | Association of MMP-12 and SDF1 with Symptomatic and Asymptomatic Atherosclerosis

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Background: Matrix metalloproteinase-12 (MMP-12) can lead to plaque destabilization and rupture of atherosclerotic plaques. Upregulation of stromal cell-derived factor (SDF1) has been shown to play a role in various cardiovascular processes.

Aims: The goal of this study was to determine SDF1 and MMP12 to evaluate cardiovascular risk and atherotrombotic events in patients with cardiovascular risk factors.

Methods: We enrolled 298 patients, median age: 66 year-old (range 45-90 years old), 70.8% male. Cardiovascular risk factors, presence of atherosclerotic plaques by arterial ultrasound (carotid, femoral and aorta), and previous cardiovascular events: heart attack, stroke, peripheral artery disease (PAD) and nephropathy were recorded. Patients were classified in two groups according to the presence or absence of previous cardiovascular events.

Serum MMP12 and SDF1 were determined by ELISA (LSBio and R&D respectively) and CRP on automated analyzer. Firstly, we evaluated the correlation of classical inflammatory biomarkers and new biomarkers: MMP12 and SDF1. Thereafter, we established the association between this parameters and cardiovascular events.

Medical ethics committee approved the study and all patients signed informed consent.

Results: We found a correlation between SDF1 and PCR ($r=0.42$, $p< 0.001$) and MMP12 and CRP ($r= 0.31$; $p< 0.001$). Compared with asymptomatic subjects (that includes patients with and without atherosclerotic plaques), those patients who had a previous event had higher levels of SDF1 (2706 pg/mL vs 2309 pg/mL, $p=0.009$) and MMP12 (501 pg/mL vs 357 pg/mL, $p=0.016$). Association was detected between SDF1 and stroke ($p=0.042$) and with nephropathy ($p=0.004$) and between MMP12 and nephropathy ($p=0.05$), after adjusting by age and sex. Finally, those patients who had plaques in three vascular territories at the same time presented higher levels of SDF1 (2364 pg/mL vs 2538 pg/mL, $p=0.025$), independently of age and sex.

Conclusions: SDF1 and MMP12 were associated with clinical atherosclerosis, thus, offering additional information on top of classical inflammatory biomarkers.

PB0029 | Antioxidant Defense Gene Polymorphism in the Nenets Population

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Background: Currently, there is a large amount of literature data indicating the violation of antioxidant balance and the development of oxidative stress in various diseases. It is known that in certain forms of cardiovascular disease, the activity of antioxidant enzymes, such as superoxide dismutase, increases, which plays a key role in reducing oxidative stress in vivo, that is, it is a protective factor. Impaired function of this enzyme leads to an increase in the number of free radicals in the cell. The article was prepared as part of a study supported by a grant from the Russian Foundation for Basic Research, project No. 18-00-00814-COMFI (18-00-00478).
Aims: To study the polymorphism of the SOD1 G7958A and SOD2 Ala16Val gene in the Nenets population.
Methods: The object of study is the indigenous population of the island village of Varnek (n = 44). The age of the sample is 25 [17.3; 51.6] years.
The determination of gene polymorphism was carried out during molecular genetic analysis using the polymerase chain reaction method using allele-specific primers with agarose gel electrophoresis detection.
Results: In our study, the polymorphism of the SOD1 G7958A and SOD2 Ala16Val genes was analyzed in a sample of the indigenous ethnic group (n = 44).
In this population, the GG genotype of the SOD1 G7958A gene is found in 79.5% of the sample, GA - 18.2%, AA - 2.3%. The Ala / Ala genotype of the SOD2 gene is found in 12%, Ala / Val - 31.2%, Val / Val - 56.8%.
Conclusions: Peculiarities of the prevalence of SOD enzyme polymorphism in the island population of Nenets were revealed.

PB0030 | Diabetes Mellitus and Risk of Intracranial Atherosclerosis: A Meta-Analysis Using Real-World Evidence

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Background: Intracranial atherosclerosis is one of the most common cause of stroke worldwide. There is strong association exists between diabetes and cardiovascular disease. Coronary artery disease due to atherosclerosis is the leading cause of death in diabetic patients and imposes significant burden on the patients and the society. Evaluation and prevention of intracranial atherosclerosis is important.
Aims: Therefore, in this study, we are exploring the risk of intracranial atherosclerosis due to diabetes mellitus.
Methods: The literature search was performed in databases including PubMed and Embase from inception to December 2019. Two investigators independently retrieved the literature, extracted the data and assess the study quality using the Newcastle-Ottawa scale. The primary outcome was to assess the association between diabetes mellitus and risk of intracranial atherosclerosis. The secondary outcomes include subgroup analysis based on ethnicity, study populations, and co-morbidities. Review Manager version 5.3 was used for the statistical analysis.
Results: Finally, twelve studies qualified for quantitative analysis with a total of 18067 patients. Included studies were of high quality. A non-significantly higher association was observed for the association between diabetes mellitus and risk of intracranial atherosclerosis with a pooled relative risk (RR) of 1.98 (95% CI: 1.67-2.30), p =0.07 as compared to patients without diabetes mellitus. A significant association was observed among community-dwelling diabetes mellitus patients with a RR of 1.95 (95% CI: 1.53-2.50), p = 0.01. Subgroup analysis based on stroke patients also revealed no significant association with a pooled risk ratio of 2.14 (95% CI: 1.74-2.52), p = 0.96.
Conclusions: Diabetes mellitus might be a risk factor for intracranial atherosclerosis. A significant association was observed in community-dwelling people with diabetes. Further relevant research is needed to make the evidence transparent.

PB0031 | In vitro Occlusive Clot Growth and Retraction

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Background: Occlusive thrombi can rapidly form under high shear rates to cause a heart attack or stroke. The growth (Casa and Ku (2017)) and contraction (Muthard and Diamond (2012)) of thrombi have been investigated in previous studies and can occur when an atherosclerotic plaque ruptures within a few minutes making it an acute life-threatening process.
Aims: We study the influence of shear rate and the presence of red blood cells on the platelet adhesion, aggregation and clot retraction.
Methods: Two microfluidic flow systems with a stenosis were perfused with porcine whole blood (WB) or platelet-rich plasma (PRP) at high shear rates (3,000-13,000 s^{-1}). The systems were driven by a constant pressure and coated with type I collagen. Outflow mass data and microscopic images were used to determine the lag times, occlusion times, rapid platelet accumulation (RPA) rates, and retraction rates.

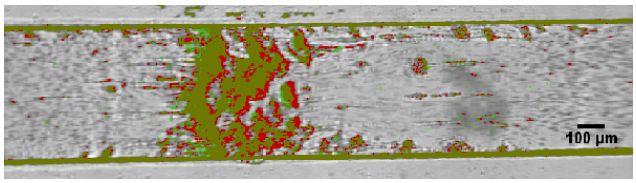

FIGURE 1 A thrombus at the time of occlusion (red) and at the end of the experiment (green). It retracted mostly downstream, where the red color is present

Results: WB occlusion times were similar to previously reported values. PRP showed a significantly longer lag time and lower RPA rate compared to WB in both devices that resulted in a long occlusion time ($p < 0.01$). In PRP, retraction of the clot was clearly visible upon flow arrest, being more pronounced downstream with a retraction rate of $7.2 \cdot 10^{-4}\ s^{-1}$ (see Fig. 1).

Conclusions: The longer lag time and lower RPA rate in PRP may indicate that margination plays an important role in the lag and RPA phase of thrombus formation. The retraction rate was equal for all PRP experiments which suggests retraction is independent of the flow environment in which the clot has been formed.

PB0032 | Selective Targeting of Shear Gradient Activated Von Willebrand Factor by the Novel Single-Chain Antibody Scfv-A1 Reduces Occlusive Thrombus Formation

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Background: A major limitation of current antithrombotic drugs is their inability to distinguish between normal haemostasis and thrombosis resulting in limited efficacy or bleeding complications. Recent studies have shown that the prothrombotic state of large intraluminal thrombi is in part driven by the local blood shear rate gradients (SRGs). SRGs are generated by the thrombus itself due to its three-dimensional geometry and are significantly more prothrombotic than constant shear rates. SRG-driven pathological thrombus formation is mediated by a mechanosensitive interaction between Von Willebrand Factor (VWF) A1 domain and platelet receptor GPIbα.

Aims: To selectively prevent thrombus formation without interfering with haemostasis by designing a single-chain antibody (scFv) targeting a shear-gradient specific conformation of the VWF-A1 domain.

Methods: Microfluidic flow channels containing straight and circular stenotic sections were fabricated in-house to generate constant shear and shear rate gradients respectively during blood perfusion. The ability of our SRG-sensitive single-chain antibody (scFv-A1) to target SRG-specific VWF-GPIbα interactions and inhibition of thrombus formation was evaluated.

Results: Enhanced VWF-GPIbα binding was observed at sites of SRGs compared to sites with constant shear rates of the same magnitude. ScFv-A1 reduced VWF-GPIbα binding and thrombus formation selectively at sites of SRGs without attenuating platelet aggregation in channel sections with constant shear rates unlike the control GPIbα inhibitor OS-1 which inhibited platelet aggregation under all shear conditions. Notably, the antibody did not directly bind to VWF or inhibited the VWF-GPIbα interaction in the absence of shear as observed in ristocetin agglutination assays and BLItz biolayer interferometry.

Conclusions: Building on the knowledge of prothrombotic effects of blood shear rates, we have produced an SRG-sensitive single-chain antibody (scFv-A1) that inhibits thrombus formation particularly under SRGs barring constant shear rates. These studies support the concept that thrombosis may be mechanically uncoupled from haemostasis and presents scFv-A1 as a candidate for more efficacious and safer anti-thrombotic therapy.

PB0033 | Trash Feet: Is Anticoagulation Therapy Useful?

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Background: Trash feet is a rare, but serious, disorder occuring in patients with advanced atherosclerosis due to cholesterol crystal embolisation. Given the relatively low incidence of the condition, and the lack of evidence, there is no consensus on the optimal management of this condition. We herein describe a patient who presented with trash feet and was successfully treated with anticoagulation.

Aims: We aim to illustrate the importance of promptly recognising the condition, review the literature and discuss the current treatment options available.

Methods: We report here a 78-year old man who presented with acute-onset spontaneous purplish discolouration of multiple toes of bilateral feet. He had a significant medical history of hypertension, dyslipidemia, ischemic heart disease and chronic kidney disease. On examination he had hypertension (BP 167/93 mmHg), intact bilateral femoral and dorsals pedis arterial pulsations. There was evidence of livedo reticularis over his legs with purplish discolouration of multiple toes of both feet. A CT scan abdominal aortogram done in the recent past was suggestive of severe diffuse atherosclerotic plaques involving thoracic-abdominal aorta and an ulcerated atherosclerotic plaque in descending abdominal aorta.

Results: He was initially started on aspirin and enoxaparin, and was subsequently transitioned to warfarin, with which the feet symptoms got better, and serum creatinine improved to baseline. On follow-up at two weeks, serum creatinine had remained stable and the discolouration of the toes had improved significantly.

Conclusions: Trash feet is a rare disorder, which is frequently underdiagnosed. Prompt diagnosis is necessary, and is aided by strong clinical suspicion. Although treatment with anticoagulation has been shown to be controversial, it appears from this report that anticoagulation has a positive role in the management of patients during the acute phase of cholesterol embolisation syndrome.

PB0034 | Impact of Gut Microbiota on Late Atherosclerosis and Atherothrombosis: A Study with Germ-Free Mice

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Background: Dependent on age, Western lifestyle is associated with the onset of cardiovascular diseases and with a dysbiotic gut microbiota. Excessive cholesterol uptake from the diet drives the formation of atherosclerotic lesions. The rupture of an atherosclerotic plaque, with subsequent thrombus formation, is defined as atherothrombosis. Although commensal microbes promote cholesterol excretion, a dysbiotic gut microbiota can trigger low grade inflammation. So far, the effect of this environmental factor on atherosclerosis is poorly investigated.
Aims: To study the impact of the gut microbiota on late atherosclerosis and its major complication atherothrombosis using a germ-free (GF) mouse model of hypercholesterolemia.
Methods: Low-density lipoprotein receptor-deficient (*Ldlr*$^{-/-}$) GF mice were compared to conventionally raised (CONV-R) *Ldlr*$^{-/-}$ controls, and kept on a high-fat diet (HFD) for 16 weeks. Thereafter, lipoprotein profiles were measured by liquid chromatography and atherosclerotic plaques size in the common carotid artery, aortic root and aortic arch was evaluated by histology. Platelet adhesion and adhesion-induced platelet activation was quantified *ex vivo*, with a standardized flow chamber model, perfusing anticoagulated blood over collagen coatings. Statistical analyses were performed with the independent samples Student's t-test (age- and sex-matched group).
Results: At HFD conditions, the gut microbiota did not affect total cholesterol levels. Accordingly, lesion sizes in the common carotid artery, but also in the aortic root and the aortic arch of GF and CONV-R *Ldlr*$^{-/-}$ mice were comparable in late atherosclerosis. In contrast, the intestinal microbiota promoted atherothrombosis, as CONV-R *Ldlr*$^{-/-}$ mice exhibited a vascular inflammatory phenotype and increased atherosclerotic plaque thrombogenicity. In the flow chamber model, increased platelet deposition was paralleled by enhanced adhesion-induced platelet activation on type III collagen coatings.
Conclusions: Our study demonstrates a prothrombotic role of the gut microbiota in late atherosclerosis. Targeting the metabolic capacity of the gut microbiota to modulate platelet function could result in new therapeutic interventions to reduce atherotrombotic complications.

PB0035 | *In vitro* Investigation of the Impact of BCR-ABL Tyrosine Kinase Inhibitors on Endothelial Cells

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Background: New-generation BCR-ABL tyrosine kinase inhibitors (TKIs) used in chronic myeloid leukemia (CML) induce cardiovascular events. However, the mechanism(s) by which these treatments induce these events is currently not clear. First *in vitro* investigations indicate that they influence the viability of endothelial cells, potentially explaining the occurrence of cardiovascular events.
Aims: This research aims to specify the mechanism(s) by which BCR-ABL TKIs affect endothelial cell viability.
Methods: Three concentration of the five BCR-ABL TKIs have been tested on Human Umbilical Venous Endothelial Cells (HUVECs). All experiments have been performed in media with 10% dialyzed FBS in order to minimize the effect of plasma protein binding. MTS and LDH assays were performed to assess the effect of TKI on cell viability. Rate of apoptotic and necrotic cells have been measured using Annexin V staining, and BrdU assay assessed the effect on cell proliferation. Generation of reactive oxygen species (ROS) was evaluated using a specific fluorescent probe (CM-H2DCFDA) by flow cytometry.
Results: All BCR-ABL TKIs affect endothelial cell viability after 72h of treatment. However, their impact on HUVECs are different according to the TKI. Ponatinib induces apoptosis and necrosis of endothelial cells (Figure). Nilotinib induces necrosis but at high dose only (2μM). Dasatinib increases the generation of ROS by HUVEC. Finally, all BCR-ABL TKIs dose-dependently influence HUVEC proliferation as early as 24h of treatment (decrease of number of cells in S-phase and increase of HUVEC in G0-G1 phase). However, this impact is significant only at the highest concentration for imatinib, nilotinib and bosutinib.
Conclusions: Cell death within the arterial wall has already been recognized in atherosclerosis, and it appears plausible that dasatinib,

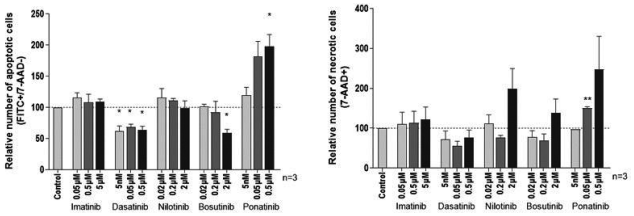

FIGURE 1 Apoptosis and necrosis of HUVEC after treatment with BCR-ABL TKIs

nilotinib and ponatinib facilitate atherosclerosis development through alteration of endothelial cell viability.

PB0036 | *In vitro* Evaluation of the Impact of BCR-ABL Tyrosine Kinase Inhibitors on Atherosclerosis Plaque Rupture

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Background: Some BCR-ABL TKIs (dasatinib, nilotinib and ponatinib) increase the risk of arterial thromboembolism in CML patients compared to the first generation TKI, imatinib. The pathophysiology of these events remains poorly understood but clinical data suggest that these treatments might induce atherosclerotic plaque rupture. The stability of atherosclerotic plaque correlates to the thickness of its fibrous cap. A major mechanism inducing fibrous cap thickening is the production of matrix metalloproteinases (MMPs) by macrophages that degrade fibrous cap components.
Aims: This research aims to determine the effect of BCR-ABL TKIs on the production of two MMPs highly involved in atherosclerotic plaque rupture (MMP-2 and MMP-9).
Methods: The THP-1 cell line differentiated to macrophages using PMA at 100ng/mL during 24h was used to assess the effect of TKIs on human macrophages. The THP-1 cells were treated with the 5 BCR-ABL TKIs at 3 clinically relevant concentrations during 24h and 48h. Macrophage viability was assessed using MTS and LDH assays and the production and activity of MMP-2 and MMP-9 were evaluated by gelatin zymography.
Results: The 5 BCR-ABL TKIs possess few impact on THP-1 viability. MTS and LDH assays demonstrate decreased macrophage viability with nilotinib at high concentration (2μM).
Gelatin zymography demonstrates no impact of TKI on MMP-2 and MMP-9 production after 24h of treatment. After 48h, imatinib at the highest dosage (5μM) increases production of activated MMP-9. Treatment with nilotinib at 2μM decreases the production of activated MMP-9, which might be the consequence of decreased macrophage viability. Other BCR-ABL TKIs do not affect the production and activity of MMP-2 and MMP-9 by THP-1.
Conclusions: BCR-ABL TKIs have little impact on macrophage survival as well as on MMP production and activity and suggest that vascular occlusive events are probably not mediated by impact on macrophages.

PB0037 | Evaluation of the Selectivity of BCR-ABL Tyrosine Kinase Inhibitors (TKIs): A Systematic Review

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Background: Although chronic myeloid leukemia is now a treatable chronic disease, patients need to take BCR-ABL tyrosine kinase inhibitors (TKIs) over long periods. Regrettably, there is growing evidence of cardiovascular adverse events associated with new-generation BCR-ABK TKIs. Due to the lack of specificity for BCR-ABL, especially for new-generation TKIs, acquiring selectivity profiles is from the utmost importance.
Aims: This systematic review aims to summarize all available data from the literature related to the selectivity of BCR-ABL TKIs to emit hypotheses about inhibited tyrosine kinases that might be involved in cardiovascular events.
Methods: Two independent reviewers screened titles and abstracts according to a predefined search strategy through Pubmed, Scopus and Cochrane Library. Included articles are those describing at least one TKI off-target for at least one of the five BCR-ABL TKI. Extraction of the results of the included studies have been performed by the two reviewers independently in a standardized extraction form.
Results: The screening of 2,850 articles led to the identification of 27 studies that assessed at least one BCR-ABL TKI off-target. Through these 27 studies, 465 kinases have been tested. Each BCR-ABL TKI has a specific inhibitory profile. Imatinib is the most selective and the most studied. This review pointed out discrepancy on 210 tyrosine kinases and considerable diversity in methods used to study TKI specificity.
This review identifies tyrosine kinases potentially implicated in the cardiovascular diseases that occurred during new-generations TKIs treatment. Among the tyrosine kinases inhibited by the new-generation TKIs but not by imatinib, the ephrin family and the tyrosine kinase HCK are implicated in atherosclerosis: EphA2 promotes endothelial cell inflammatory response whereas HCK is implicated in atherosclerosis plaque formation.
Conclusions: The review points out discrepancy in inhibitory profiles of BCR-ABL TKIs from 27 experimental studies and considerable diversity in methods used to study TKI specificity.

PB0039 | The Role of ERp44 in the Atherosclerosis

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Clinical Lab of Ruijin Hospital, Shanghai, China

Background: The endoplasmic reticulum (ER) is an important organelle involved in protein synthesis and folding, calcium homeostasis, and lipid biosynthesis. Recent findings indicate that atherosclerosis may be associated with impaired function of the ER. The ER resident protein 44 (ERp44) is a member of the protein disulfide isomerase family, which is induced by ER stress. However, the physiological role of ERp44 in atherosclerosis is still unclear.

Aims: To study the role of ERp44 in the atherosclerosis processes.

Methods: Thirty C57BL/6 mice and 90 adult ApoE$^{-/-}$ C57BL/6 mice were used as animal model in immunohistochemistry, real-time PCR and western blot (WB). HUVEC, THP-1 and HASMC were used as *in vitro* cell models in apoptosis, angiogenesis and migration assay.

Results: Immunohistochemical staining showed that a large amount of ERp44 expression in the aortic root of ApoE$^{-/-}$ high-fat diet mice at 6 weeks (Fig 1A). Similar results were detected by PCR and WB (Fig 1BC).

Apoptosis induced by ox-LDL was inhibited in HUVECs with high expression of ERp44 significantly. It showed that ERp44 inhibited the apoptosis by inhibiting Bax and p53 and promoting the expression of Bcl-2 (Fig 2A).

It showed that angiogenesis inhibited by ox-LDL could be partially rescued by ERp44 through promoting the expression of PDGFRβ, VEGFR2 and EGFR (Fig 2B).

It showed that over-expression of ERp44 in THP-1 could reduce the number of foam cells by promoting expression of cholesterol reverse proteins (ABCA1 and ABCG1) (Fig 2C).

The increasing expression of cdc42 and AAMP might be the main reason for the enhancement of THP-1migration by ERp44 (Fig 2D).

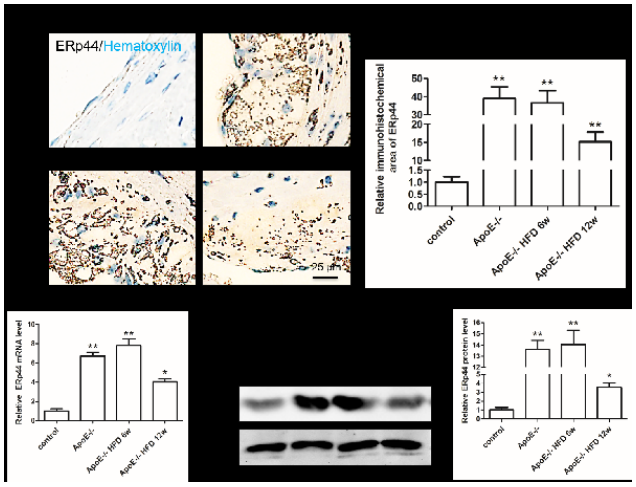

FIGURE 1 ERp44 expression in the aortic root of ApoE-/- high-fat diet mice

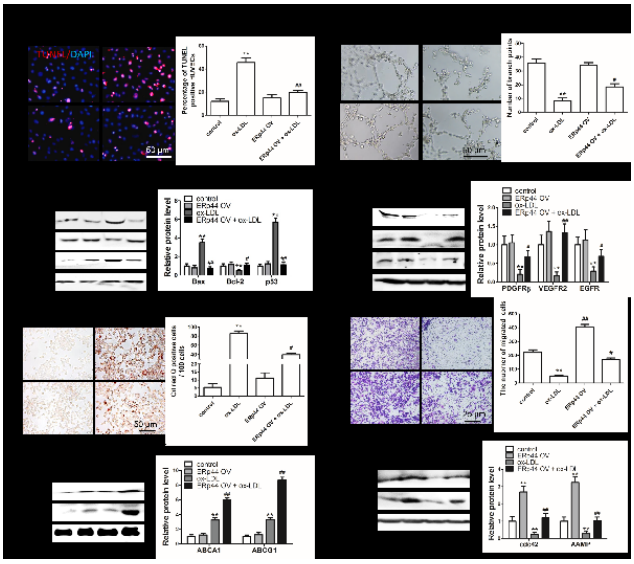

FIGURE 2 In vitro cell assays

TUNEL staining showed over-expression of ERp44 significantly reduced the apoptosis of HASMC induced by ox-LDL, which was achieved by inhibiting Bax and p53, and promoting the expression of Bcl-2.

Conclusions: We found that ERp44 played an active role in the early and middle stages of atherosclerosis formation.

PB0040 | Importance of Arginase-1 in Erythrocytes for Smooth Muscle Cell Calcification and Atherosclerosis

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Background: Experimental evidence suggest that erythrocytes (red blood cells, RBCs) participate in vascular homeostasis and disease, however, the mediators underlying their erythrocrine activities are largely unknown. Our group has recently shown that nitric oxide (NO) released from erythrocytes promotes vascular calcification. Production of NO by eNOS is counteracted by arginase-1 (Arg1) and competition for their common substrate, L-arginine.

Aims: To study the importance of erythrocyte arginase-1 in atherosclerosis.

Methods: Mice lacking Arg1 in erythrocytes were generated by crossing mice expressing Cre recombinase under control of the erythropoietin promoter with Arg1 flox/flox transgenic mice on the apolipoprotein E-deficient background (RBC.Arg1 knockout, KO). At 6 weeks-of-age, mice were switched to Western type diet for 20 weeks.

Results: Molecular imaging of the aortic tree revealed a greater osteogenic activity in RBC.Arg1-KO mice compared to RBC.Arg1 wild-type littermates. Alizarin S staining confirmed increased vascular calcification in the absence of Arg1 in erythrocytes, and co-incubation of smooth muscle cells with RBC.Arg1-KO erythrocytes increased their osteogenic differentiation in a NO-dependent manner. On the other hand, Oil Red O staining of whole aorta *en face* preparations revealed a reduced total atherosclerotic plaque burden in RBC.Arg1-KO compared to RBC.Arg1-WT mice. In this regard, mice lacking Arg1 in erythrocytes exhibited lower blood pressure levels compared to wild-type controls, and quantitative *real-time* PCR analysis of primary endothelial cells revealed differential regulation of genes involved in the control of blood pressure, leucocyte trafficking, inflammation and coagulation.

Conclusions: Our findings identify an important contribution of arginase-1 and erythrocyte-derived NO to atherosclerotic lesion calcification. They also underline the close interaction and cross-talk between erythrocytes and endothelial cells at the interface of the blood and vessel wall.

PB0041 | Absence of Protein Tyrosine Phosphatase-1B in Smooth Muscle Cells Promotes Perivascular Fibrosis: Role of Receptor Tyrosine Kinase Signaling

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Background: Perivascular fibrosis, the deposition of extracellular matrix around blood vessels, reduces vascular compliance and impairs blood flow thus contributing to cardiac and vascular dysfunction and disease. Targeting perivascular fibrosis might have therapeutic potential for cardiovascular disease processes. However, the mechanism underlying perivascular fibrosis are incompletely understood.

Aims: To determine the importance of protein tyrosine phosphatase-1B (PTP1B), a negative regulator of tyrosine kinase receptors, for perivascular fibrosis.

Methods: Mice with smooth muscle cell-specific deletion of PTP1B (SMC.PTP1B-KO) were generated by crossing mice transgenic for Cre.ERT2 recombinase under control of the SMMHC promotor with PTP1B flox/flox transgenic mice. Vascular injury and neointima formation was induced in 12-16 week-old male mice at the carotid artery using 10% ferric chloride. For functional and mechanistic analyses in vitro, primary smooth muscle cells were isolated from the aorta of male SMMHC.Cre wild-type mice.

Results: Histochemical analyses revealed that absence of PTP1B in SMMHC-positive cells resulted in a significant increase in the adventitia area (P=0.004) and thickness (P< 0.001) following vascular injury. Moreover, a significant increase in the total number of adventitial cells (P=0.002) and higher numbers of PCNA-positive, proliferating cells (P=0.009) were observed. Histochemical characterization revealed a greater percentage of cells expressing smooth muscle alpha-actin (SMA; P< 0.0001) and platelet-derived growth factor (PDGF) receptor-beta (P=0.004) in the adventitia of SMC.PTP1B-KO mice. Also, larger amounts of interstitial collagen (P=0.012) were observed, whereas the number of cells expressing the pericyte marker NG2 did not differ (P=0.460). In vitro, exposure of murine primary aortic smooth muscle to hypoxia in the presence of a chemical PTP1B inhibitor resulted in increased HIF1alpha and PDGF protein levels.

Conclusions: Our findings suggest that absence of PTP1B in smooth muscle cells may promote adventitia enlargement and perivascular fibrosis under hypoxic stress by increasing the expression of PDGF and subsequent proliferation and differentiation of smooth muscle (progenitor) cells.

PB0042 | Relationship between Distal Venous Thrombosis and Asymptomatic Atherosclerotic Disease

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Background: Several studies have been describing a link between venous and arterial thrombosis, particularly patients with spontaneous VTE have been found to have a higher prevalence of atherosclerosis than patients with secondary VTE. Moreover, VTE and cardiovascular disorders seem to share common risk factors.

Aims: To evaluate patients with isolated distal venous thrombosis who were often excluded from the previous studies. While it is still debating whether to anticoagulate these patients or not, if this condition is related to a higher risk of atherosclerosis is unknown.

Methods: We assessed the ultrasonography of the carotid arteries and the ankle-brachial index (ABI) measurement in 116 patients who had deep venous distal or proximal thrombosis of the legs without symptomatic atherosclerosis and in 57 control subjects. Each group has been assessed for plaques, intima-media thickness (IMT) and ABI.

Results: The mean intima-media thickness of the common carotid arteries was 1.00 ± 0.20 mm in patients with distal spontaneous thrombosis, 0.98 ± 0.16 in those with proximal spontaneous thrombosis, and 0.88 ± 0.15 in the control subjects.

Patients with a distal spontaneous thrombotic history showed an abnormal mean IMT (> 0.9mm) compared to the controls (odds ratio 2.22; 95 percent confidence interval 1.04 to 4.74; P=0.0374), similar results for the comparison between the proximal spontaneous thrombosis and the controls (odds ratio 2.75; 95 percent confidence interval 1.28 to 5.91; P=0.009). When taking into account the presence of carotidal plaques or a pathologic ABI we found an higher prevalence in patients with a history of thrombosis, regardless whether distal or proximal, even though not statistical significant.

Conclusions: There is an association between spontaneous venous thrombosis and atherosclerotic disease, even in case of a distal isolated thrombosis. Despite the debated indication for anticoagulation in these patients, it could be reasonable to assess their cardiovascular risk factors.

PB0043 | Un Untargeted Lipidomic Analysis Reveals Lactosylceramide as Mediator of Monocyte Migration in Coronary Artery Disease Patients

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Background : Atherothrombosis, defined as atherosclerotic plaque rupture with overlaid thrombosis, is the major cause of coronary artery disease. Atheroma progression involves circulating monocytes recruitment and arterial wall infiltration. Different circulating stimuli, such as lipids, may alter monocytes physiological state, inducing a cellular reprogramming and thus influencing their functionality. Several studies investigated the mechanisms by which circulating lipids and lipoproteins affects the monocyte functions, but no information about the relationship between cellular lipid composition and monocyte functions are available.

Aims: To delineate the lipidomic profile of circulating monocytes isolated from acute myocardial infarction (AMI) patients and healthy subjects (Ctrl). The possible correlation between the lipid composition and monocyte migration has been evaluated.

Methods: Mononuclear cells were obtained from whole blood of 41 AMI patients (62.9±11.7 years) and 23 Ctrl (62.0±5.1 years) by negative magnetic depletion. The lipidomic profile was evaluated through an untargeted lipidomic approach. Monocyte migration assay was performed using fetal bovine (FBS) or autologous serum as chemotactic stimuli. Study complies with the Helsinki Declaration.

Results: The lipidomic analysis revealed a different lipid profile of AMI monocytes compared to Ctrl ones. In particular, AMI monocytes showed an altered lipid composition in term of lactosylceramide, that several studies demonstrated to be involved in neutrophil migration. Furthermore, AMI patients' monocytes exhibited an increase migration as compared to that of Ctrl. Notably, a positive correlation between the monocyte migration and lactosylceramide levels was observed. Moreover, the use of an inhibitor of lactosylceramide synthase, D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP), significantly reduced the monocyte migration induced by FBS.

Conclusions: The results evidence that AMI patients' monocytes have an altered lipidome with high level of lactosylceramide, which is involved in monocytes migration. These preliminary data may be useful to define new biochemical pathway involved in the progression of atherosclerosis, that could be considered as a target for treatment and/or prevention of coronary heart diseases.

PB0044 | Effect of BTK Inhibition on Platelet-mediated Inflammation in an Obese Rhesus Macaque Model of Early Atherosclerosis

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Background: Atherosclerosis not only results from lipid accumulation within the arterial wall but is also a chronic inflammatory disease. Aberrant leukocyte recruitment plays a pivotal role in atherogenesis; we posit that platelets promote leukocyte adhesion during inflammation and encourage inappropriate leukocyte recruitment in atherosclerosis. We hypothesize that selective targeting of kinase pathways of platelet activation may reduce inflammation associated with atherogenesis.

Aims: Investigate the effects of TEC kinase inhibition with the BTK inhibitor, ibrutinib. on endothelial inflammation and platelet-endothelial interactions in a non-human primate atherosclerosis model.

Methods: Two obese rhesus macaques on a high-fat diet for 2 years with detectable carotid intimal thickening were administered ibrutinib orally (10mg/kg/d) for 1 week. We performed *in vivo* molecular imaging of VCAM-1 and platelet GPIbα as surrogates for endothelial inflammation and platelet activation, respectively. Whole blood was collected on days 0, 2, and 7 for flow cytometry studies. Platelet aggregation in response to the GPVI-agonist CRP was measured using aggregometry. Complete blood counts, lipid levels, and hemostatic assessments were recorded.

Results: After 7 days of ibrutinib administration, molecular imaging of the bilateral carotid arteries showed dramatically decreased carotid signal for both VCAM-1 and GPIbα compared to vehicle. Flow cytometry showed reduced systemic platelet activation and secretion in response to CRP following ibrutinib administration, while platelet aggregation in response to CRP was abrogated compared to baseline. Ibrutinib had no measurable effect on complete blood counts; lipid levels; or prothrombin time and activated partial thromboplastin times.

Conclusions: In a non-human primate model of early atherosclerosis, BTK inhibition reduced endothelial inflammation and platelet activation and endothelial interaction *in vivo*, and decreased platelet activation and GPVI-mediated aggregation *in vitro*. These finding suggest that BTK may play a role in driving platelet-mediated inflammation contributing to atherogenesis. Targeting BTK may confer beneficial effects on both the inflammatory and thrombotic contributors to atherosclerosis.

PB0045 | Effect of Photobiomodulation Therapy on Arterial Biomechanical Parameters in a Rabbit Carotid Artery Model of Early Stage Atherosclerosis

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Background: Atherosclerotic plaque rupture, which gives rise to thrombosis, is the main cause of acute cardiovascular and cerebrovascular syndromes. It is critical to ensure from the treatment of atherosclerosis at the early stages and prevent disease progression. Several protocols of atherosclerosis treatment are introduced which can be categorized as two main groups: invasive and non-invasive. Besides, detection and evaluation methods are also different, divided into the above two same groups.
Aims: Taken together, current study was aimed to propose a non-invasive treatment (photobiomodulation therapy) protocol for early stage atherosclerosis. In order to evaluate this treatment protocol we used a non-invasive method based on computerized ultrasound image processing.
Methods: To begin, we first induced early stage atherosclerosis in the common carotid artery of New Zealand white rabbits by arterial injury, using high frequency electrical current accompanied by a 1.5% cholesterol- rich diet for eight weeks. Induction of early stage atherosclerosis was verified by pathology results in all of the rabbits′ arteries. After eight weeks, the treatment group received extracorporeal red laser (λ= 632 nm, P= 500 mw) irradiation for 20 minutes (photobiomodulation therapy) at the injury region. In the next step, the cholesterol- rich diet was terminated and the rabbits were given care for 4 weeks. Arterial biomechanical parameters at the site of injury, to be evaluated by a new computer based image processing and ultrasound were: resistance index, pulse index, radial strain and longitudinal strain.
Results: Our findings suggest the statistically meaningful decrease of resistance and pulse indexes and elevated radial and longitudinal strain of the treatment group in comparison with the other groups ($p< 0.05$).
Conclusions: Photobiomodulation therapy of early stage atherosclerosis diminishes inflammation and lipids at the site of injury combined with elevated nitric oxide (NO) synthesis. In this regard, this method recovers the endothelial performance and arterial biomechanical parameters.

PB0046 | Effect of Photodynamic Therapy on Arterial Biomechanical Parameters in a Rabbit Carotid Artery Model of Neointimal Hyperplasia

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Background: Smooth muscle cell proliferation and migration from the medial layer into the intima, following an intravascular damage like angioplasty, causes neointimal hyperplasia.
It is great importance to treat neointimal hyperplasia through early stages and vascular restenosis should be prevented. There exists a range of treatment protocols for neointimal hyperplasia which can be divided into two main groups: invasive and non-invasive. Accordingly, detection and evaluation methods are again too vast, divided into the above same main groups.
Aims: In this study, we aimed to introduce a novel non-invasive treatment (Photodynamic therapy) for neointimal hyperplasia reduction, for which the evaluation procedure (a new computerized ultrasound image processing) is be based on a non-invasive method.
Methods: In the current study, we first induced neointimal hyperplasia in the common carotid artery of New Zealand white rabbits by arterial injury following balloon angioplasty for two minutes. After 4 weeks pathology results confirmed the induction of neointimal hyperplasia in all the rabbits′ arteries. At the end of four weeks, we performed extracorporeal red laser (λ= 632 nm, P= 500 mw) irradiation for 20 minutes, accompanied by sensitive drug, 5-aminolevulinic acid (10mg/Kg) administration (Photodynamic therapy) at the injury site of treatment group. Next, the rabbits were care given for two weeks. Arterial biomechanical parameters at the site of injury to be evaluated by a new computer based image processing and ultrasound were: resistance index, pulse index, radial strain and longitudinal strain.
Results: Our findings suggest the statistically meaningful decrease of resistance and pulse indexes and elevated radial and longitudinal strain of the treatment group in comparison with the other groups ($p< 0.05$).
Conclusions: Photodynamic therapy recovers arterial biomechanical parameters by ameliorating inflammation and smooth muscle cells at the injury site.

CARDIOVASCULAR RISK FACTORS

PB0048 | The Level of SPECAM-1 and von Willebrand Factor during the Supraventricular Arrhytmia Episodes in Patients with Arterial Hypertension

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Background: The coexistence of any kind of arrhythmia and high level of the blood pressure may increase the risk of the thrombotic complication. Thus, thrombus formation is accompanied by the

changes of the levels of biomarkers of vascular affection, cell adhesion molecules, von Willebrand factor (vWF) etc.

Aims: To define the character of interrelationship between the episodes of supraventricular arrhythmias (SVT) and paroxysmal atrial fibrillation (PAF) and the changes of level and activity of von Willebrand factor (vWF) and soluble platelet endothelium cell adhesion molecule-1 (SPECAM-1) in high riskarterial hypertensive (AH) patients.

Methods: We have examined 594 patients with the 5 years period of bservation. Episodes of SVT and PAF were fixed in protocol. During the arrhythmical episodes, the initial levels of SPECAM-1 and vWF were measured.

Results: We have set the reliable increase of the probability of PAF (9.93% [7.98-12.19]) than the SVT (3.87% [2.47-575]) development, OR=2.74 [1.64-4.72] in patients with clinical course of arterial hypertension. There was not found the reliable changes in the levels of SPECAM-1 in the subgroups of SVT, PAF and AH without arrhythmia. However, multivariate ANOVA showed statistically significant differences in initial levels of vWF depending on the presence of arrhythmia. This parameter was significally higher in the group of AH and PAF than in the group of AH and SVT. In these both groups the initial level of vWF was significally higher than in the group with AH only
($p < 0.01$).

Conclusions: Study provides evidence that episode both SVT and PAF on the basis of AH are followed by the growth of coagulation activity which may affect the changes of functional state of endothelium and forward to the severity of vessel wall damage which contributes in acute cardiovascular complications.

PB0049 | The State of Carotids and the von Willebrand Factor Levels in Patients with Arterial Hypertension

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Background: ATHEROREMO-IVUS trial has proved that the increased level of von Willebrand Factor (vWF) is associated with the growth of cardiac and non-cardiac mortality in chronic coronary artery disease (CAD). In addition, the investigation showed the reliable links between the activity of vWF and the atherosclerotic plaque state in coronary vessels.

Aims: To found possible association between the basal vWF activity and the local state of vessel wall in patients with arterial hypertension without concomitant CAD.

Methods: We have studied the intima-media thickness (IMT) in external and internal carotids in 146 patients with arterial hypertension. We have measured the activity of vWF on prognostic level of IMT>0.9 mm. In patients with the normal level of IMT we additionally measured the local stiffness indexes: distensibility coefficient (DC), elasticity module (EM) and one point pulse wave velocity (OPPWV).

Results: Using the ANOVA we have found a reliable increase of basal activity of vWF in the group of patients with IMT>0.9 mm, but only in internal carotids (107.1±12.5% (< 0.9 mm) and 118.2±13.9% (>1.0 mm); F=9,114 p=0.004 in the left side and 110.9±13.4% (< 0.9 mm) and 118.0±14.5% (>1.0 mm); F=3.62 p=0.055 in the right side). We have not set the dependency between the increase of the vWF and IMT of external carotids. Analysis of patients with normal IMT dimension showed the reliable interactions between the vWF level and the local stiffness. Thus, the we have identified the negative weak correlation between DC and vWF (R=−0.37; p=0.03) and positive weak correlation between EM and vWF (R=0.39; p=0.03) and OPPWV and vWF
(R=0.41; p=0.02).

Conclusions: The activity of vWF in plasma of patients with arterial hypertension can represent the coagulative activation state of the muscle-resistive type vessels even without clear morphological changes and need the additional investigation on the association of the hypertensive thrombotic outcomes.

PB0050 | Assessing the Impact of Switching to the Tobacco Heating System on Cardiovascular Disease: Translating Basic Science into Clinical Benefit

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Background: Cigarette smoke (CS) is causally linked to the development of cardiovascular diseases (CVD). Tobacco harm reduction, by virtue of substituting cigarettes with less harmful products, is a complementary approach to current strategies for smokers who would otherwise continue to smoke. The Tobacco Heating System (THS) 2.2 is a novel tobacco product that heats tobacco instead of burning it, never allowing the temperature to exceed 350°C, thereby preventing the combustion process from occurring and producing substantially lower levels of toxicants than CS.

Aims: Our assessment program aims to demonstrate that switching to THS has the potential to reduce the risk of smoking-related diseases compared with continued smoking.

Methods: The program includes in vitro/in vivo toxicology testing methods that follow OECD guidelines and Good Laboratory Practice, a systems toxicology approach, and randomized, controlled clinical studies that follow the principles of Good Clinical Practice.

Results: The results of the THS assessment program demonstrated that cardiovascular toxicants are reduced by an average of >92% in

THS aerosol relative to CS and that THS aerosol contains no solid carbon-based nanoparticles.

The effects of THS aerosol on the adhesion of monocytic cells to human coronary endothelial cells in vitro are significantly reduced. Switching to THS halted the progression of CS-induced atherosclerotic changes in ApoE-/- mice in vivo.

Biomarkers linked to the development of smoking-related diseases were analyzed following a 6-month randomized, controlled clinical study with THS, which demonstrated a consistent improvement of biomarkers in different pathophysiologic pathways leading to atherosclerosis.

Conclusions: The evidence available to date indicates that switching to THS has the potential to reduce the risk of smoking-related diseases such as CVD.

As a next step, we will complement our THS assessment program with cardiovascular outcome studies intended to further support the clinical benefits of switching to THS over continuous smoking.

PB0051 | Comparison and Association of Fibrinogen, von Willibrand Factor and Factor VIII Levels with Risk of Cardiovascular Disease in Cigarette Smokers Residing in Calabar Metropolis, Nigeria

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Background: Cigarette smoking has assumed public health problem and is known to be the leading cause of preventable deaths in many countries of the world, 39% of these deaths is from cardiovascular disease. Eighty percent of the world 1.1 billion smokers live in developing countries.

Aims: The aim is to assess the effect of cigarette smoke on VWF, fibrinogen, factor VIII, VWF:CBA, APTT and PT levels of cigarette smokers in Calabar metropolis.

Methods: A cross sectional study involving 100 smokers (smoked between 1 and 30 years) and 80 non-smokers between the ages of 18-60 years. Ethical approval was obtained from the CRS MOH, Informed consent and a questionnaire from the studied participants. Blood samples was collected into K_2EDTA bottle for VWF, VWF:CBA, FV VIII and fibrinogen, carried out with ELISA kit (Bioassay Technology laboratory, Shangai, China) and trisodium citrate for the assay of APTT and PT (Technochlone Diagnostic, Vienna, Austria), stored at −80°C until ready to assay. The data were analysed using SPSS version 22 and MS excel.

Results: Fibrinogen (FIB) (2.39 g/L) was significantly higher as Von Willibrand Factor (VWF) and factor VIII (FVIII), (29.53 ng/ml and 30.40 mg/dl) dropped significantly in the smokers when compared to the non-smokers (P< 0.05). Whereas VWF:CBA, PT and APTT were not significant (P>0.05). FIB showed a significant decrease in 18-25 years (1.98g/L) when compared with 26-40 years (2.85g/L) P< 0.05, other parameters were not significant. There was a mild positive and negative correlation between VWF and FVIII (r=0.145, P< 0.05) and FVIII and APTT (r=0.214, P< 0.05) respectively.

TABLE 1 Mean ± SD of the haemostatic parameters of smokers when compared with non-smokers

Parameters/ unit	Smokers (test) n=100	Non-Smokers (control) n=80	P value	Remark
FIB (g/L)	2.39±1.71	1.14±1.17	0.000	S
VWF (ng/ml)	29.53±41.11	46.15±35.56	0.006	S
FVIII (mg/dl)	30.40±20.30	34.2±21.67	0.000	S
VWF:CBA (IU/L)	1.51±0.83	1.66±0.52	0.140	NS
APTT (secs)	36.88±3.97	35.49±3.65	0.180	NS
PT (secs)	13.2±1.68	13.65±1.73	0.81	NS

TABLE 2 Mean ± SD of the haemostatic parameters of smokers based on the effect of age

Parameters/ units	Group1 (18–25 years) n=45	Group 11 (26–40 years) n=45	Group 111 (41–60years) n=10	F value	P value	Remark
FIB (g/L)	1.98±0.98	2.85±2.0*	2.48±0.61	3.29	0.04	S
VWF (ng/ml)	20.60±20.01	40.17±61.99	25.94±21.12	1.90	0.16	NS
FVIII (mg/dl)	13.27±23.60	13.54±22.35	13.86±6.34	0.03	0.99	NS
APTT (secs)	36.58±3.9	36.45±3.9	38.86±1.1	1.28	0.28	NS
PT (secs)	12.76±1.7	13.57±1.58	14.29±8.1	3.10	0.05	NS

* Significantly different from Group 1 at P>0.05 S = Significant

Conclusions: It can therefore be deduced that in smokers, there is a slight raise and dropped in FIB, VWF and FVII, which show a possibility of predisposing smokers to CVD. It will also help in the proper management of clinical conditions arising from cigarette smoking.

PB0052 | A Study on Pressure Overload-Induced Cardiac Hypertrophy Model in Mice by Applying Transverse Aortic Constriction and O-Ring Ascending Aortic Banding Technique: A Comparative Analysis

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Background: Vascular diseases are the major cause of mortality and morbidity and associated with high societal costs. The understanding of the pathological mechanism's underlying cardiovascular remodeling; although critical but essential for the development of innovative therapies for vascular diseases. Left-ventricular cardiac

hypertrophy is a well-established precursor of heart failure with the ominous prognosis.

Aims: To develop the surgical procedure that can easily and efficiently replicate this condition is too critical, and is significantly important for the better understanding of the pathophysiological mechanisms associated with this vascular disease.

Methods: Constriction of the aorta in mice is a highly accepted method for the creation of pressure overload-induced cardiac hypertrophy model. The two microsurgical procedures available for the successful development of the disease model are aortic constriction at the level of transverse aorta and o-ring aortic banding using the ascending aorta.

Results: The transverse aortic constriction has many advantages over the other available technique which is significantly highlighted in this paper. Using transverse aorta, though the degree of constriction is widely dependent on the diameter of the gauge needle. Our main purpose is to establish a comparison between these two surgical procedures to highlight the highly reproducible and precise method to study the mechanism of the disease progression as well as cardiac remodeling.

Conclusions: To summarize, both techniques have some advantages and disadvantages to one and other. Many studies reports that TAC is more efficient and it generates consistent and homogenous stenosis of aortic arch. Therefore, this technique provides stable and fully controlled aortic constriction. So, this microsurgical procedure can be easily completed with basic surgical understanding.

PB0053 | Factor V Leiden but Not Factor II 20210G>A Is a Risk Factor in a Large Cohort of Iranian Patients with Premature Coronary Artery Disease

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Background: Factor V Leiden (FVL) and factor II 20210G>A (FII20210) are the most common genetic risk factors for venous thromboembolism. The contribution of these markers to coronary artery disease (CAD) is less clear, with reported increased risks of small-moderate entity, or confined to patients with concomitant cardiovascular risk factors.

Aims: To investigate the association between FVL, FII20210 and premature CAD.

Methods: We performed a genetic case-control association study in the frame of the premature CAD Milano-Iran (MIran) study, a cohort of consecutive young patients (females < 55, males < 45 years) undergoing coronary angiography at the Tehran Heart Centre (Iran) in 2009-2012. Cases had coronary artery stenosis ≥50% and no history of myocardial infarction. Controls were age-, sex-matched individuals with no CAD history. FVL and FII20210 were genotyped using TaqMan SNP genotyping assays. Association testing was performed using logistic regression adjusting for matching factors and ethnicity. The modifier effect of sex and major cardiovascular risk factors (smoking, metabolic [at least one among obesity, hypertension, hyperlipidaemia, diabetes]) was also assessed.

Results: We genotyped 944 cases and 1081 controls (Table 1). The risk of premature CAD in FVL carriers was increased by 50% (OR 1.54 [95% CI 0.95-2.48]). Conversely, in FII20210 carriers it was decreased by almost 30% (OR 0.72 [95% CI 0.41-1.28]) (Table 2). These

TABLE 1 Baseline characteristics of study subjects

	Cases (n=944)	Controls (n=1081)
Female sex, n (%)	616 (65)	756 (70)
Age, years, median (IQR)	47 (43–52)	45 (40–51)
Family history of CAD, n (%)	411 (44)	242 (23)
BMI, Kg/m^2, median (IQR)	29.2 (26.3–32.2)	27.5 (24.8–31.0)
Diabetes mellitus, n (%)	340 (36)	125 (12)
Hyperlipidemia, n (%)	707 (75)	278 (26)
Hypertension, n (%)	529 (56)	159 (15)
Cigarette smoking, n (%)	263 (28)	139 (13)

TABLE 2 Association of FVL and FII20210 variants with the occurrence of premature CAD

	Genotype	Cases (n=911)	Controls (n=1081)	OR (95% CI)	OR[1] (95% CI)
FVL	GG	870 (95.5)	1049 (97.0)	Reference	Reference
	AG	39 (4.3)	31 (2.9)	1.52 [0.94 to 2.45]	1.49 [0.92 to 2.43]
	AA	2 (0.2)	1 (0.1)	2.41 [0.22 to 26.64]	3.05 [0.27 to 34.66]
	Carriers (AG + AA)	41 (4.5)	32 (3.0)	1.55 [0.97 to 2.47]	1.54 [0.95 to 2.48]
FII20210	GG	916 (97.8)	1050 (97.1)	Reference	Reference
	AG	19 (2.0)	31 (2.9)	0.70 [0.39 to 1.25]	0.64 [0.35 to 1.16]
	AA	2 (0.2)	0 (0.0)	-	-
	Carriers (AG + AA)	21 (2.2)	31 (2.9)	0.78 [0.44 to 1.36]	0.72 [0.41 to 1.28]

OR[1]: adjusted for sex, age and ethnicity.

effects were more pronounced in women (FVL: OR 1.66 [95% CI 0.95-2.89]; FII20210: OR 0.61 [95% CI 0.30-1.25]) than men (FVL: OR 1.25 [95% CI 0.48-3.31]; FII20210: OR 1.07 [95% CI 0.39-2.91]). The risk of premature CAD was substantially increased when FVL and at least one major cardiovascular risk factor were present simultaneously, compared with non-carriers without cardiovascular risk factors (smoking: OR 4.44 [95% CI 1.17-16.86], metabolic: OR 24.60 [95% CI 12.27-49.34]).

Conclusions: FVL but not FII20210 is associated with an increased risk of CAD in young Iranians. This risk increases considerably when combined to other cardiovascular risk factors.

PB0054 | Thrombin-Induced Platelet Fibrin Clot Strength Identified by Thromboelastography: A Potential Marker of Increased Thrombotic Risk in Older Adults with Coronary Artery Disease

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Background: Older adults (age>65 years) are at an increased risk of acute coronary syndrome and stroke. High thrombogenicity measured by Thromboelastography has previously been shown to be associated with poor clinical outcomes. Thrombogenicity markers in older adults are unexplored.

Aims: To analyze differences in viscoelastic properties of clot in older adults using Thromboelastography.

Methods: In this post-hoc analysis of the prospective cohort MAGMA study (NCT01276678), we included 443 consecutive patients with moderate to severe coronary artery disease (luminal diameter stenosis >25%) undergoing elective cardiac catheterization and measured viscoelastic properties using Thromboelastography.

Results: Of the total cohort, 217 (49%) and 226 (51%) patients were >65 and ≤65 years, respectively. In the overall population, majority were men and Caucasians, the gender and race were equally distributed between the two groups. Older adults had a lower BMI (median 30 vs. 32, p=0.002). Among cardiovascular comorbidities, older adults had higher rates of CABG and CHF (22% vs 13%, p=0.01 and 12% vs 3%, p< 0.001, respectively). Other comorbidities including hypertension, hyperlipidemia, diabetes, CAD, PCI, PVD and stroke were similar between the two groups. Both groups had similar aspirin, P2Y12 inhibitor and DAPT usage. In older adults, thrombin-induced platelet fibrin clot strength (TIP-FCS) was found to be significantly elevated (67±6 vs. 64±6, p=0.001) (Figure 1). Using previously described cut-point for high thrombotic risk (>67 mm), 50% older adults (vs. 33%) had high TIP-FCS (p=0.002). Only TIP-FCS retained statistical significance after linear and logistic regressions for the predefined cut-point in older adults.

Conclusions: Older adults with moderate to severe CAD had a significantly higher tensile strength of platelet-fibrin clots. The higher

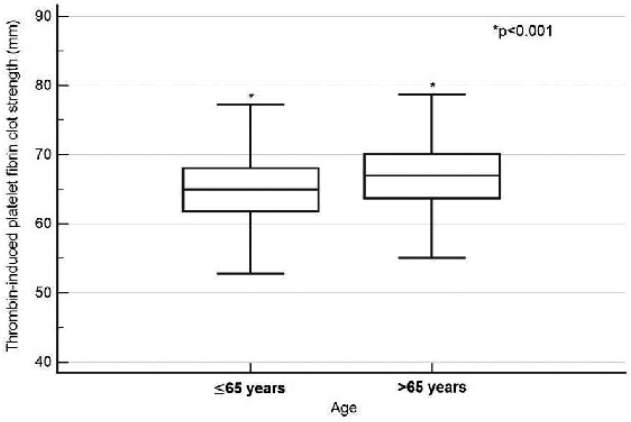

FIGURE 1 The influence of age on TIP-FCS (mm)

TIP-FCS could be attributable to the elevated risk and be a measure of predicting a high-risk thrombotic event in this group.

PB0055 | Tissue Factor Cytoplasmic Tail Promotes Inflammatory Myeloid Cell Derived Superoxide Formation and TGF-β1 Driven Cardiac Remodeling in Myocardial Infarction

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Background: The inflammatory transition from tissue factor (TF)-dependent signaling to TGF-β1 driven cardiac remodeling in myocardial infarction (MI) is incompletely understood.

Aims: We tested the hypothesis that the signaling function of the cytoplasmic tail of TF promotes inflammatory myeloid cell activation and oxidative stress mediated TGF-β1 activation in subacute MI.

Methods: MI was induced in 9 to 12 weeks old male C57BL/6J mice (B6J), mice specifically lacking CT of TF ($TF^{\Delta ct}$) and $TF^{fl/fl}$LysMCre+/- mice by permanent ligation of left anterior descending (LAD) coronary artery. Left ventricular function was assessed by echocardiography 1, 7,14,21 and 28 days (d) after MI. Infiltration of immune cells was investigated by performing flow cytometry analysis after enzymatic digestion of the myocardium. Superoxide specific product 2-hydroxy ethidium (2-HE) from dihydroethidium (DHE) was measured by HPLC (high performance liquid chromotography).

Results: MI results in phosphorylation of the cytoplasmic tail (CT) of TF within myeloid cells. Mice with conditional knockout of TF on myeloid cells ($TF^{fl/fl}$LysMCre+/-) or $TF^{\Delta CT}$ mice, we found that this regulatory intracellular domain of TF within myeloid cells is required for cardiac infiltration of inflammatory $Ly6C^{high}$ TF^{+} monocytes, Rac-1 GTPase and NADPH-oxidase activation as well as superoxide formation of $gp91^{phox+}$ myeloid cells in MI. TGF-β1 dependent SMAD2 activation and cardiac collagen deposition as late sequel of MI was reduced in $TF^{\Delta CT}$ mice, resulting in attenuated cardiac dysfunction and reduced mortality. Circulating monocytes in patients with sub-acute MI showed increased nitrosative stress as well as increased phosphorylation of TF CT along with TGF-β1 and NF-kB inflammatory activation, which was recapitulated in cardiac tissue of end-stage heart failure patients with chronic MI. Therefore, TF CT drives NADPH-oxidase derived superoxide formation, thrombo-inflammation and cardiac fibrosis.

Conclusions: We conclude, that TF CT phosphorylation might serve as putative biomarker and risk predictor in MI.

PB0056 | Crosstalk between Platelet and Endothelial Dysfunction in Survivors of Allogeneic Hematopoietic Cell Transplantation Independently of Traditional Cardiovascular Risk Assessment

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Background: Cardiovascular events emerge as a major cause of morbidity and mortality among allogeneic hematopoietic cell transplantation (alloHCT) survivors. Platelet and endothelial dysfunction complicate HCT. Nevertheless, it has been scarcely investigated whether these processes persist long after HCT, subsequently predisposing to cardiovascular events.

Aims: We investigated whether platelet and endothelial dysfunction are evident in alloHCT survivors using subtle markers indicative of subclinical dysfunction.

Methods: We enrolled consecutive adult alloHCT survivors from our HCT Clinic (January-December 2019). We excluded patients with established cardiovascular disease, chronic graft-versus-host disease under immunosuppressive treatment (GVHD), active malignancy or relapse. We also enrolled consecutive otherwise healthy control individuals from our Hypertension Clinic, matched for traditional cardiovascular risk factors (age, hypertension, diabetes, dyslipidemia, obesity, smoking). Detailed history, physical examination and blood sampling were performed. Subclinical cardiovascular damage was assessed by pulse-wave velocity (PWV)and carotid intima-media thickness (IMT). Platelet activation was evaluated by measurement of platelet microvesicles (PMVs) as previously published. Endothelial dysfunction was dynamically assessed by Laser Speckle Contrast Analysis (LASCA), evaluating microvascular blood flow of the skin forearm following ischemic occlusion (PeriCam PSI NR System, Perimed Järfälla, Sweden).

TABLE 1 Baseline characteristics, surrogate markers of cardiovascular disease and indices of platelet and endothelial dysfunction in alloHCT survivors and mat

Table. Baseline characteristics, surrogate markers of cardiovascular disease and indices of platelet and endothelial dysfunction in alloHCT survivors and matched controls

	alloHCT (n=45)	Controls (n=45)	p-value
Age (years)	46±18	47±9	0.514
Office SBP (mmHg)	121±16	122±14	0.770
Office DBP (mmHg)	80±12	77±12	0.194
Heart rate (/min)	78 (16)	75 (14)	0.125
Total cholesterol (mg/dl)	208±70	199±35	0.435
LDL-C (mg/dl)	140±30	137±25	0.267
HDL-C (mg/dl)	55±10	48±10	0.118
Triglycerides (mg/dl)	141±51	127±78	0.102
Glucose (mg/dl)	92±10	88±10	0.113
BMI (kg/m^2)	26 (7)	28 (6)	0.605
Smoking (%)	4	7	0.824
Pulse-wave velocity (m/s)	5.8 (3.3)	5.9 (1.6)	0.715
Left Carotid IMT (mm)	0.57 (0.18)	0.59 (0.16)	0.694
Right Carotid IMT (mm)	0.55 (0.16)	0.54 (0.12)	0.397
Baseline CVC (%)	40.6 (16)	39.6 (15)	0.204
Peak CVC (%)	328	315	**<0.001**
Time to peak (sec)	9.3 (7.7)	11 (6.5)	**0.007**
Base to peak (%change)	82±65	137±80	**<0.001**
Platelet microvesicles (/μl)	253 (432)	267 (205)	**0.059**

alloHCT: allogeneic hematopoietic cell transplantation; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-C: High Density lipoprotein Cholesterol, LDL-C: Low Density lipoprotein Cholesterol; IMT: intima media thickness, CVC: Cutaneous Vascular Conductance. Continuous variables are presented as mean±SD or median (interquartile range) according to normality tests.

Results: We studied 45 patients after a median of 2.3 (range 1.1-13.2) years from alloHCT, the majority of whom had suffered from acute (44%) and/or chronic GVHD (66%), and 45 controls. Traditional cardiovascular risk factors and surrogate markers of cardiovascular disease did not differ between groups (Figure). Nevertheless, alloHCT survivors showed significantly increased PMVs and altered indices of endothelial dysfunction assessed with LASCA.

In alloHCT survivors, PMVs were significantly associated with base to peak change (r=0.321, p=0.046).

Conclusions: Endothelial and platelet dysfunction are present in alloHCT survivors long after HCT, independently of traditional cardiovascular risk assessment. An apparent synergism exists that may be strongly implicated in the subsequent establishment of cardiovascular disease in these patients.

PB0057 | Study of Fluctuations in the Endothelin Concentration of the Crew Trans-Latitude Journey Transarctica-2019

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Background: Every day, interest in the Arctic is increasing, more and more people are arriving in adverse climatic and geographical conditions of the Far North. Therefore, there is a need to study

the adaptation of vascular endothelium to the conditions of the far north.

Aims: To assess the change in the concentration of endothelin in the sailors of the transarctic flight "TRANSARCTICA-2019".

Methods: The subjects of the study were 33 sailors (29 men, 4 women) average age 39 years (range: 20-63). Sampling was carried out at zero point - Arkhangelsk N64°33' E40°32', and at a high point - island Hayes N80°34' E57°41'. The parameters were determined gender, age, the concentration of endothelin-1 (determined by enzyme immunoassay). Statistics. The article was prepared as part of a study supported by a grant from the RFBR project No. 18-00-00814-COMFI (18-00-00478).

Results: The concentration of endothelin-1 in the crew of the transarctic flight TRANSARCTICA-2019 - Minimum concentration (pg / ml) - 0.814-2.321, The maximum concentration (pg / ml) 8.369-12.662, Mean value (M) (pg / ml) and standard deviation (SD) Mean value (M) (pg / ml) and standard deviation (SD).

M = 4. 79 SD = 2.10. Statistically significant differences were obtained in the average concentration of endothelin in sailors at the zero and high points in the population

(t = −3.6532, df = 31, p = 0.0009).

Conclusions: It was shown that under the conditions of a trans-latitudinal flight, the concentration of endothelin-1 increases. This may indicate the formation of a state of maladaptation of the vascular endothelium with a tendency to vasoconstriction in high latitudes. Further research is needed on changes in the parameters of endothelial dysfunction during adaptation to the climate of the Far North.

PB0058 | Study of Thrombine Generation Assay Parameters of the Crew Trans-Latitude Journey Transartika-2019

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Background: Interest in the Arctic territories and number of scientific expeditions is increasing. Therefore, there is a need to study the adaptive capabilities of the human hemostatic system in the North. One method that can evaluate hyper- and hypocoagulation conditions is the thrombin generation assay (TGA).

Aims: To evaluate the change in the TGA parameters of the crew in the transarctic journey TRANSARCTICA-2019.

Methods: The subjects were 54 crew members of the journey "TRANSARCTIC-2019" (35 men and 19 women), average age 36 years (range: 20-70). Sampling was carried out at zero point - Arkhangelsk N64°33' E40°32', and at a high point - island Hayes N80°34' E57°41'. TGA parameters were determined: lag phase time (tLag), thrombin peak time (tPeak), thrombin peak (Peak), endogenous thrombin potential (AUC). Statistic were performed using R. The study supported by a grant from the Russian Foundation for Basic Research, project №18-00-00814-COMFI

(18-00-00478).

Results: At a high point, tLag is statistically significantly less (Student's test, t = 4.358, p-value = $6.364e^{-5}$), tPeak is statistically significantly less (Student's test, t = 4.0495, p-value = 0.0002). AUC is statistically significantly higher at a high point (Student's test, t = −3.3305, p-value = 0.0016). Peak did not change (Mann-Whitney test V = 593, p-value = 0.3845).

Conclusions: There was shown that under conditions of a trans-latitudinal journey, the time of the lag phase and the time of the formation of a thrombin peak decrease, and the endogenous potential of thrombin increases, which may indicate the formation of a state of hypercoagulation in expedition members at high latitudes. There are needed the further research of the hemostatic system during adaptation to the north climate.

PB0059 | Monitoring the Safety and Effectiveness of Antiplatelet Therapy

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Background: The Verify-Now system can evalua teplatelet reactivity results in minutes, providing antiplatelet therapy guidance in many clinical settings. Understanding the patient's unique response to antiplatelet drugs is important for making effective treatment decisions.

Aims: To evaluate the effectiveness and safety of antiplatelet therapy in patients receiving antiplatelet therapy.

Methods: A retrospective analysis of the evaluation of residual platelet reactivity (ORT) of P2Y12 PRU and ARU by bedside aggregatometry using the Verify-Now system was performed in 170 patients admitted with a diagnosis of coronary heart disease.

Results: We examined 115 a men and 55 a women mediana (Me) age 64 years who received aspirin and/or clopidogrel, ticagrelol. At 116 patients received aspirin therapy the ARU index was Me 400 (95% 423.9; 457.5) units. Clopidogrel therapy was assigned at 133 patients, 27 patients received ticagrelor, form them 79 received DAT. Indicator PRU of the clopidogrel therapy has made Me 126 (83.4; 178,4). ORT for plavix was Me 99 (89.9; 131.4), for zilt - Me 190.0 (160.8; 215.1), for ticagrelor - Me 17.0 (2.23; 149) Stroke (24.7%), thrombosis stent (11.8%), bleeding (11.2%), unstable angina (15.3%), recurrent ACS (27.1%) and were indications for the ORT analysis. Thrombosis stent developed on the therapy of P2Y12 inhibitor (original plavix, n=9) PRU made Me 105.4 (92.5; 136.1), Me 17.0 (2.23; 149) of the reception of generic clopidogrel (n=8), ticagrelor (n=3),

which indicated low compliance of therapy and required pharmacogenetics analysis.

Conclusions: Cases of resistance to antiplatelet drugs and compliance problems can lead to a recurrence of vascular events and to create schools for patients receiving antiplatelet drugs.

PB0060 | Aspirin Antiplatelet Effects Are Associated with Body Weight

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Background: Aspirin is indispensable in secondary prevention of ischemic events. Recently, it was reported that clinical aspirin effects are hampered in patients above 70 kg body weight. It is well known that a plethora of reasons beside obesity is associated with increased platelet reactivity and insufficient aspirin effects (HTPR).

Aims: However, data regarding an association between pharmacodynamic response to aspirin and body weight are missing.

Methods: In this pilot study, we included 59 patients from University Hospital Duesseldorf. Impedance aggregometry was used to assess pharmacodynamic response to aspirin.

Results: AA-induced platelet reactivity was significantly higher in patients above 70kg (< 70kg: 28.27 ± 26.33 vs. >70kg: 45.93 ± 27.1, p=0.035) and correlated well with the bodyweight of patients in this study (r=0.33, R^2=0.09, p=0.016). According to this, insufficient pharmacodynamic response (HTPR) to aspirin was significantly more frequent in patients over 70kg (< 70kg: 25% vs. >70kg: 43%, p=0.035).

Conclusions: Insufficient pharmacodynamic response to aspirin is associated with body weight. This finding may play a role in the impaired clinical efficacy of aspirin in patients > 70 kg. An optimal aspirin regime in these patients needs to be evaluated in large scale trials.

PB0061 | Overexpression of FXII as a Risk of Thrombosis in Patients with SAMTER Syndrome, Angioneurotic Edema, and its Association with Smoking Rate and Hematological Parameters

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Background: SAMTER syndrome (SSx), with its triad bronchial asthma, nasal polyps and aspirin allergy; It is an entity that must be recognized since many patients with asthma may have other risk factors for thrombotic events, complicating the use of antiplatelet therapy. In the world we only find 5 clinics that study SSx, in addition, a strong association between angioneurotic edema, SSx and an over-expression of coagulation FXII has been found.

Aims: To study in our SAMTER clinic the expression of FXII in patients who have developed a picture of angioedema and assess their risk of thrombosis.

Methods: A cohort of 21 patients with SxS was studied, with manifestations of angioneurotic edema secondary to aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), whose clinical history, search for thrombosis factors, samples were taken for the measurement of blood count (baseline and follow-up) and FXII factor. Patients were classified as hyperactive and with normal levels of FXII. Descriptive statistics were performed on quantitative variables and frequency measurements on qualitative variables. Clinical history and hematological parameters were compared between the two study groups.

Results: A total of 21 patients were studied, 6 men and 15 women, 38% (8) were classified as hyperactive to FXII, while 71% (15) were smokers.

A strongly significant association was found between smoking rate and Hyperactivity to FXII, with respect to hematological variables: Erythrocytes hemoglobin, hematocrit and lymphocytes showing an increase in their average in hyperactives compared with those not hyperactive to FXII (Table 1, graphs 1,2).

Conclusions: There is a strong association between smoking rate, hematocrit, hemoglobin and erythrocytes and factor XII hyperactivity in a small cohort of patients with SAMTER syndrome and angioneurotic edema.

TABLE 1 Results between patient groups with hyperactivity of FXII and factor XII normal

LABORATORY FINDINGS	FACTOR XII ACTIVITY									
	ALL			HYPERACTIVITY FACTOR XII			NORMAL FACTOR XII			P-VALUE*
	MEDIAN	Q1	Q3	MEDIAN	Q1	Q3	MEDIAN	Q1	Q3	
AGE	48.500	43.000	60	48.000	45.250	64.000	49.000	39.500	77.000	.697
TOBACCO INDEX	7.850	0.825	18.25	17.000	13.500	22.500	1.200	0.000	6.525	**.003**
LEUCOCYTES	6.900	5.550	9.15	8.650	6.750	9.825	6.400	5.225	7.425	.083
	6.350	5.175	8.125	6.350	5.100	9.075	6.350	5.575	8.125	.897
NEUTROPHILOS	63.450	49.625	71.025	65.950	54.225	69.350	55.350	47.375	83.625	.897
	66.600	54.400	78.175	60.650	54.400	76.300	70.650	54.500	85.000	.515
LIMPHOCYTES	23.900	15.400	29.875	20.200	17.800	27.425	27.850	9.675	31.150	.515
	21.750	9.925	31.175	28.400	17.450	35.000	15.350	7.675	26.700	**.055**
MONOCYTES	7.750	4.875	11.375	6.650	5.150	11.525	7.900	4.875	11.300	.696
	7.050	4.400	11.125	6.150	4.500	9.425	8.250	4.350	13.575	.315
EOSINOPHILS	3.600	1.000	9.125	3.600	1.175	7.100	3.000	.875	13.575	.762
	2.750	1.175	4.975	2.500	.725	4.400	2.750	1.875	6.475	.515
BASOPHILS	.650	.300	1.025	.650	.400	1.075	.650	.200	.975	.515
	.600	.275	0.75	.600	.325	.675	.500	.200	.900	.762
ERYTHROCYTES	5.0300	4.6150	5.4825	5.2300	4.9050	6.0925	4.8100	4.4775	5.2800	.146
	5.2350	4.5525	5.6825	5.5600	5.4700	6.3525	4.6950	4.2450	5.0775	**.004**
HEMOGLOBIN	16.150	14.150	16.9	16.600	15.775	19.075	14.500	13.000	16.575	.068
	17.200	14.350	18.125	17.950	17.225	18.575	14.850	12.750	16.675	.003
HEMATOCRITE	48.400	41.875	49.575	48.500	47.400	55.650	43.050	38.800	49.250	.268
	49.200	41.350	53.55	53.050	50.100	55.650	42.300	37.550	49.150	**.006**
PLATES	138.00	206.50	267.75	259.00	208.25	266.50	236.00	201.00	313.75	.965
	4.8900	188.7500	277	248.5000	205.7500	277.0000	235.0000	162.5000	270.7500	.573

PB0062 | Folate Exchange in Athletes of North-West Russia

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Background: The study of folate exchange in individuals with high physical activity living in the north harsh conditions is important for maintaining the athletes health. Climatic conditions, an evolutionarily formed genotype translates changes in folate metabolism.

Aims: Due to athletic activity, an increased level of homocysteine c an le ad to the severe cardiovascular system work disruption.

Methods: The study involved 98 athlet es living in the North-West of Russia. We studied the genes regulating folate metabolism and the blood homocysteine level.

Results: The enzyme methylenetetrahydrofolate reductase C677T gen e variant was found in 45.2% (95% CI: 34.1-56.5%), the homozygous variant - in 5% (95% CI: 1.5-12%) of cases. The genetic variant MTHFR (glu429ala) was observed in 48% of athletes (95% CI: 36-59%), the homozygous allele 1298C occurred in 3.6% (95% CI: 0.8-10, 3%). The genetic marker MTRR 66G methionine synthase reductase had a frequency of 76% (95% CI: 65-84%). Homozygous variant was observed in 23% (95% CI: 15-34%). Hidden hyperhomocysteinemia (10.5-13.5 μmol / l) was detected in 40.2% [95% CI: 29.3-51.7%] of cases. Explicit hyperhomocysteinemia (from 13.5 μmol / L) was in 38.9% [95% CI: 28-50%] of cases. In 20.9% [95% CI 13.3-32.5] the level of homocysteine was within physiological values (up to 10.5 μmol / l).

Conclusions: The examined athletes genotype carries a number of o ptions that lead to disruptions in the normal functioning of the enzymes involved in the conversion of folic acid. Such changes can lead to the accumulation of homocysteine, manifested by hyperhomocysteinemia, observed in athletes.

The reported study was funded by RFBR according to the research project No18-00-00814-KOMFI (18-00-0047818).

PB0063 | Changes in the Hemostasis System in Athletes

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Background: The adequate functioning of the hemostatic system determines the cardiovascular system health. Under the influence of high physical exertion, the heart and blood vessels experience tremendous stress, compensation is possible only under conditions of all systems harmonious operation.

Aims: The study included a grou p of athletes engaged in various sports.

Methods: Among 98 athletes, a study of the hemostasis system proteins genes was conducted, and the functional state of the fibrinolytic system was also assessed. The genetic variant of Leiden Factor V is found in 3.6% of athletes (95% CI: 0.6-8.5%). In 77.4% of cases (95% CI: 67.9-85.7%), an SERPINE1 −675 4G gene allelic variant was found.

Results: The allelic variant of the fibrinogen gene −455A was noted in 40.5% of athletes (95% CI: 30.3-51.1%). The integrin beta-3 protein gene 1565C variant was 25% (95% CI: 16.3-34.8%). Due to evaluation of fibrinolytic activity, a significant decrease in the plasminogen tissue activator level (M = 0.73 [0.61-0.88]), an increase in the plasminogen activator inhibitor level of the first type PAI-1 (Me = 29.89 [22.3; 47.3]) were revealed. A direct correlation was found between the presence of the allelic variant of the SERPINE1-675 4G gene carriage and the level of PAI-1 in the blood plasma (rs = 0.29, $p \leq 0.05$).

Conclusions: Indicators of fibrinolytic status defin e the inhibition of fibrinolysis activity. A higher prevalence of allelic variants of genes determining the increased risk of developing thrombophilic conditions in athletes compared with the population of the North-West of the Russian Federation was shown. The reported study was funded by RFBR according to the research project No18-00-00814-KOMFI (18-00-0047818).

PB0064 | The Antidepressant Drug Desipramine Prevents Arterial Thrombosis in a Mouse Model Carrying the Human BDNFVal66Met Polymorphism

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Background: Depression represents an independent risk factor for acute coronary syndrome (ACS) and guidelines strongly suggest its management. However, whether and how antidepressant drugs have beneficial effect(s) in the reduction of cardiac mortality and of clinical events is still unclear.

Interestingly, the BDNFVal66Met polymorphism, related to mood disorders and to selective antidepressant drug response, was associated to ACS.

Aims: This study investigated whether desipramine antidepressant treatment concomitantly influenced behavioural impairment and prothrombotic phenotype in humanized BDNFVal66Met mouse. In addition, we explored the prothrombotic features in Met carrier homozygous patients with coronary artery disease (CAD).

Methods: Wild-type $BDNF^{Val/Val}$ and homozygous mutant $BDNF^{Met/Met}$ mice were treated for three weeks with desipramine (DES; 20 mg/kg/die), then drug efficacy was assessed by novelty-suppressed feeding test, and $FeCl_3$ induced carotid artery thrombosis and bone marrow megakaryocytes (MKs) number were assessed. Pro-platelet generation was evaluated in MKs obtained from circulating $CD45^+$ cells of $BDNF^{Val/Val}$ and $BDNF^{Met/Met}$ CAD patients (n=13/group). Human and mouse platelets were characterized analysing P-selectin and GPIIbIIIa expression, and platelet/leukocyte aggregates by flow cytometry. The study complies Helsinki declaration and was approved by Centro Cardiologico Monzino Ethical Committee. All partecipants provided written informed consent.

Results: In $BDNF^{Met/Met}$ mice DES treatment relapsed the behavioral impairment and concomitantly normalized the number of blood leukocytes and platelets, and of bone marrow MKs. DES reduced platelet hyper-reactivity, leading to a total reversion of thrombosis, without any affect on $BDNF^{Val/Val}$ mice. $BDNF^{Met/Met}$ patients displayed an activated platelet profile similar to that observed in mutant mice, despite standard antiplatelet treatment. The greater percentage of MKs-releasing pro-platelets reflected the higher platelet number detected in $BDNF^{Met/Met}$ patients.

Conclusions: Antidepressant therapy reverts not only the behavioral impairment but also the prothrombotic phenotype in $BDNF^{Met/Met}$ mice. Human studies will be crucial to understand if this approach will ameliorate the hyper-reactive platelet profile in BDNFVal66Met CAD patients.

PB0065 | Gender-Associated Cardiometabolic Biomarkers in Young Adults

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Background: The worldwide prevalence of cardiometabolic disorders among young adults has increased in the last years. However, gender differences in cardiometabolic risk factors not well understood.

Aims: To compare serum biomarkers of cardiometabolic risk factors in young men and women.

Methods: A cross-sectional study was conducted in men and women of outpatient department. Inclusion criteria: age 18-44 years, consent to participate. Exclusion criteria: secondary hypertension, diabetes mellitus. 251 adults were divided into 2 groups: gr. 1-124 men (49.4%) aged 27 [21-36] years (BMI 26.3 [25.1-27.8] kg/m^2, WC 95 [91.6-96.4] cm); gr. 2-127 women (50.6%) aged 35 [26-41] years (BMI 24.5 [23.1-25.9] kg/m^2, WC 82 [78-85.1] cm). The frequency of hypertension, abdominal obesity, dyslipidemia and impaired carbohydrate metabolism was similar among man and women. Leptin, adiponectin, interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), endothelin-1, angiotensin II, fibrinogen, type 1 plasminogen activator inhibitor (PAI-1) were determined by ELISA. The data are presents as M±SD and Me [Q25-Q75]. Statistical significance was defined as $p< 0.05$.

Results: Women had statistically higher levels of leptin (34.6 [13.8-49.1] vs 15.5 [4.4-16.2] ng/L, $p< 0.001$), adiponectin (8.9 [7.5-11.7] vs 7.9 [4.9-10.0], $p=0.01$) μm/L and fibrinogen (3.9 [3.4-4.5] vs 3.4 [2.8-4.0], $p< 0.001$) g/l compared to men. Men had significantly higher concentrations of angiotensin II (9.1 [6.4-24.4] vs 8.5 [5.0-15.5], $p=0.04$) ng/ml and PAI-1 (399.7 [264.1-597.9] vs 342.3 [221.9-453.5], $p=0.02$) ng/ml and IL-10 (6.3 [5.7-6.9] vs 5.2 [4.1-5.5], $p=0.03$) pg/ml. We did not find any other gender significant differences between IL-1β, IL-6, TNF-α and endothelin-1.

Conclusions: In young persons we found gender differences between adipokines (leptin, adiponectin), anti-inflammatory cytokines (IL-10), vasopressors (angiotensin II) and prothrombotic factors (fibrinogen, PAI-1). Further research is encouraged to determine the efficacy of applying these biomarkers to diagnosis and treatment in a clinical setting.

PB0066 | MiR-146a Mediates Neutrophil Extracellular Traps Formation in Acute Myocardial Infarction (AMI) in Young Patients

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Background: AMI has a high incidence in general population and high morbidity and mortality. Although the infarct process is multifactorial, atherosclerosis and inflammation play a relevant role. In this context, neutrophils and their extracellular traps (NETs) are crucial. Our group has described that miR-146a rs2431697 genotype influences NET formation.

Aims: We aimed to determine if young AMI patients have enhanced NETosis and evaluated their relationship with miR-146a genotype.

Methods: 300 AMI patients (41±4 years, 90% men) and 50 healthy blood donors (40±4 years, 90% men) < 45 year-old were recruited. The informed consent was obtained for each subject and the study was approved by local ethics committee. NETs in plasma were evaluated by quantifying both, cell-free DNA (cfDNA) using SYTOX green and citrullinated histone-3 (citH3)/

DNA by ELISA. Rs2431697 was genotyped using TaqMan probes. Finally, plasma DNaseI activity was determined in healthy donors (n=16) and in patients with normal (n=10) or high (more than 1.5-fold) levels of both cfDNA and citH3/DNA (n=47) respect to controls by ELISA.

Results: CfDNA and citH3/DNA levels in AMI patients were higher than in controls (p< 0.0001, and p< 0.0005, respectively) (Fig. 1A-B). These levels were increased in patients carrying the T allele of rs2431687 in comparison with CC patients (p< 0.05) (Fig. 1C). DNaseI activity was measured in patients with the highest cfDNA levels and citH3/DNA. Interestingly, DNaseI activity was significantly elevated in patients compared to controls. Additionally, 4 out of 47 AMI patients display low DNaseI activity compared to controls (1 with 50%, 2 with 25%, 1 with 5%).

Conclusions: These data show that plasma NET levels are significantly elevated in young AMI patients. On the other hand, miR-146a rs2431697 T allele may influences NET formation in these patients. Further investigation is required to determine the role of low DNaseI activity in AMI.

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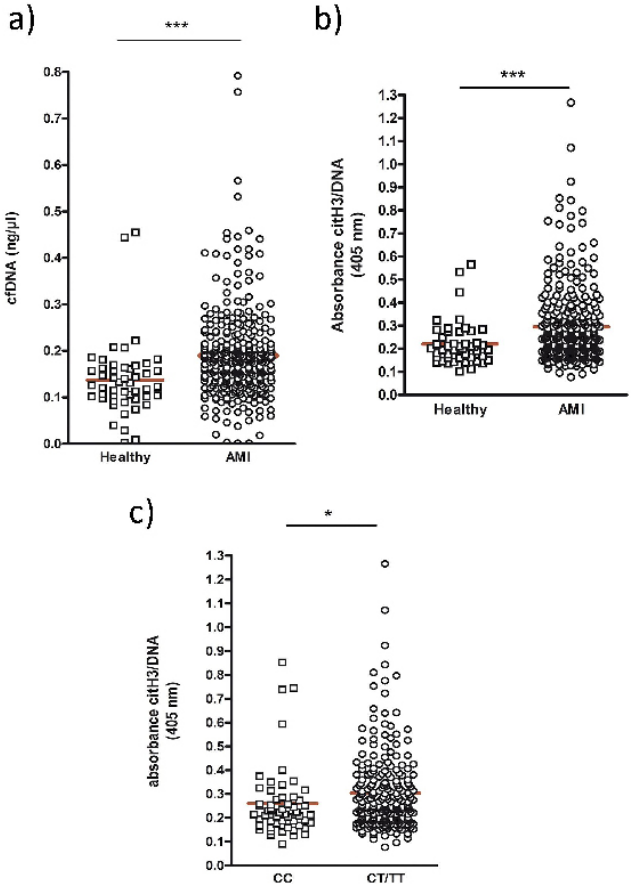

FIGURE 1

PB0069 | Study of the Endothelial Dysfuncion in Patients with Cancer: Preliminary Results

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Background: Emerging evidences suggest physio-pathologic relationship between cardiovascular (CV) diseases and cancer. In fact, oxidative stress and inflammation play a leading role both in atherogenesis and cancer genesis.

Aims: We conducted a case-control study to evaluate the presence of endothelial dysfunction and early vascular disease in patients with cancer.

Methods: Patients with a first diagnosis of solid or hematologic cancer were consecutively enrolled (cases). Sex- and age-matched patients without history of cancer acted as controls. Exclusion criteria: previous venous or arterial thrombosis, ongoing anticoagulation, age >80 years, previous radio- or chemotherapy. Endothelial dysfunction was evaluated by ankle-brachial index (ABI) and intima-media thickness (IMT) or presence of carotid plaques by ultrasound. ABI ≤0.9 means presence of peripheral artery disease; carotid IMT was consider abnormal when > 0.9 mm.

Results: 64 cases (16 hematologic and 48 solid cancers; M/F 36/28; mean age 68 ± 9 years) and 70 matched- controls (M/F 38/32; mean age 64 ± 11 years) were enrolled. The CV risk profile (measured by American College of Cardiology/American Heart Association [ACC/AHA] score) was comparable between groups, as well as the proportion of patients taking antiplatelet drugs and statins. A significantly higher proportion of cases (n 62, 97%) showed carotid IMT compared to controls (n 50, 71%.; OR 12.8 [95% CI 2.8-57.3]). No difference in the proportion of abnormal ABI and presence of carotid plaque was detected. Interestingly, more cases with advanced solid cancer (n 22, 73%) had carotid plaque compared with cases with local solid tumors (n 6, 33%; OR 5.5 [95% CI 1.5-19.6]).

Conclusions: Patients with cancers, especially advanced, presented at diagnosis with endothelial dysfunction and asymptomatic vascular disease. This may be due to the inflammatory response towards the neoplasm and may led to a higher CV risk profile.

PB0070 | Platelet Glycoprotein IIIa Leu 33 Pro Polymorphism and Susceptibility to ST-segmented and Non-ST-segmented Myocardial Infraction

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Background: The platelet glycoprotein IIb/IIIa receptor plays an important role in platelets aggregation. The IIIa polypeptide is

polymorphic due to a single base change at position 1565, resulting in either leucine or proline at position 33 of the protein. This polymorphism has been investigated in several thrombotic diseases, and suggested to play a role in thrombotic cardiovascular diseases.

Aims: This study aimed to investigate the association between platelet glycoprotein IIIa Leu 33 Pro gent polymorphism and risk of myocardial infarction (MI).

Methods: This was an analytical case-control study, in which a total of 100 patients with confirmed diagnosis of MI, 60 (60%) of them with ST-elevated myocardial infraction (STEMI) and 40 (40%) with non-ST-elevated myocardial infraction (NSTEMI) were enrolled. Further 100 age- and sex- matched apparently healthy volunteers were recruited as a control group. Genomic DNA was extracted from peripheral blood leucocytes by salting out method. The platelet glycoprotein IIIa gene Le 33 Pro polymorphism was analysed using polymerase chain reaction- restriction fragments length polymorphism (PCR-RFLP). The study was approved by scientific research committee, faculty of medical laboratory sciences, Al Neelain University, Khartoum, Sudan. Informed verbal consent was obtained from all participants before sample collection.

Results: The genotype Le/Le was the most frequent in both patients and control groups (44% and 80%) followed by Le/Pro (41% and 10%)) and Pro/Pro (15% and 10%). While the genotypes Le/Le was found to have a protective effect on both types of MI (OR=0.191; 95% CI: 0.094-0.338, *P. value*: 0.000 and OR=0.205; 95% CI: .093-.452, *P. value*= 0.000), the genotype Le/Pro was associated with increased risk (~6 folds) of both STEMI and NSTEMI (OR=6.0, 95% CI: 2.609-13.799, *P. value*= 0.000 and OR=6.652, 95% CI: 2.690-16.45, *P. value*= 0.000 respectively).

Conclusions: The GP IIIa Le 33 Pro is significantly associated with the risk of both STEMI and NSTEMI.

PB0071 | Cardiovascular Risk in Systemic Lupus Erythematosus

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Background: Patients with systemic lupus erythematosus (SLE) seem to have higher cardiovascular risks (CVR) as they combine traditional and non- traditional CVR factors.

Aims: To determine the CVR in SLE patients and its correlation with disease activity and systemic manifestations.

Methods: Medical records of 89 patients diagnosed with SLE according to the modified ACR criteria of 1997 were retrospectively analyzed. CVR was assessed using the Framingham Risk Score to estimate the 10 years CVR. It's considered low if less than 10%; moderate if it's between 10 à 20% and high if over 20%.

Results: The mean age was 35 years with a sex ratio M/F= 0.1. About 3.4% of the patients were smokers, 4.5% diabetics, 10.1% with high blood pressure and 3.4% dyslipidemic. The mean Body mass index was 25.9 kg/m^2. Mean cholesterol levels were 4.38 mmol/l, Triglyceride 2.04, HDL 1.38 and LDL 1.74. Mean SLEDAI score was 9 and mean Framingham score was 2.58%. The CVR was considered low in 55.6% of the patients and moderate in 44.4%. Framingham score was inversely correlated with renal clearance (p=0.037) and neutrophile to lymphocyte ratio (p=0.06) without reaching the significant level. There was no correlation with disease activity, inflammatory parameters or systemic manifestations.

Conclusions: Despite the high disease activity in our patients and the high levels of inflammatory parameters, no one of our patients was presenting with high CVR according to Framingham score. Thus, the latter seems not suitable to estimate the CVR in inflammatory diseases especially SLE.

PB0072 | Apolipoprotein A-IV Is an Endogenous Inhibitor of Thrombosis: The Roles of Polymorphisms in the Risk of Cardiovascular Disease

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Background: Platelets are essential in atherothrombosis, a common cause of heart attack and stroke. We recently identified apolipoprotein A-IV (apoA-IV) as an endogenous inhibitor of atherothrombosis through competitive blockade of the αIIbβ3 platelet integrin (Nat. Commun. 2018). Mutational studies suggest the D5 and D13 N-terminal residues are essential in this interaction. Structural analysis indicates an interface between the N- and C-termini that may partially mask its platelet binding site. Interestingly, common apoA-IV C-terminal polymorphisms, Q360H and T347S, are linked to an increased risk of cardiovascular disease as high as 2-fold, however, the mechanism behind this phenomenon remains unknown.

Aims: To determine whether apoA-IV C-terminal polymorphisms, Q360H and T347S, reduces its affinity for the αIIbβ3 integrin and thus, its endogenous inhibition of thrombosis.

Methods: Site-directed mutagenesis was used to generate Q360H and T347S apoA-IV plasmids and proteins were subsequently expressed in E. coli BL21 cells. Light transmittance aggregometry was used to measure platelet aggregation following either ADP or collagen agonist in human samples of platelet-rich plasma (PRP) or gel-filtered platelets. Thrombus formation was measured under shear conditions by flowing human whole blood across collagen-coated microcapillary perfusion chambers under a real-time fluorescence microscope.

Results: Our data demonstrates the apoA-IV inhibitory effect is completely abrogated in the Q360H polymorphism. Our preliminary data also suggests this effect is significantly reduced in the T347S polymorphism. These results were consistent using ADP or

collagen-induced platelet aggregation in either PRP or gel-filtered platelets and in human whole-blood under high shear.

Conclusions: These data identify a novel mechanism that may explain the elevated risk of cardiovascular disease in individuals with apoA-IV polymorphisms, Q360H and T347S. Our findings also contribute to our understanding of the link between lipid metabolism and cardiovascular disease, our collective understanding of pathologies leading to atherothrombosis, and may guide personalized based treatments of cardiovascular disease.

* denotes equal contribution.

PB0073 | Von Willebrand Factor in Stroke and Non-valvular Atrial Fibrillation Patients

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Background: Von Willebrand Factor (vWF) is a biomarker of endothelial dysfunction. High molecular weight (HMWM) multimers have high thrombogenic capacity. ADAMTS-13 metalloprotease regulates the size of vWF circulating multimers, which are directly related to their hemostatic activity. The vWF /ADAMTS-13 inverse ratio is a potential risk factor for thromboembolism. Atrial fibrillation (AF) is associated with thrombosis secondary to vascular damage and with cardioembolic stroke. On the other hand, the Embolic Stroke of Undetermined Source (ESUS) even though involves an embolic mechanism shows no evidence of apparent cardioembolic origin.

Aims: To study von Willebrand Factor and its protease ADAMTS 13 in patients with atrial fibrillation and stroke.

Methods: 124 patients were included in four groups: AF, AF/Stroke, ESUS patients and healthy subjects as control group. VWF and ADAMTS-13 plasma concentrations by ELISA, multimeric structure by Western Blot. CHADS-Vasc assessed for thrombotic risk. Kolmogorov-Smirnov to assess normality, accordingly ANOVA or Kruskal-Wallis to compare groups and Spearman for correlations. Significance was $p< 0.05$.

Results: When compared to healthy subjects, AF/Stroke and ESUS patients showed a significant decrease in ADAMTS-13 plasma concentrations. (p=0.031) and (P-0.000) respectively, AF patients showed higher vWF concentrations (P-0.005) and vWF HMWM (P-0.006). There was a direct correlation between the amount of HMWM in relation to the plasma concentration of vWF (P=0.002). The risk of CVD assessed by CHA2DS2VASc correlates with higher vWF concentrations (P=0.000) and HMWM (P=0.018) as well as decreased concentrations of ADAMTS-13 (P-0.000). ESUS and AF show very similar behavior in relation to these biomarkers.

Conclusions: The vWF/ADAMTS-13 inverse ratio is associated to the presence of procoagulant high molecular weight multimer structures, altogether represent a risk factor for thrombotic events in patients with FA and could be associated to ESUS. These results suggest that the mechanism involved in thromboembolism could be intimately linked to the FVW/ADAMTS-13 relationship making them a potential therapeutic target.

PB0074 | PCSK9 Regulates Nox2-Mediated Platelet Activation via CD36 Receptor in Patients with Atrial Fibrillation

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Background: High circulating levels of proprotein convertase subtilisin/kexin 9 (PCSK9) are predictive of cardiovascular events (CVEs) in patients with atrial fibrillation (AF).

Aims: We hypothesized that PCSK9 may directly induce platelet activation (PA) and investigated possible mechanisms involved.

Methods: Among 1135 AF we selected 88 patients having PCSK9 values < (n=44) or >(n=44) 1.2 ng/ml (the median values of the entire cohort), balanced for age, sex and cardiovascular risk factors. PA was assessed by in vitro aggregation tests, platelet recruitment, serum TxB_2 formation and ex vivo by soluble P-selectin plasma levels. Nox2 activation as assessed by soluble Nox2-dp, serum H_2O_2, urinary isoprostanes and oxLDL were used as markers of oxidative stress (OS). Furthermore we investigated if normal (n=5) platelets incubated with PCSK9 (1.0-2.0 ng/ml) alone or in combination with LDL (50 µg/ml) displayed changes of PA and OS and down-stream signalling including p38, p47 and PLA_2 phosphorylation.

Results: PA and OS markers were significantly higher in patients with PCSK9 levels >1.2 ng/ml compared to those with values < 1.2 ng/ml ($p< 0.05$). Plasma levels of PCSK9 significantly correlated with markers of PA and OS. Platelet incubation with PCSK9 dose-dependently increased PA and OS coincidentally with enhanced phosphorylation of p38,p47 and PLA_2. All these changes were amplified by coincubation with LDL and were blunted by CD36 or Nox2 inhibitors. Co-immunoprecipitation analysis revealed an immune complex of PCSK9 with CD36.

Conclusions: Here we provide the first evidence that PCSK9, at concentration commonly found in human circulation of AF patients, directly interacts with platelets via CD36 receptor and eventually Nox2 activation: this effect is amplified in presence of LDL.

PB0075 | Systemic Aspirin Esterification in Subjects with Cardiovascular Disease: Impact of Body Size

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Background: Obesity is one of the major and growing health problem worldwide and it is associated with an increased incidence of cardiovascular disease. Obese subjects display a chronic pro-inflammatory state that may affect vascular endothelium, platelets, and other circulating cells. Nevertheless, in obese setting low-dose aspirin responsiveness is reduced and may be influenced by several factors; among them, body size and plasma esterase activity may play a key role.

Aims: To characterize the activity of aspirin esterase in relation to body size.

Methods: In this study, 84 patients with stable coronary artery disease (CAD) were enrolled and evaluated before (t0) and after 6 months of Mediterranean diet (t3). Plasma aspirin esterase activity was assessed using a fluorimetric method developed in our laboratory (Porro B et al., Biochem Pharmacol. 2019:163:119-127). Levels of urinary prostacyclin (PGIM) and thromboxane (TXM) metabolites were measured by liquid chromatography tandem mass spectrometry methods. Study complies with the Helsinki Declaration.

Results: At baseline, the activity of aspirin esterase in CAD patients with body mass index (BMI)>25 kg/m^2 was increased compared to normal-weight ones (34.5±7.1 *vs* 30.9±4.9 µmol/ml SA / min (mean±SD), *p= 0.03*). This result goes in parallel to increased levels of prostacyclin (150.8±119.7 *vs* 111.2±29.8 pg/mg of creatinine (mean±SD), *p= 0.04*), a pro-adipogenic prostanoid. When we compared enzyme activity after 6 months of Mediterranean diet (t3), we highlighted a decrease (Figure 1a, *p= 0.002*) and this variation correlated with BMI reduction (Figure 1b, *p=0.04*). In line with this data, PGIM and TXM were reduced at t3 (Figure 2, *p= 0.03* and

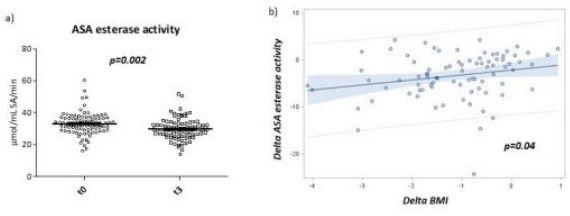

Figure 1. Plasma ASA esterase activity was measured in patients with coronary artery disease (CAD, n=84) (**a**) before (t0) and after 6 months of Mediterranean diet (t3). **b**) Correlation between the variation (t3-t0) of ASA esterase activity and body mass index (BMI) in the same setting was assessed.

FIGURE 1

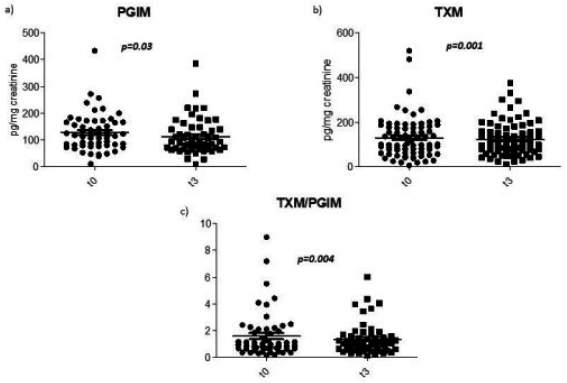

Figure 2. Urinary levels of prostacyclin (PGIM, **a**) and thromboxane (TXM, **b**) metabolites and their ratio, index of vascular homeostasis (**c**), were measured in patients with coronary artery disease (CAD, n=84) before (t0) and after 6 months of Mediterranean diet (t3).

FIGURE 2

p=0.001, respectively), evidencing an improvement also in vascular homeostasis.

Conclusions: The data here presented highlighted the presence of a correlation between aspirin esterase activity and body size in CAD setting. Overall, the results of this study should be considered in designing the cardiovascular prevention strategies in obese patients.

PB0076 | Endogenous PCSK9 Is Related to Circulating CD34^{bright}/CD146^{-} Cells in Patients with Type 2 Diabetes Mellitus

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Background: Protease proprotein convertase subtilisin/kexin type 9 (PCSK9) is a regulator of low-density lipoprotein (LDL)-cholesterol clearance and has been associated with cardiovascular disease risk, platelet function and blood coagulation. PCSK9 knockout mice develop less venous and arterial thrombosis and show reduced in vivo platelet activation upon arterial injury. In vivo PCSK9 inhibitors, besides the cholesterol-lowering effect, increase circulating endothelial progenitor cells (cEPCs). cEPCs are a subtype of immature cells with a role in ongoing endothelial repair. EPC administration accelerates the resolution of arterial thrombus in mice while their depletion may contribute to endothelial dysfunction and cardiovascular disease progression. Circulating CD34^{bright} and their subpopulation CD34^{bright}/CD146^{-} cells include both mature Endothelial Cells (ECs) and EPCs.

Aims: The aim of this study was to evaluate whether the effect of PCSK9 on vascular homeostasis and atherothrombosis may be mediated by EPC in patients with or without Type 2 Diabetes Mellitus (T2DM).

Methods: Fifty-five patients (33 with and 22 without T2DM) were enrolled at the Diabetes Clinics of Chieti University Hospitals. All patients were treated with low dose ASA (100 mg day) for

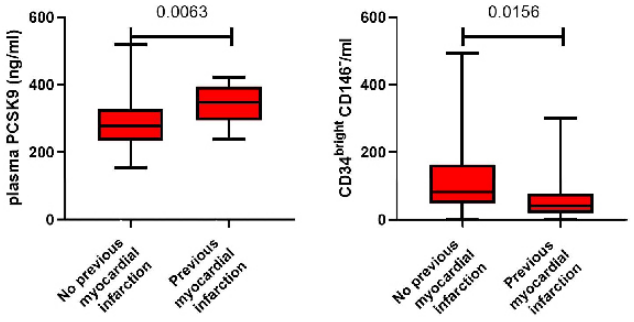

FIGURE 1

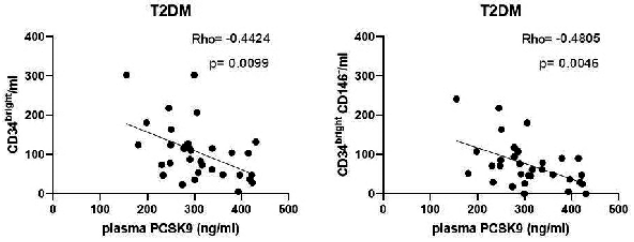

FIGURE 2

cardiovascular prevention. Twelve had a previous myocardial infarction. Plasma PCSK9 was measured by enzyme-linked immunosorbent assays (ELISA) (#DPC900, R&D). Circulating CD34^{bright} and CD34^{bright}/CD146^{-} cells were analyzed by a standardized flow cytometry method.

Results: PCSK9 levels were comparable between patients with or without T2DM. Circulating CD34^{bright} and CD34^{bright}/CD146^{-} cells were significantly lower in patients with T2DM (p=0.018 and p=0.039 respectively). Patients with a previous myocardial infarction had higher levels of PCSK9 (p=0.006) and lower levels of circulating CD34^{bright}/CD146^{-} (p=0.016). Only in patients with T2DM, PCSK9 correlated inversely with both CD34^{bright} (Rho=−0.442, p=0.010) and CD34^{bright}/CD146^{-} (Rho=−0.480, p=0.005).

Conclusions: Our results suggest that in high-risk patients with T2DM, endogenous levels of PCSK9 may have a detrimental effect on CD34^{bright}/CD146^{-} by reducing the endothelial repair and worsening the progression of atherothrombosis.

PB0077 | Clinical Implications of White Blood Cell Count and Platelet Indices in Individuals with Risk Factor of Coronary Artery Disease

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Background: Hypertension, dyslipidemia, diabetes mellitus and obesity are the major risk factors of coronary artery disease (CAD). It has been known that atherosclerosis is a major cause of CAD. It has been reported that white blood cells (WBC) count and platelet indices play the important roles in the promotion of vascular injury and endothelial cell damage in individuals with risk factors of CAD.

Aims: Therefore, this study aimed to evaluate the association of hematological parameters including WBC and platelet with the risk of CAD risk factors along with other biological parameters.

Methods: A case-control study was performed in 220 individuals who underwent to Dokkhamtai hospital, Phayao province, Thailand for annual health check-up since 2015-2018. Based on baseline characteristics, participants were classified into two groups; healthy control (n=106) and individuals with risk factors (n=114).

Results: The results show that participants with CAD risk factors had higher levels of WBC count, mean platelet volume (MPV) and platelet distribution width (PDW) than healthy groups (P< 0.05). Moreover, WBC count was associated with BMI, SBP, uric acid, and triglyceride. In addition, PDW was related to SBP (p=0.018). Thus, these results indicated that WBC count and PDW may contribute to obesity, hypertension and dyslipidemia. Furthermore, high MPV levels (OR [95% CI]=2.72 [1.18-6.24], P< 0.001) and PDW (OR [95% CI]=1.83 [1.08-3.10], P< 0.001) were also associated with increased risk of obesity and hypertension, respectively. However, the correlation of hematological parameters with diabetes mellitus was not observed.

Conclusions: In conclusion, this study suggested that hematological parameters were associated with risk of major risk factor for CAD which may indicate that these hematological parameters can be used to evaluate the risk of major risk factors and CAD in healthy individuals.

PB0078 | Proteases from Pathogenic and Non-pathogenic Bacteria Activate Prothrombin (ProT) in Human Plasma: Implications in Thrombotic Diseases

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Background: Clinical evidences accumulated so far indicate that Aging and Inflammatory Bowel Diseases (IBDs), both associated with enhanced gut permeability, are positively related to remarkably increased thrombotic risk. Similar conclusions can also be drawn for severe thrombotic complications in Infectious Diseases (IDs). We have recently discovered that subtilisin, a protease secreted from *B. subtilis* in the gut microbiota, is able to clot human blood by converting ProT into active thrombin species [Pontarollo et al., JBC (2017)].

Aims: Extend our previous work to other proteases from both non-pathogenic (*Bacillus subtilis*) and pathogenic (*Staphylococcus aureus*) bacteria to address novel molecular mechanisms of thrombotic diseases.

Methods: Biochemical techniques: limited proteolysis, enzymatic chromogenic assay, mass spectrometry; coagulation assays: fibrin generation and platelet aggregation; intestinal permeability assay.

Results: Neutral protease (NP), the second most abundant protease secreted by *B. subtilis*, cleaves ProT at Asn162-Leu163, Ala470-Asn471 in the γ-loop, and Arg320-Ile321 in the activation loop. Unlike subtilisin, NP can generate fibrin with purified fibrinogen but not in plasma samples. Addition of *B. subtilis* culture medium to human plasma can efficiently induce clot formation and, importantly, fluorescein-labelled subtilisin and NP can translocate across a CaCo-2 cells simulated intestinal epithelium, with a rate of 25-35% after 7 hrs.

Among the four *S. aureus* extracellular serine proteases tested, only EpiP induced fibrin clotting in human plasma. Notably, EpiP cleaves ProT at the very same cleavage sites as factor Xa (i.e. Arg155-Ser156, Arg271-Thr272, Arg320-Ile321), generating mature αT which in turn triggers fibrin generation and platelets aggregation.

Conclusions: The identification of ProT-cleaving proteases in pathogenic/non-pathogenic bacteria explains how unrelated diseases (Aging, IDs and IBDs) are all complicated by increased thrombotic risk. Whereas in IFs the pathogen finds its own route to the blood, in Aging and IBDs the enhanced permeability of the gut mucosa allow translocation of extracellular bacterial proteases into the bloodstream.

PB0080 | Evaluation of Biological Effects of Airborne / Urban Pollution Particles

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Good Biomarker Sciences, Leiden, the Netherlands

Background: Contact activation is an essential aspect of biocompatibility. It plays a role in blood collection because of the material of the tubes and can cause a low-grade coagulation activation. For airborne / urban particulate matter it may be a factor in causing lung disease and other health problems.

Aims: We tested laboratory materials, air particulate matter for causing contact activation in plasma.

Methods: Kallikrein generation and NAPTT in plasma. Aerosil was the calibrator

Results: In the kallikrein generation, some of the laboratory material showed strong reactions (Eppendorf tubes, Greiner Elisa, Nunc High Bind and Coater black plates) and were excluded. Various APTT- silica reagents were tested and had variable potency: 336-696 μg/ml aerosil-equivalents: the highest was Synthasil. Actin FS showed 135 ug/ml aerosil-equivalents. For solutions with 400 μg/ml: asbestos was equivalent to 169 μg/ml aerosil-eq; Cerium oxide and diesel exhaust to 75 μg/ml aerosil-eq and Beach sand dust to 70 μg/ml aerosil-eq. Air particulate matter was harvested on building surfaces and roadsides and mixed with fluid to 400 μg/ml. Along roads the potency was around 3-7 μg/ml aerosil-eq. with a peak of 19 and trough of 1.4 μg/ml aerosil-eq. All particles were tested after uptake in water and tested for size distribution by dynamic light scattering: all were between 200-700 nm hydrodynamic radius. Comparing contact activation of kallikrein with activation of factor XI showed that some air borne particles were inactive in factor XIa activation. When both kallikrein and factor XIa were formed the potency showed a good correlation.

Conclusions: Airborne pollution particles have a range of potencies to activate the contact system, and specific activation of kallikrein is possible. It suggests that next to number, also quality of the particles may be relevant for inducing health problems.

PB0082 | The Clinical Predictors of Coronary Artery Disease: A Population-based Study from Developing Country

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Background: Coronary artery disease (CAD) was the second leading (9%) of overall mortality in Indonesia. However, Indonesia has limited data regarding cardiovascular disease (CVD) predictors.

Aims: To identify clinical predictors of CAD among the population of Central Jakarta City, Indonesia.

Methods: Population-based retrospective study was conducted to evaluate the prevalence and predictors of CAD. We examined 87.895 participants (15-99 years old [y.o]) in community-based health services located in Central-Jakarta, Indonesia. We collected data of blood pressure, body mass index (BMI), age, sex, personal (PH) and family history (FH) of CVD risk factors including smoking, diabetes mellitus (DM), stroke, CAD, dyslipidemia using standardized protocol. Data were analyzed using SPSS to estimate the odds ratio (OR) with logistic regression.

Results: Prevalence of CAD was 1.06%. Premature CAD in participants (15-45 y.o) was counted 0.48%. The mean age of participants was 42.1±6.4 y.o. Multivariate analysis showed that odds for developing CAD increased along with increasing age (OR=4.3,p< 0.0001,p-trend< 0.0001), and BMI (OR=1.3,p< 0.05), p-trend< 0.05), male (OR=1.4,p< 0.0001), presence of FH of CAD (OR=7.2,p< 0.0001), FH of DM (OR=1.9,p< 0.0001, PH of DM (OR=2,p< 0.0001), PH of hypertension (OR=3.7,p< 0.0001), PH of dyslipidemia (OR=1.4,p< 0.0001). Stratified analysis showed that the odds of CAD was also higher in the female with PH of DM (OR=2,p< 0.0001), and increasing age (OR=4.9,p< 0.0001) in comparison with the male. In contrast, male with FH of CAD (OR=7.7,p< 0.0001), PH of hypertension (OR=4.4,p< 0.0001) has higher odds of developing CAD compared

with female. Female with menopause also has greater odds of having CAD in comparison with reproductive age (OR=1.7,p< 0.05).

Conclusions: Increasing age and BMI, male, FH of CAD and DM, PH of DM, dyslipidemia, and hypertension were independent predictors for CAD among the population of Central-Jakarta, Indonesia. Public health initiatives engaging communities should be escalated to prevent the burden of CAD.

PB0083 | African American Women with Diabetes Undergoing Catheterization Represent Most Prothrombotic Subgroup: An Explanation for the Platinum Diversity Study Results?

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Background: Recent studies, such as PLATINUM Diversity, have shown that African American (AA) women have a higher risk of myocardial infarction after percutaneous coronary intervention (PCI); this risk may be increased by concomitant diabetes (DM). Disparities in post-PCI outcomes may be explained by differences in thrombogenicity among race and gender and DM.

Aims: To investigate the influence of race and gender on thrombogenicty in AA and Caucasians (C).

Methods: We included AA and Caucasian (C) men (M) and women (W) with and without DM undergoing elective coronary angiography (n=1,031). The viscoelastic properties of platelet-fibrin clot were measured pre-procedure using the TEG 5000 Hemostasis System. Thrombotic risk was defined as thrombin induced platelet-fibrin clot strength (TIP-FCS) >68mm.

Results: Baseline clinical characteristics were similar across the eight groups, however AA-W were more likely to have BMI > 35. There was greater TIP-FCS (P< 0.001 by
ANOVA) in AA vs C, W vs M and, DM vs non DM, respectively. AA-W with DM had the greatest thrombogenicity compared to all other groups (Figure). Multivariate

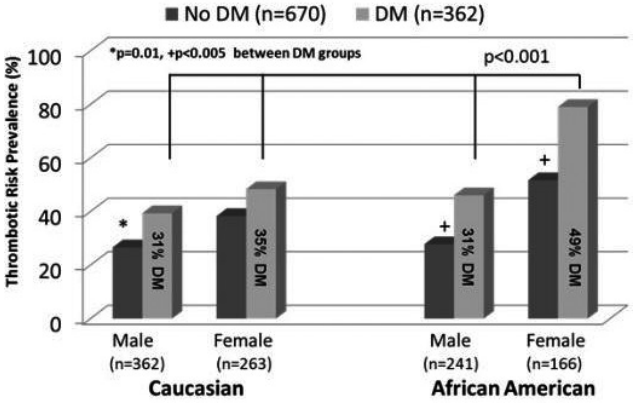

FIGURE 1 Thrombotic Risk Prevalence

analysis showed that race and gender, DM, morbid obesity, and WBC count were independent predictors of thrombotic risk.

Conclusions: Thrombogenicity is a novel measurable cardiovascular risk factor that varies by race, gender and DM and is greatest in AA women with DM. These findings provide a plausible biologic mechanism for the gender-, race- and diabetes- related variances observed in the post PCI risk of ischemic events.

PB0085 | Pulmonary Thromboembolism in General Practice

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Background: Timely diagnosis of pulmonary thromboembolism (PTE) is remaining problem in general practice and emergency department. Deep vein thrombosis (DVT) less often precedes PTE.

Aims: Aim of our study was to determine the most frequent reasons of PTE in general practice.

Methods: We observed 15 patients with acute respiratory insufficiency suspected for PTE. Besides common investigations was studied D-dimer, all patients were estimated according to revised Geneva clinical prediction rule for PTE. Patients with high or intermediate clinical probability of PTE were studied by computed tomographic pulmonary angiography (CTPA).

Results: Though in all these patients D-dimer was above age-adjusted cut-off level (1200-7520 μg/L), CTPA study confirmed PTE only in 10 patients. Among them echocardiologic evaluation revealed right ventricular enlargement, highly or severely increased PASP only in 6 patients, decreased TAPSE in 3 patients. DVP preceded PTE only in 2 cases (one 70-year-old woman with atherosclerosis, one 24-year-old woman with congenital MTHFR gene C677T homozygous mutation). The rest 8 patients were at age of 59-86 year (2men, 6-women) with arterial hypertension, atherosclerosis and/or heart failure as comorbidities and acute pneumonia, that appeared main trigger for the development of PTE. 2 patients were hemodynamically unstable during first 2 days. In coagulation tests INR and APPT was at the low level of normal range, fibrinogen was more than 4g/L in 5 patients (2 had >6g/L). All patients improved. Besides symptomatic appropriate therapy (antibiotics in cases of pneumonia) in acute stage patients were treated with UF Heparin on the 1st day, then with LMW Heparin for 7-10 days, continued with Rivaroxaban 30mg/day for a month and 20mg/day for 2-3months.

Conclusions: Our study shows that in all elderly patients with acute pneumonia, especially with high or intermediate clinical probability of PTE besides echocardiography, coagulation tests and D-dimmer should be investigated CTPA to precise diagnosis, give adequate treatment and escape complications.

CEREBROVASCULAR DISORDERS

PB0086 | Thromboembolism in Thalassemia Patients: Is Aspirin Protective against Brain Ischemia over Three Years Follow Up in Beta Thalassemia Patients?

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Background: Silent brain lesions might be associated with overt cerebrovascular accident over time in beta thalassemia major (BTM) and intermediate (BTI) patients.
Aims: we evaluated brain MRI in thalassemia patients who had already performed brain MRI in order to see whether aspirin is protective or not over three years.
Methods: In this historical cohort study, 44 thalassemia patients (22 BTI and 22 BTM) were randomly selected by simple random sampling method from 46 BTM and 49 BTI patients who had performed brain MRI at baseline, 3 years before the end of the study. The inclusion criteria were no overt neurological defects and the patients underwent second MRI after three years follow up. The dose of aspirin was 80-100 mg once daily.
Results: Median age of the patients was 32 years and ranged from 8 to 42 years. Overall frequency of white matter lesions (WMLs) in the first MRI was 10 patients (28.6%). After 3 years, 3 patients developed new lesions and the frequency of WMLs was 13 patients (37.1%) in the second MRI. Moreover, in 3 patients, number of WMLs increased. Patients with new lesions or more lesions compared to the baseline were significantly older than the other group (median age 36.5 years vs. 31 years). Only 1 patient (16.7%) of those patients with new lesions was using aspirin compared to 10 (34.5%) of the other group.
Conclusions: Increased numbers of silent cerebral ischemia are found both in multi transfused and non-transfused patients with β-thalassemia. The high-risk patients with thrombocytosis, splenectomy, severe iron overload, and older age (>30 years) should be under close follow-up and evaluated on a regular periodic basis as well as brain MRI at least once every 3 years. Aspirin could be protective against new or progressive brain lesions so that low-dose aspirin is recommended in high-risk thalassemia patients.

FIGURE 1 CT-scan of brain lesion in asymptomatic beta thalassemia intermediate patient: Hypodense ischemic i

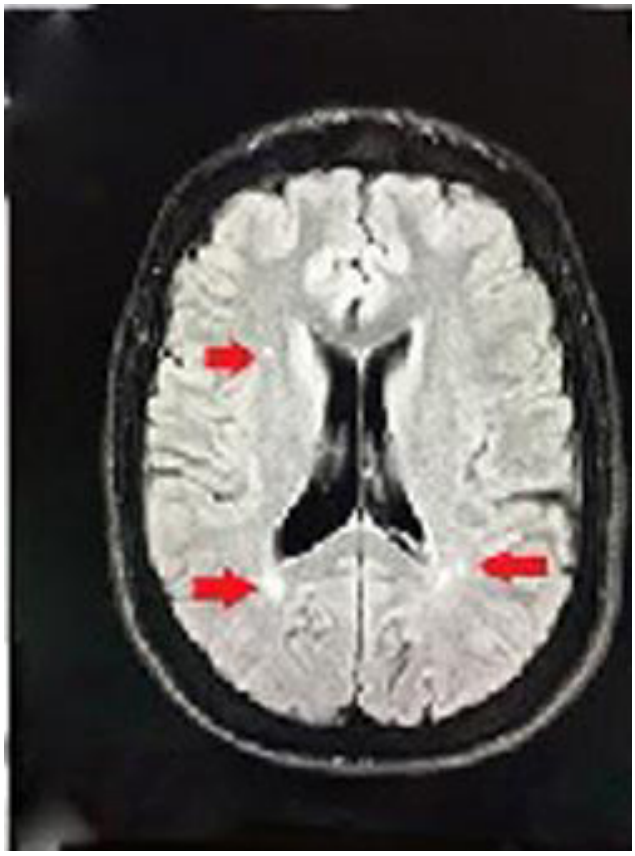

FIGURE 2 A 25-year-old male BTM patient not splenectomized on regular blood transfusion Cranial MR axial Flai

PB0087 | Silent Brain Ischemia in Thalassemia Patients: Breaking the Silence

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Background: Despite advances in understanding silent cerebral ischemia (SCI) in sickle cell anemia, data from thalassemia has only recently started to emerge. Cerebral venous and arterial thromboembolism have been reported both in non-transfusion and transfusion-dependent thalassemia (NTDT and TDT).

Aims: We highlight advances in understanding the prevalence of SCI in thalassemia, discuss the potential underlying risk factors, and determine required efforts to achieve optimal prevention and treatment.

Methods: Several studies have been conducted on 135 neurologically intact thalassemia patients (49 NTDT and 86 TDT) from 2008 to 2018. Diffusion-weighted imaging brain MRI (1.5 tesla) was performed.

Results: The microthrombosis in the brain in asymptomatic patients with NTDT and TDT has been shown changes in the white matter suggestive of ischemia and small infarctions (figure1).

Overall, white matter lesions (WMLs) was found in 30 of 135 TDT and NTDT patients (22.2%) including 11 of 49 NTDT (22.4%) and 19 of 86 TDT (22%). Splenectomy, age >30 years old, high serum ferritin levels, platelet counts > 500000/mm^3 and hemoglobin level < 9 g/dl are risk factors for SCI.

In another study, brain MRI in 35 NTDT and TDT patients was evaluated over 3 years. The frequency of WMLs was increased from 10 patients (28.6%) in the first MRI to 13 patients (37.1%) in the second MRI after 3 years.

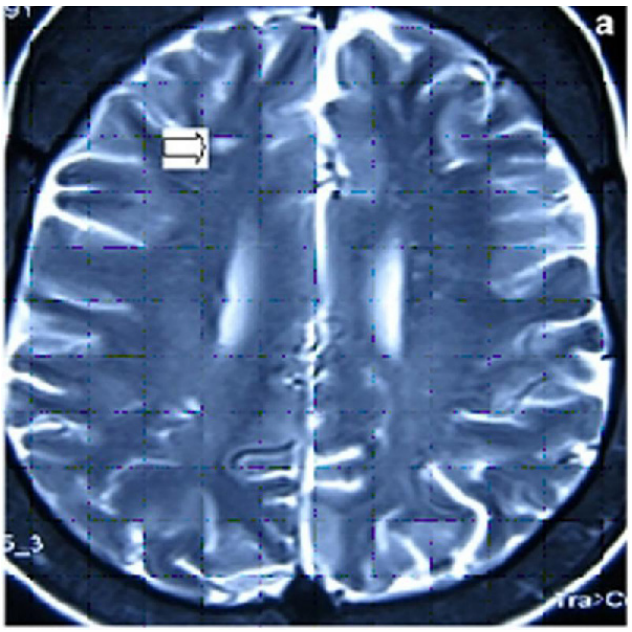

FIGURE 1 Axial T2-weighted. Image shows hyperintense foci in both cerebral hemi-spheres suggesting small isc

Conclusions: The long-term sequelae of such covert findings are unknown, although experience from patients with sickle cell anemia confirms their association with subsequent overt stroke and neurocognitive deficits. The high-risk patients with thrombocytosis, splenectomy, severe iron overload, and older age should be under close follow-up and evaluated regularly as well as brain MRI. Aspirin, keep the Hb levels ≥9 g/dL, regular iron chelation regimen and try to preserve the spleen are the management strategies to prevent new or progressive silent brain lesions.

PB0089 | Hereditary Thrombophilia in Arterial Ischemic Stroke: A Study from the South of Tunisia

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Background: Arterial ischemic stroke (AIS) is the second most common cause of death in Tunisia in the last 15 years and whose etiological investigation is still challenging. Some recent reports have noticed a probable accountability of common prothrombotic risk factors in the AIS's occurrence.

Aims: Our study was aimed to describe epidemiological and biological characteristics of AIS in a young Tunisian population.

Methods: This study was carried out in 253 adults younger than 55 years old who had presented an arterial ischemic stroke (AIS) and were referred to our laboratory from 2012 to 2018. Biological exploration included the PS, PC and APC resistance (APC-R) analysis by coagulometric methods (STACLOT® PROTEIN S; STACLOT® PROTEIN C;STACLOT® APC-R diagnostica) and the AT analysis by a chromogenic method (STA-CHROM-AT diagnostica).

Results: The mean age of our patients was 38 ± 12 years old and the sex ratio was about 1. Our study revealed the presence of 35 cases (13%) congenital thrombophilic markers as follows: PS deficiency was detected in three patients/206 (1.5%), PC defect noticed in two cases/209 (1%), ATIII deficiency detected in three patients/231 (1.3%) and the APC-R found in 27 cases/225 (12%). In addition, combined disorders were noticed in 0.8%: PS defect and APC-R; AT and APC-R deficiencies.

Conclusions: According to some recent research papers, we consider that thrombophilia in AIS is rather frequent in the South of Tunisia. The most prominent effect has been awarded to the APC-R which was slightly increased in our study. Its high rate could be explained by the geographical origin of patients and by the endogamy's concept. Added to that, it is worth mentioning that combined defects do exist in our series. Then, it would be necessary to enlarge the number of samples to understand the thrombophilic characteristics of young Tunisian adults who suffered from AIS to decide the anticoagulant therapy.

PB0090 | Histological Analysis of a Thrombectomy-Resistant Thrombus in Acute Ischemic Stroke

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Background: An important challenge in mechanical thrombectomy is that thrombi differ in removability and that approximately 10-20% of them are irretrievable. The reasons for thrombectomy resistance are often unclear, but thrombus composition might be an important factor. Multiple removal attempts are associated with worse clinical outcome, stressing the importance of first pass success.
Aims: The aim of this study was to histologically analyze a thrombus nearly irretrievable by mechanical thrombectomy.
Methods: Thrombus material was histologically analyzed using hematoxylin and eosin, Martius Scarlet Blue staining (red blood cells (RBCs) and fibrin), Feulgen staining (DNA), von Kossa staining (calcifications) and immunohistochemical analysis of von Willebrand factor (VWF), platelets (GPIb), leukocytes (CD45) and neutrophil extracellular DNA traps (H3Cit; NETs).
Results: In a patient that was admitted with a right MCA M1 occlusion and treated with mechanical thrombectomy (Groeninge Hospital, Kortrijk, Belgium), 11 attempts using various thrombectomy devices and techniques were required for removal, representing a nearly irretrievable thrombus. This rare case of achieving thrombus material from a nearly irretrievable thrombus allowed us to perform an in-depth histopathological study of the retrieved material. Histological analysis revealed abnormally high amounts of extracellular DNA (exDNA), leukocytes, VWF and calcifications. ExDNA stained positive for markers of leukocytes and NETs, indicating that the source of exDNA is at least partially derived from NETs.
Conclusions: In this unique case of a nearly thrombectomy-resistant stroke thrombus, our study showed an atypical composition compared to the common structural features found in ischemic stroke thrombi. The bulk of the retrieved thrombus consisted of exDNA that colocalized with VWF and microcalcifications. This result supports the hypothesis that VWF, NETs and microcalcifications contribute to thrombectomy resistance. Such information may be important to optimize removal protocols and technologies that enhance first pass success rates.

PB0091 | Impact of Protease Nexin-1 in Cerebral Ischemia

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INSERM U 1148-LVTS, Paris, France

Background: Ischemic strokes result from the consequences of a cerebral artery obstruction by thromboembolism. Protease Nexin-1 (PN-1) is a cellular serpin present in both nervous and vascular systems, and is hypothesized to display a neuroprotective role in the brain parenchyma, through its ability to inhibit proteases of the coagulation and fibrinolytic system.
Aims: The project aims to demonstrate the impact of PN-1 in ischemic stroke development, and in particular on the blood brain barrier (BBB) that separates blood flow and the central nervous system.
Methods: A transformed human cell line (hCMEC/D3), an endothelial line isolated from the brain micro-vessels, was used as a model for the study of the BBB. Lentiviral vector-mediated knockdown of PN-1 in D3 cells cultured under ischemic conditions (oxygen and glucose deprivation for 24h, OGD) was used to determine the impact of PN-1:

1) on BBB permeability by using the real-time cellular analysis system xCELLIgence,
2) on pericellular proteolysis by western blotting of junction proteins, and
3) on cell death using LDH dosage. Moreover, wild-type and PN-1$^{-/-}$ mice were subjected to the transient middle cerebral artery occlusion (tMCAO) stroke model for brain infarct volume evaluation.

Results: Proteolysis of junction proteins (VE-cadherin, ZO-1 and Claudin-5) induced by OGD was accentuated after PN-1 extinction. Both D3 cell permeability and D3 cell death induced by OGD were increased in PN-1-knockdown D3 cells. In vivo data showed that brain infarct volumes observed 24 h after tMCAO, in TTC-stained brain sections, were larger in PN-1$^{-/-}$ compared to wild-type mice.
Conclusions: PN-1 preserves the integrity of the BBB by limiting the proteolysis of junction proteins and cell death in BBB endothelial cells. These data explain how PN-1 expressed by the BBB can display its protective role against cerebral ischemia/reperfusion.

PB0092 | Hemorrhagic and Ischemic Stroke in Patients after Allogeneic Stem Cell Transplantation

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative therapy for many hematological disorders.

TABLE 1 Risk Score Points of Stroke after Allo-HSCT

Table 1. Risk Score Points of Stroke after Allo-HSCT

Predictors	HR (95%CI)	P value	Points
High risk disease	1.975 (1.289-3.027)	0.024	1
ALL	1.798 (1.226-2.637)	0.012	1
Central nervous system leukemia	1.910 (1.144-3.191)	0.004	2
Grade III–IV aGVHD	1.948 (1.245-3.048)	0.004	2
TMA	2.896 (1.631-5.143)	< 0.001	3
Delayed platelet engraftment	2.658 (1.842-3.837)	< 0.001	2

Recently, there have been new insights into the pathophysiology of stroke.

Aims: Here, we conducted a retrospective nested case-control study to investigate the incidence, clinical characteristics, risk factors and transplant outcomes of stroke following allo-HSCT.

Methods: A total of 6123 consecutive patients received allo-HSCT at Peking University People's Hospital from 2007 to 2018. Patients diagnosed with stroke were enrolled in our study, including hemorrhagic and ischemic stroke.

Results: Overall, 2.3% of patients were diagnosed with stroke after allo-HSCT (hemorrhagic: 1.0%, ischemic: 1.3%). The median time to hemorrhagic and ischemic stroke after HSCT was 161 days and 137 days. In total, 8.4% of patients experienced neurological sequelae. The stroke led to death in 25.9% of patients. The cumulative incidence of 11-year relapse was 30.8% and 24.1% in patients with and without stroke. The incidence of relapse was comparable between the two groups. The 11-year cumulative incidence of overall survival (OS) for patients diagnosed with stroke was 26.8% compared with 46.5% in the control group. OS was much worse in patients with stroke than in control patients ($p < 0.001$). The cumulative incidence of transplant-related mortality (TRM) at 11 years was 28.9% and 54.1% for patients with and without stroke ($p < 0.001$). Our analysis showed that significant independent risk factors for the occurrence of stroke were high risk disease, acute lymphoblastic leukemia (ALL), central nervous system leukemia, grade III-IV acute graft-versus-host disease (aGVHD), thrombotic microangiopathy (TMA), and delayed platelet engraftment. Using these risk factors, a risk score model was constructed to predict the risk of stroke after allo-HSCT (Figure).

Conclusions: Stroke is a relatively significant and life-threatening complication after allo-HSCT. The risk score model provides a practical tool to predict the risk of stroke.

PB0093 | A Recurrent Ischaemic Stroke Case with Type 1 von Willebrand Disease

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Background: An elevated level of von Willebrand factor (VWF) is associated with an increased risk for coronary heart disease and ischemic stroke.

Aims: The objective of the study to present a rare case Type 1 von Willebrand disease with ischaemic stroke.

Methods: 18 year old, male patient who has mild combine faktör 8 deficiency (%22) and von willebrand factör deficiency (%10) apply to emergency department with aphasia and inability to walk. As possible stroke risk factörs are high homosistein levels (45), O Rh (+), ANA (+,granüler,sitoplasmic pattern). His echocardiography and ecg is normal. Do not use regularly aspirin and factor 8 and von Willebrand prophylaxis. After 2 years, again ischeamic stroke happened.

Results: After factor replacement with aspirin treatment, clinical outcome better. He could be able to speak and walk.

Conclusions: Type 1 von Willebrand disease with remittant ischeamic stroke is a rare case in the literatüre that might have better prognosis with antiagregan treatment and factor replacement.

PB0094 | Plasma Calprotectin as a Novel Independent Predictor of Mortality in Acute Ischemic Stroke

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Background: Inflammatory response plays an important role in several processes related to acute ischemic stroke (AIS) secondary injury.

Aims: The purpose of this study is to evaluate circulating plasmatic calprotectin levels as a prognosis biomarker in AIS patients.

Methods: We collected a total of 748 AIS patients admitted to our Stroke Unit between 2015 and 2017 in which acute established ischemic injury was confirmed by neuroimage in 467. Those patients with systemic inflammation or infection at onset (including active cancer, post-operative patients and active infections) were excluded. Plasma calprotectin was measured by ELISA in blood samples obtained within the first 24 h (Total, n=413). Univariate and multivariate logistic regression models were performed.

Results: Higher calprotectin levels were observed in patients who died at 90 days [median, (IQR) µg/mL; 2.81, (2.06-4.26) vs 1.58, (1.00-2.29), $p< 0.001$], and in those patients who developed an intracranial haemorrhage (ICH) after AIS [1.99, (1.36-3.32) vs 1.72, (1.10-2.56), $p=0.030$], whereas significant lower calprotectin levels were found in patients with 3-month functional independence (FI) [1.49, (0.97-2.23) vs 2.2, (1.41-3.38), $p< 0.001$]. In multivariate

predictive models, 1-point increase in plasma calprotectin log-value was independently associated with higher odds of 3-month mortality [OR (CI 95%); 3.00 (1.71-5.26)]. Patients with a 24 h calprotectin ≥ 2.26 μg/mL were 4.14 times more likely to die than AIS patients with levels < 2.26 μg/mL Nevertheless, calprotectin was not a significant predictor of FI or ICH in adjusted models.

Conclusions: Plasma calprotectin is an independent predictor of 3-month mortality in AIS patients; therefore it may be considered in future predictive models to identify patients with high risk of poor prognosis to improve stroke patient management.

PB0097 | Effect of Pulsatility on Thrombosis Modelling in Cerebral Aneurysms

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Background: There are a growing number of computational models of thrombosis in cerebral aneurysms that have been designed with consideration towards clinical use. Many of these computational models rely on assumptions to simplify what would otherwise be computationally expensive and slow simulations. A common assumption for these models is that because the average flow-rate is the same, steady flow is an acceptable simplification of pulsatile flow. There is, however, limited evidence suggesting whether steady flow is an acceptable approximation when considering clot formation within a flowing environment.

Aims: Determine whether the use of pulsatile flow in a computational thrombosis model results in a significantly different outcomes than steady flow.

Methods: A pulsatile-flow thrombosis-model has been created in ANSYS® Fluent (ANSYS, Lebanon, NH) using a transient-solution implicit pressure-based solver, wherein an idealized aneurysm geometry is applied, with patient-data-derived pulsatile volumetric flow function supplied at the inlet. A scalar function, derived from Wagenvoord et al., is supplied at the aneurysm wall, and simulates the release of thrombin. The output of an otherwise identical steady-state model is validated with the output of a steady-flow, Particle-Image-Velocimetry (PIV) experiment utilizing an identical geometry, wherein thrombin is injected at the aneurysm, and tracked via a proflavine dye. The validated steady-state numerical model is then altered to include pulsatility, and is then used to determine whether clotting outcomes of a model by Ngoepe and Ventikos, amongst others, differs with pulsatile flow.

Results: This comparison reveals that activated thrombin accumulates more quickly and dissipates more slowly when utilizing pulsatile flow over steady flow. Pulsatile flow creates unsteady flow patterns within the aneurysm, which create an environment where less thrombin is carried out of the aneurysmal sac and into the regular bloodstream.

Conclusions: The results suggest that pulsatile-flow can significantly increase the duration of clotting, which may have a significant effect on clotting outcomes.

PB0098 | Temporary Hypofibrinogenemia in Reversible Cerebral Vasoconstriction Syndrome (SRVC) Associated a Cannabis Overdose

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Background: More than 5000 drug-related emergencies are attended annually in Spain.

Aims: We report a case of transitory hypofibrinogenemia in a young woman with SRVC secondary to cannabis overdose with an uncommon clinical presentation.

Methods: A 19-year-old female patient with episodes of recurrent vomiting presented a generalized tonic-clonic seizure followed by intense holocranial headache, and high blood pressure. CT scan and cranial MRI were performed visualizing acute ischemic bilateral frontoparietal subcortical lesions with vasogenic edema, as well as chronic hemorrhagic deposits in both posteroparietal regions. Infectious, autoimmune, porphyria and vasculitic etiologies were excluded. A Transcranial Doppler study showed segmental stenosis in both MCAs. A coagulation study revealed normal prothrombin, activated partial thromboplastin and low fibrinogen levels (Clauss method)). The patient confessed that in the last days she has been consumming high doses of cannabis during festival days (10 cigars per day).

Results: Given the suspicion of SRVC, an intracraneal arteriorgraphy was done, showing multiple vasospasms in segmental distal territories of both ACA and left PCA, with lesser involvement of MCA. Toxic in urine was positive for Cannabis. Prothrombin time-derived fibrinogen activity was 1.8 g/L and Clauss fibrinogen was 1.2 g/L. Finally, she was discharged asymptomatic in the third week after treatment with metilprednisolone, analgesics and levetiracetam, with fibrinogen normalization.

Conclusions: Hemorrhagic or ischemic stroke, aneurysmal subarachnoid hemorrhage and SRVC following high doses of cannabis consumption have been described. It is unclear whether have associated cannabis use in the regulation of inflammatory responses (inverse association between cannabis use and levels fibrinogen was described). Ensuring optimal levels of real fibrinogen, not derived, is important in these patients and especially if they go to fibrinolytic therapy. We highlight the unusual initial clinical presentation and fibrinogen decreased after taking Cannabis, one of the most consumed drugs by our teenagers.

PB0099 | Secondary Brain Damage in Mouse Models of Focal and Global Ischaemia and Haemorrhagic Stroke: A Comparative Study

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Background: Stroke is caused due to obstructed blood flow (ischaemia) or unrestricted bleeding in the brain (intracerebral haemorrhage; ICH). Global brain ischaemia occurs when cerebral blood flow is restricted e.g. during cardiac arrest. Following ischaemic injury, restoration of blood flow causes ischaemia-reperfusion (I/R) injury which further worsens outcome.
Aims: To study secondary injury mechanisms in global and focal ischaemia and to compare it with ICH using standardised readouts for inflammation, endothelial dysfunction, blood-brain barrier (BBB) damage and apoptosis.
Methods: We compared the conventionally used model of I/R injury (middle cerebral artery occlusion; MCAo) with ICH (collagenase model) and a newly developed model of global I/R injury (dual carotid artery ligation; DCAL).
Results: MRI revealed that DCAL produced smaller bilateral striatal lesions, whereas MCAo produced a larger focal corticostriatal lesion. Striatal lesion volume was relatively smaller after ICH. After global ischaemia mice had worse overall neurological scores, although quantitative locomotor assessment showed that MCAo and ICH had significantly worsened mobility. BBB breakdown was seen in both hemispheres of the brain after DCAL whereas apoptotic activity was highest in the haemorrhage model. VCAM-1 upregulation was specific to ischaemia, but increased complement receptor (C5aR) mRNA was detected in all three conditions. Differential transcriptional upregulation of pro-inflammatory chemokines and cytokines was seen in the three models.
Conclusions: Given the lack of success in addressing neuroprotection in stroke, laboratory studies comparing more than one ischaemic stroke model or ischaemic and haemorrhagic stroke models are important, but uncommon. The establishment of multiple readouts for different pathophysiological processes can help pinpoint drugs that might be effective under conditions of both ischaemic and haemorrhagic stroke, thereby also eliminating requirement for sophisticated neuroimaging prior to administration. This work also offers strategies to identify drugs that might cause haemorrhagic transformation when developed for treatment of ischaemic stroke.

PB0101 | Characterization and Antithrombotic Treatment of Patients with Acute Ischemic Stroke and No Atrial Fibrillation: A Real-world Study

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Background: Anticoagulants are indicated for the prevention of acute ischemic stroke (AIS) in patients with atrial fibrillation (AF). For AIS patients without AF, antiplatelets are recommended for the treatment and prevention of recurrent events. However, their actual use in clinical practice remains unclear.
Aims: This study characterized AIS patients without AF and the pattern of antithrombotic treatment in current clinical practice.
Methods: Hospitalized AIS patients without AF were identified from 4 US administrative claims databases, IBM MarketScan® Commercial Claims and Encounters (CCAE, aged 18-65 years), Multistate Medicaid (MDCD, aged ≥18 years), Medicare Supplemental Beneficiaries (MDCR, aged ≥65 years), and Optum's de-identified Clinformatics® Data Mart Database (Optum, aged ≥18 years) during the period January-1-2008 to December-31-2018. Demographics and comorbidities in 6 months prior to hospitalization and treatment with antiplatelets (P2Y12 inhibitors [P2Y12i] and aggrenox) and anticoagulants (warfarin and direct oral anticoagulants [DOACs]) in 30 days after hospitalization were examined. Use of over-the-counter aspirin and in-hospital drug exposure were not captured in these databases.

TABLE 1 Distribution of antithrombotic treatment patterns 30 days after hospitalized acute ischemic stroke without atrial fibrillation

	CCAE		MDCD		MDCR		Optum	
	N	%	N	%	N	%	N	%
All	122,317	100%	77,979	100%	127,046	100%	172,415	100%
No P2Y12i, Aggrenox, warfarin, or DOACs	90817	74.2%	69974	89.7%	99458	78.3%	136192	79.0%
P2Y12i mono-treatment	19805	16.2%	5349	6.9%	18009	14.2%	25987	15.1%
Aggrenox mono-treatment	3048	2.5%	826	1.1%	4090	3.2%	3906	2.3%
Warfarin mono-treatment	6174	5.0%	1181	1.5%	3437	2.7%	4145	2.4%
DOACs mono-treatment	1443	1.2%	465	0.6%	1177	0.9%	1199	0.7%
More than 1 treatment	1030	0.8%	184	0.2%	875	0.7%	986	0.6%

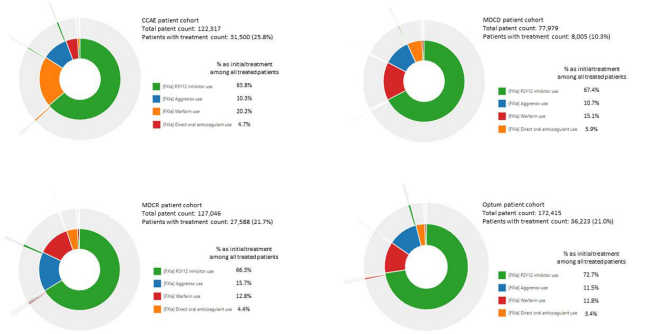

FIGURE 1 Pathway analysis among patients receiving antithrombotic treatment 30 days after hospitalized acute ischemic stroke without atrial fibrillation

Results: A total of 122,317, 77,979, 127,046, 172,415 AIS patients without AF were identified from CCAE, MDCD, MDCR, and Optum, with mean ages of 54.0, 61.6, 78.9, 70.0 years, respectively. In the 6 months prior to AIS, common comorbidities were hypertension, inflammation, hyperlipidemia, diabetes, heart and peripheral vascular disease. In the 30 days after AIS, 74.2-89.7% patients received no treatment of P2Y12i, aggrenox, warfarin, or DOACs, 6.9-16.2% received P2Y12i mono-treatment, 1.1-3.2% received aggrenox mono-treatment, 1.5-5.0% received warfarin mono-treatment, and 0.6-1.2% received DOACs mono-treatment.(Table) Among those receiving >1 treatment (0.2-0.8%), P2Y12i was the most common initial treatment, with warfarin as concurrent or subsequent treatment. (Figure)

Conclusions:: The large majority of patients do not receive P2Y12i, aggrenox, or anticoagulant treatment within 30 days after AIS. Among those treated, use of P2Y12i is the most common initial treatment. Future studies may evaluate outcomes associated with these treatments.

PB0102 | Plasma Concentration of Apolipoprotein C-III is Associated with Ischemic Cerebrovascular Events in High-Risk Cardiovascular Patients Taking Anticoagulant Therapy with Warfarin

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Background: Apolipoprotein C-III (Apo C-III) is a crucial regulator of lipoprotein metabolism and a recognized risk factor for cardiovascular diseases. High plasma concentration of Apo C-III has been associated with increased levels of coagulation factors, an enhanced thrombin generation, and prothrombotic diathesis. In an earlier work we suggest that high Apo C-III plasma concentration may predict an increased risk of ischemic stroke/TIA in cardiovascular patients.

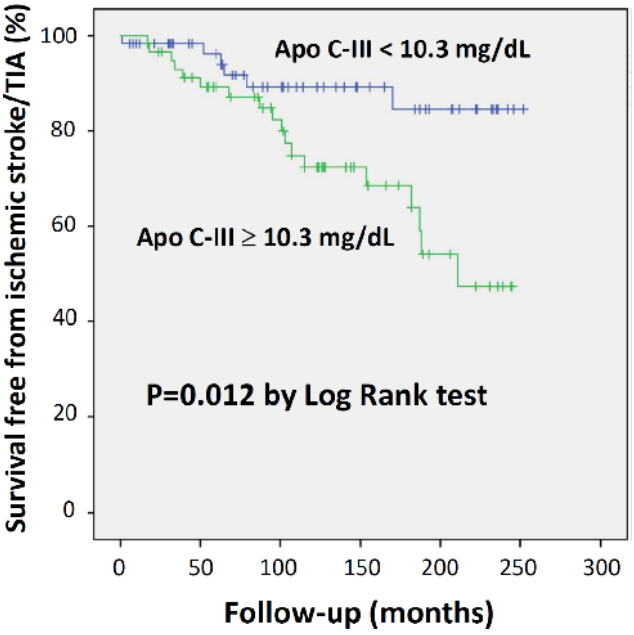

FIGURE 1 Kaplan-Meier survival curve for ischemic stroke/TIA in the study population stratified on the basis of apolipoprotein C-III plasma concentration

Aims: To examine the possible association between Apo C-III plasma concentration and ischemic cerebrovascular events in the specific setting of high-risk cardiovascular patients taking anticoagulant drugs.

Methods: Out of the original study cohort of 950 subjects with or without angiographically defined coronary artery disease (CAD) and with long-term follow-up data for cerebral ischemic events, we selected 118 subjects (66.9% males, mean age 66.1±11.7 years, 52.5% CAD) taking anticoagulant therapy with warfarin for either mechanical prosthetic heart valves or non-valvular atrial fibrillation. Complete plasma lipid and apolipoprotein profiles, including Apo A-I, B, C-III, and E, were available for all subjects at enrolment.

Results: After a median follow-up of 128 months, 24 subjects (20.3%) suffered ischemic stroke/transient ischemic attack (TIA) events. Stratifying the study population on the basis of Apo C-III plasma concentration, the incidence rate of ischemic stroke/TIA rate increased from the lowest to the highest Apo C-III quartiles (13.8%→6.7%→33.3%→27.6%, P=0.040). Subjects with Apo CIII levels above the median value (10.3 mg/dL) exhibited an approximately three-fold increased risk of stroke/TIA (HR 3.08, 95% CI 1.22-7.77 - Figure), even after adjustment for sex, age, and CAD diagnosis (HR 2.86, 95% CI 1.09-7.53), as well as after adjustment for CHA_2DS_2-VASc Score (HR 2.63, 95% CI 1.03-6.71) and plasma lipids/apolipoproteins (HR 4.45, 95% CI 1.51-13.12).

Conclusions: High Apo C-III plasma concentration may predict an increased risk of ischemic stroke/TIA in high-risk cardiovascular patients taking anticoagulant therapy with warfarin.

PB0103 | PI3KC2β Deficiency Stabilizes Adherens Junctions and Preserves Vascular Integrity in Stroke Models

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Background: Internalization and degradation of VE-cadherin, a major constituent of adherens junctions, lead to vascular permeability, a situation which can be dramatic in pathological situations such as ischemic stroke. This pathological context is characterized by a profound inflammatory response followed by a rupture of the blood-brain barrier with endothelial junction opening, leukocyte infiltration, edema formation and neuronal death. Therefore, the possibility to manipulate the levels of VE-cadherin at vascular adherens junctions is of potential therapeutic interest. Class II PI3KC2β is mainly thought to produce phosphatidylinositol 3-phosphate (PI3P), a phosphoinositide known to control intracellular trafficking. However, among the 3 isoforms of this class of PI3K, the function of the beta isoform (PI3KC2β) remains poorly investigated and it's role in trafficking is poorly known.
Aims: To investigate whether PI3KC2β and its lipid product could influence VE-cadherin trafficking and endothelial permeability under the pathological conditions of ischemic stroke.
Methods: For this study we used human cerebral microvascular endothelial cells (hCMEC/D3) knock-down for PI3KC2β and PI3KC2β$^{D1212A/D1212A}$ kinase-dead knock-in mice and WT littermates.
Results: We showed that the knock-down of PI3KC2β in hCMEC/D3 cells increased the stability of homotypic cell-cell junctions and enhanced VE-cadherin delivery to the plasma membrane. Interestingly, PI3KC2β inactivation prevented the production of a specific pool of PI3P, resulting in a decrease in early endosomal maturation towards lysosomal degradation and an increase in endosomal recycling. In vivo, we found that genetic inactivation of PI3KC2β conferred a remarkable protection against ischemic stroke in two mouse models. Indeed, inhibition of PI3KC2β led to a stabilization of the blood-brain barrier resulting in a reduction of infarct volume, edema and inflammation.
Conclusions: These data identify the PI3KC2β as an attractive therapeutic target in ischemic stroke and reperfusion. This is of relevance to the increasing global incidence of human diseases with compromised homeostasis of the blood-brain barrier.

PB0104 | Antiaggregation Evaluation of Aspirin and Clopidogrel in Endovascular Treatment of Intracranial Aneurysms with Flow Diverter Stents

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Background: Flow diverters represent a major paradigm shift in the endovascular treatment of intracranial aneurysms. Periprocedural adherence to strict pharmacological protocol is important to decrease thrombosis and/or bleeding complications. In recent years controversy has arise concerning the less-than-expected effect of antiplatelet treatments, mainly P2Y12 blockers but also aspirin and its clinical implications.
Aims: To analyse the efficiency of antiplatelet treatments in patients subjected to the imposition of a flow diverter stent.
Methods: Ninety-two consecutive patients were included. All patients were taken aspirin (100 mg) and clopidogrel (75 mg) for at least a week previously to platelet reactivity studies. Platelet reactivity was assayed by light transmission aggregometry (Chronolog) (LTA) with arachidonic acid (1 mM), ADP (20 mM) and TRAP-6 (20 mM) in PRP, whole blood impedance aggregometry (Multiplate) (arachidonic acid (0.5 mM), ADP (6.4 mM) and TRAP-6 (32 mM), VerifyNow (Aspirin and P2Y12) (Werfen) in whole blood and phosphorylation of VASP by flow cytometry (Biocytex). A patient was considered to be insufficiently inhibited when at least 2 probes were below limit of optimal inhibition as previously published (1)
(1) Pharmacological inhibition of platelet reactivity. Clinical and pharmacodynamic effects. Vallés J, et al. Curr Vasc Pharmacol. 2013 Jul;11:431-47.
Results: Aspirin-related inhibition of platelet reactivity was optimal in all tested patients. In contrast, in 38 patients (42%) inhibition with clopidogrel was less than expected. Change to a higher dose of clopidogrel (150 mg/day) was enough to obtain an optimal inhibition in 26 patients (68%). The remaining patients (n=12) required treatment with ticagrelor (90 mg/twice daily) to optimally blocked P2Y12-related platelet reactivity.
Conclusions: Inadequate antiplatelet inhibition with clopidogrel appears in an important percentage (42%) of these patients and could be corrected with a higher dose of clopidogrel or switching to ticagrelor. Tailored antiplatelet treatment could be an interesting approach in these high risk patients.

PB0106 | Thrombin Generation in Patients with Non-traumatic Intracerebral Hemorrhage

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Background: Non-traumatic intracerebral hemorrhage (ICH) accounts for 10-15% of all strokes and results in a higher rate of mortality as compared to ischemic strokes.

Aims: In the IRONHEART study, we aimed to find out whether the thrombin generation assay (TGA) could predict outcomes in patients with ICH.

Methods: In this prospective, observational study, 57 consecutive patients with ICH of a single stroke center and 120 healthy controls were enrolled. Exclusion criteria included aneurysm rupture, cancer, liver or kidney failure, hemorrhagic diathesis or anticoagulant therapy. Detailed clinical and laboratory investigations were performed on admission. Baseline traditional cerebrovascular risk factors were registered in both groups. TGA was performed on stored platelet poor plasma obtained on admission. Bleeding volume was estimated in patients based on CT performed on admission, day 1 and 2. Short- and long-term outcomes of ICH were defined at 14 days and 3 months post-event according to NIHSS and the modified Rankin Scale (mRS), respectively. All patients or relatives and controls provided written informed consent.

Results: Endogen thrombin potential (ETP) was significantly higher in patients as compared to controls (1719±358 vs. 1495±271 nM*min, respectively, p< 0.0001). Peak thrombin was significantly higher, while lag time and time-to-peak parameters were significantly longer in patients vs. controls. Elevated ETP was associated with worse long-term outcomes (mRS 0-1:1586±299 vs. mRS 2-5:1983±476 and mRS 6:1765±288 nM*min, p=0.019). Peak thrombin showed similar, significant association (mRS 0-1:321.1±55.9 vs. mRS 2-5:411.4±75.5 and mRS 6:308.1±148.8 nM, p=0.022). Time-to-peak showed a modest, significant negative correlation with admission intracerebral bleeding volume (r=−0.29, p=0.040).

Conclusions: Patients with ICH showed increased thrombin generation as compared to controls, which might be explained by the presence of more cerebrovascular risk factors in patients. Elevated ETP and peak thrombin was associated with unfavorable outcomes, indicating that TGA might be useful to predict outcomes in patients with ICH.

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PB0107 | Characteristics and Recurrence of Acute Ischemic Stroke in Children: An Examination Using Real World Data

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Background: Acute ischemic stroke (AIS) in children has not been well studied. Its management and clinical characteristics remain incompletely understood.

Aims: This study examines characteristics, antithrombotic treatment, and recurrence among pediatric subjects with AIS using healthcare claims databases.

Methods: Using three US healthcare claims databases (IBM MarketScan® Commercial (CCAE), Multi-state Medicaid (MDCD), and Optum® De-Identified Clinformatics® Data Mart Database - Socio-Economic Status (SES)) pediatric (< 18 years) subjects with hospitalized AIS were identified between 2008-01-01 and 2018-12-31. Demographics, comorbidities, and prescription drug exposures six months prior to hospitalization and treatment with anticoagulants (warfarin and direct anticoagulants (DOACs)) and antiplatelets (P2Y12 inhibitors (P2Y12i)) 30 days after hospitalization were examined. Crude incidence rates (IR, /1000 person-years) in 1 year after first AIS were examined and stratified by gender and age group (0-1; 2-5; 6-11; and 12-17 years).

Results: Among the pediatric AIS subjects identified from 3 databases (N=1240 (CCAE); N=1120 (MDCD); N=423 (SES)) the mean age was 10.5, 9.2, and 9.9 years respectively. The majority of pediatric AIS subjects were not treated with DOACs, warfarin, or P2Y12i medications in 30 days after AIS (range 96-98%). The rates of recurrent AIS (IR) in 1 year after first AIS were (148.0, 140.7, 76.6/1000 person-years). AIS recurrence (IR) were higher among females compared to males in CCAE (189.4 vs. 114.9/1000 person-years) and SES (86.6 vs. 68.7/1000 subject-years) but not in MDCD (139.8 vs. 141.6/1000 person-years). AIS recurrence (IR) in 1 year were higher among the 2-5 years age group (range 112.6-207.6/1000 person-years) compared to all other age groups.

TABLE 1 Unadjusted overall, sex, and age 1-year incidence of recurrent stroke among US pediatric subjects with AIS

Strata	CCAE						MDCD						SES					
	Patients	Events	%	PYs	IR	IP	Patients	Events	%	PYs	IR	IP	Patients	Events	%	PYs	IR	IP
Overall	1,240	153	12.3	1,033	148.0	123.4	1120	135	12.1	959	140.7	120.5	423	28	6.6	365	76.6	66.2
Female	564	87	15.4	459	189.4	154.3	537	65	12.1	465	139.8	121.0	192	14	7.3	161	86.6	72.9
Male	676	66	9.8	574	114.9	97.6	583	70	12.0	494	141.6	120.1	231	14	6.1	203	68.7	60.6
0-1 years	29	3	10.3	22	132.7	103.4	73	10	13.7	59	167.7	137.0	21	1	4.8	16	60.7	47.6
2-5 years	252	42	16.7	202	207.6	166.7	294	38	12.9	242	156.5	129.3	107	10	9.3	88	112.5	93.5
6-11 years	344	38	11.0	285	133.2	110.5	287	38	13.2	251	150.8	132.4	93	8	8.6	79	100.6	86.0
12-17 years	615	70	11.4	523	133.7	113.8	466	49	10.5	405	121.0	105.2	202	9	4.5	180	49.8	44.6

Key – PY: person-years; IR: incidence rate per 1000 person-years; IP: incidence proportion per 1000 persons; CCAE: IBM MarketScan® Commercial Claims and Encounters; MDCD: IBM MarketScan® Multi-state Medicaid; SES: Optum® De-Identified Clinformatics® Data Mart Database – Socio-Economic Status

Conclusions: Our study shows prescription antithrombotic therapies are rarely used in spite of relatively high AIS recurrence rates in pediatric AIS subjects. Future studies may assess whether recurrence rates differ by known risk factors.

PB0108 | The Use of in Vitro Clot Lysis Assay for Predicting Outcomes in Non-traumatic Intracerebral Hemorrhage Stroke Patients

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Background: Non-traumatic intracerebral hemorrhage (ICH) accounts for 10-15% of all strokes and results in a higher rate of mortality as compared to ischemic strokes.
Aims: In the IRONHEART study we aimed to find out whether the in vitro clot lysis assay or a modified method including the effect of neutrophil extracellular traps (NETs) might predict ICH outcomes.
Methods: In this prospective, observational study, 63 consecutive ICH patients were enrolled. Exclusion criteria included aneurysm rupture, cancer, liver or kidney failure, hemorrhagic diathesis or anticoagulant therapy. On admission, detailed clinical and laboratory investigations were performed. Bleeding volume was estimated based on CT performed on admission, day 1 and 2. In vitro clot lysis assay was carried out from stored platelet-free plasma of each patient taken on admission. A modified method was also performed including cell-free-DNA and histones in order to mimick the effect of NETs. Short- and long-term outcomes of ICH were defined at 14 days and 3 months post-event according to the NIHSS and the modified Rankin Scale (mRS), respectively. All patients or their relatives provided written informed consent.
Results: Modification of the clot lysis assay by adding cell-free-DNA and histones resulted in prolonged clot lysis times and significantly higher area-under-the-curve (AUC) values. Stroke severity was not associated with clot lysis time results of either assays. Using the modified clot lysis assay, median time to reach 50% clot lysis (50%CLT) showed a step-wise decrease in case of adverse long-term outcomes (mRS 0-1: median:62 [IQR:46-69] min; mRS 2-5: median:39 [IQR:28-46] min and mRS 6: median:36 [IQR:30-43] min, p=0.0018). Estimated intracerebral hemorrhage volume showed a negative correlation with the modified clot lysis assay lag time (r=−0.3489, p=0.0059) and 10%CLT (r=−0.3443, p=0.0066).
Conclusions: A clot-lysis assay might be useful to predict outcomes of ICH, particularly when the assay is supplemented with cell-free-DNA and histones.
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PB0109 | The Epinephrine-induced Platelet Aggregation Changes in Arterial Hypertension of Very High Risk

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Background: The crucial role in the development of atherotrombosis in arterial hypertension (AH) played thrombocyte activation. Different studies have shown controversial results in blood platelet functionality profiles due to the influence of diabetes mellitus of type 2 (DM 2) on the background of AH.
Aims: To set the types and relationship of the thrombocyte activation inducted by epinephrine in the clinical course of AH with DM 2.
Methods: We have learned 252 patients, aged 34 to 67 years: among them 124 people were with AH of high risk and 128 people were with AH with very high risk (+ DM type 2). The 3 indexes of epinephrine-induced platelet aggregation (the level, time and rate of aggregation) and the plasma levels of triglycerides (TG), total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), glucose and insulin were measured.
Results: We have set significant increase of the level and rate of aggregation ($p< 0.05$) in the group of AH with the high risk. There was significant difference between the time of aggregation in all groups ($p>0.05$). We have set a reliable positive correlation between the level of aggregation and TC (R=0.22; p=0.01) and rate of aggregation and TC (R=0.26; p=0.002). We have found the weak relation between the increase of LDL-C and the level of aggregation (R=0.21; p=0.028) and TG and the level of aggregation (R=0.22; p=0.05). But there weren't significant interrelations between the aggregation and the levels of glucose and insulin. The independent (in-group) correlation coefficients for glucose and insulin were also unreliable ($p>0.05$).
Conclusions: The AH with DM type 2 followed by the activation of thrombocytes, which didn't directly deal with glucose and insulin level, but depended on changes of lipidic profile.

PB0111 | The Optimal Treatment of Atrial Fibrillation in Patients with Cancer

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Background: Atrial fibrillation (AF) is associated with ischemic stroke and is frequent in cancer patients. Selection of antithrombotic treatment for stroke prevention in patients with cancer and AF is challenging. Vitamin K antagonists (VKA) have been the mainstay of such therapy; yet, their use in cancer patients may be problematic. In clinical practice, low-molecular-weight heparin and direct oral anticoagulants are employed in this setting. However, data regarding

anticoagulation outcomes and adverse events in such patients are lacking.

Aims: To evaluate the outcome of anticoagulation treatment, its thromboembolic and bleeding complications in cancer patients with AF.

Methods: In this retrospective study (IRB approval #300-18-RMB), Rambam electronic medical records were searched for the information regarding all patients with active malignancy who received anticoagulation for AF. The following data were analyzed: patient demographics, cancer type, anticoagulants and antiaggregants used, incidence of thromboembolic events and treatment-related bleeding.

Results: Between 2010-2019, 1538 cancer patients with AF were followed at Rambam. Patients with basal cell carcinoma were excluded (n=181) from the analysis. No data were available for 361 patients. Data on 996 patients were analyzed. Thirty-four percent (n=337) of patients did not receive anticoagulation, with 45% of them receiving antiaggregants. Out of 659 patients on anticoagulants, 341 patients received VKA, 110 - apixaban and 94 - enoxaparin. The highest percentage of bleeding events was recorded among patients treated with dabigatran and VKA (16% and 15%, respectively). Intracranial hemorrhage occurred in 2.6% of patients receiving VKA and in none of those receiving dabigatran (p=0.001). Stroke was more frequent in patients on enoxaparin than in patients on antiaggregants only (9.6% versus 0; p=0.001). The highest clinical benefit (stroke-% versus any bleeding-%) was observed in patients receiving rivaroxaban and apixaban.

Conclusions: For cancer patients with AF, treatment with Factor Xa inhibitors may be more beneficial than VKA, dabigatran or antiaggregants only.

PB0112 | Incidence of Recurrent Ischemic Stroke in Patients without Atrial Fibrillation: A Real-world Study

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Background: Anticoagulation is intended to treat and prevent thrombotic events in patients with atrial fibrillation (AF). For acute ischemic stroke (AIS) patients without AF, antiplatelets are recommended to prevent recurrence, although recurrence by certain demographic groups is not well described.

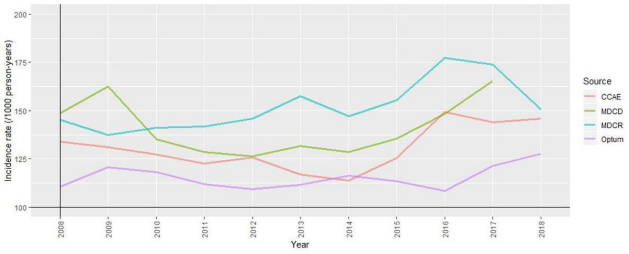
Aims: Assess incidence of ischemic stroke recurrence in patients without AF in the US by demographic groups and evaluate the trend across calendar years.

Methods: Hospitalized patients with first AIS (index date) without prior AF were identified from 4 US administrative claims databases, IBM MarketScan® Commercial Claims and Encounters (CCAE), Multi-state Medicaid (MDCD), Medicare Supplemental Beneficiaries (MDCR), and OptumInsight's Clinformatics® Datamart (Optum) from January-1-2008 to December-31-2018. Crude incidence rate (IR, /100 person-years) and incidence proportion (IP, /100 persons) of recurrent event during 1-year post-index was calculated overall and stratified by sex and age groups. Age-sex adjusted IR of recurrent event during 1-year was calculated by calendar years.

TABLE 1 Unadjusted 1-year incidence of recurrent AIS among patients with no prior AF, stratified by sex, age, and calendar year

Strata	CCAE					MDCD					MDCR					Optum				
SAS	Patients	Events	PYs	IR	IP	Patients	Events	PYs	IR	IP	Patients	Events	PYs	IR	IP	Patients	Events	PYs	IR	IP
Overall	122,317	12,464	97,406	12.8	10.2	77,979	9,147	62,698	14.6	11.7	127,046	15,011	100,534	14.9	11.8	172,415	16,442	141,687	11.6	9.5
Female	59,081	5,977	47,201	12.7	10.1	47,519	5,379	38,552	14.0	11.3	69,024	8,093	54,646	14.8	11.7	91,077	8,628	75,234	11.5	9.5
Male	63,236	6,487	50,204	12.9	10.3	30,460	3,768	24,145	15.6	12.4	58,022	6,918	45,888	15.1	11.9	81,338	7,814	66,453	11.8	9.6
18-44y	17,682	1,485	14,554	10.2	8.4	8,646	1,038	7,014	14.8	12.0	6	0	6	0.0	0.0	7,370	455	6,139	7.4	6.2
45-64y	101,104	10,703	81,611	13.1	10.6	37,850	5,096	30,667	16.6	13.5	1,988	275	1,712	16.1	13.8	43,563	3,964	35,568	11.1	9.1
>=65y	3,531	276	1,240	22.3	7.8	31,483	3,013	25,016	12.0	9.6	125,052	14,736	98,815	14.9	11.8	121,482	12,025	99,979	12.0	9.9
2008	9,879	1,079	8,167	13.2	10.9	5,539	679	4,559	14.9	12.3	14,769	1,717	11,819	14.5	11.6	12,341	1,137	10,273	11.1	9.2
2009	11,124	1,146	8,827	13.0	10.3	5,128	678	4,182	16.2	13.2	13,245	1,463	10,644	13.7	11.0	12,651	1,250	10,371	12.1	9.9
2010	11,259	1,176	9,291	12.7	10.4	5,397	607	4,486	13.5	11.2	12,913	1,538	10,899	14.1	11.9	13,542	1,312	11,099	11.8	9.7
2011	13,121	1,326	10,911	12.2	10.1	4,849	541	4,206	12.9	11.2	16,427	1,860	13,124	14.2	11.3	13,279	1,254	11,187	11.2	9.4
2012	14,755	1,365	11,012	12.4	9.3	4,747	526	4,162	12.6	11.1	15,014	1,782	12,225	14.6	11.9	14,294	1,329	12,170	10.9	9.3
2013	11,001	1,026	8,922	11.5	9.3	7,009	805	6,080	13.2	11.5	13,229	1,623	10,317	15.7	12.3	15,725	1,433	12,833	11.2	9.1
2014	11,724	1,027	9,112	11.3	8.8	6,953	749	5,820	12.9	10.8	12,625	1,445	9,818	14.7	11.4	14,492	1,397	12,020	11.6	9.6
2015	10,340	1,065	8,568	12.4	10.3	9,405	1,116	8,240	13.5	11.9	9,843	1,268	8,171	15.5	12.9	14,735	1,422	12,521	11.4	9.7
2016	10,467	1,208	8,262	14.6	11.5	11,030	1,436	9,656	14.9	13.0	9,317	1,214	6,852	17.7	13.0	16,148	1,491	13,738	10.9	9.2
2017	9,437	1,075	7,576	14.2	11.4	11,332	1,352	8,192	16.5	11.9	6,272	735	4,227	17.4	11.7	21,002	2,150	17,703	12.1	10.2
2018	9,210	971	6,751	14.4	10.5	6,590	658	3,110	21.2	10.0	3,392	366	2,432	15.0	10.8	24,206	2,267	17,766	12.8	9.4

Key – PY: person-years; IR: incidence rate per 100 person-years; IP: incidence proportion per 100 persons; CCAE: IBM MarketScan® Commercial Claims and Encounters; MDCD: IBM MarketScan® Multi-state Medicaid; MDCR: IBM MarketScan® Medicare Supplemental Beneficiaries; Optum: OptumInsight's Clinformatics® Datamart.
*Incomplete data capture for MDCR in 2018.
1 – Age strata IRs differed significantly *within* databases.
2 – Sex strata IRs did not differ significantly *within* database except for MDCD
3 – Age and sex strata differed significantly *across* databases except for: Overall MDCD vs MDCR, 45-64y MDCD vs MDCR, ≥65y MDCD vs Optum, Male MDCD vs MDCR



Key – CCAE: IBM MarketScan® Commercial Claims and Encounters; MDCD: IBM MarketScan® Multi-state Medicaid; MDCR: IBM MarketScan® Medicare Supplemental Beneficiaries; Optum: OptumInsight's Clinformatics® Datamart. *Incomplete data capture for MDCR is 2018.

FIGURE 1 Sex and age standardized 1-year incidence of recurrent AIS across calendar years 2008-2018 among patients with no prior AFFIGURE 1 Sex and age standardized 1-year incidence of recurrent AIS across calendar years 2008-2018 among patients with no prior AF

Results: CCAE, MDCD, MDCR, and Optum databases included 122,317, 77,979, 127,046, 172,415 AIS patients without AF with mean age of 54.0, 61.6, 78.9, 70.0 years, respectively. AIS recurrence IR ranged from 11.7 to 14.9 /100 person-years and IP ranged from 10.2-11.8 /100 persons. In three databases, recurrence IR did not differ by sex while increased with age (Table 1). In MDCD, recurrence IR was higher for men vs. women (difference: 1.7/100 person-years) and for patients aged 45-64y vs ≥65y (4.6) and vs 18-44y (1.8) (all P< 0.05). Age-sex adjusted IR of recurrence were generally stable over the study period (Figure 1).

Conclusions: AIS recurrence rate in patients without AF in this real-world study is higher than some previous reports. Recurrence rate increased with age, did not differ by sex in three databases. Age-sex adjusted incidence did not appreciably change during 2008-2018. Future studies may assess risk factors and treatment pattern in association with recurrence.

PERIPHERAL ARTERY DISEASE

PB0113 | Acute Leriche Syndrome in a 55-Year-Old Woman with Recurrent Central Pulmonary Embolism: A Case Report

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Background: Arterial thromboembolism of the infrarenal abdominal aorta results from aortoiliac occlusive disease or Leriche syndrome. Pulmonary embolism is usually caused by an embolic thrombus originating from a deep vein thrombosis. The underlying causes of both vascular thromboembolic diseases are multifactorial and a link between venous and arterial thrombotic disorders is still unclear.

Aims: We present a case of a 55-year-old woman suffering from Leriche syndrome after consecutive multiple pulmonary embolisms of idiopathic origin.

Methods: A 55-year-old woman presented herself in the emergency department with acute onset of dyspnea and pain in the left leg for 4 days. A formerly intake of oral anticoagulation therapy due to idiopathic pulmonary embolism was stopped already over a year before. Computed tomography revealed central pulmonary embolism with right ventricular overload. Initial treatment with heparin was initiated, the patient stabilized noticeably. Another 5 days later, the patient developed sudden onset paresthesia, paraplegia and pulselessness of both lower limbs revealing Leriche syndrome. During consecutive arterial embolectomy, the patient again became hemodynamically instable suggesting a further progression of the pulmonary embolism.

Results: Atrial fibrillation as well as a patent foramen ovale was excluded as cause for arterial embolism. Interim suspected heparin-induced thrombocytopenia was also not confirmed. Despite adipositas, no other risk factors for venous thromboembolism were known and standardized screening for thrombophilia, in particular antiphospholipid syndrome, was negative. Evaluation of JAK2 V617F, JAK 2 exon-12 and calreticulin exon-9 mutation was unremarkable. At discharge, anticoagulation therapy with phenprocoumon was initiated. No further thromboembolic events occurred within 1 year follow up.

Conclusions: Leriche syndrome and pulmonary embolism are two thromboembolic and potential life-threatening diseases with multifactorial origins. In this case no underlying cause for the thromboembolic events could be found and a link between arterial and venous thrombotic disorders remains uncertain.

PB0114 | Elevated Levels of Activated Factor XII Are Associated with an Increased Risk of Atherothrombotic Events in Peripheral Artery Disease

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Background: Peripheral artery disease (PAD) is a manifestation of systemic atherosclerosis resulting in restrictive blood flow in peripheral arteries. Patients with PAD are at increased risk of atherothrombotic events despite current optimal medical treatment. Risk stratification is necessary in clinical decision making, but identifying high-risk PAD patients remains challenging. The COMPASS-study showed that combined anticoagulant and antiplatelet therapy reduced cardiovascular (CV) mortality, illustrating the important role of coagulation in PAD.

Aims: To compare differences in coagulation status in PAD patients with or without a major CV event during follow-up.

Methods: Between May 2018 and January 2019, 120 patients objectively diagnosed with PAD using the ankle-brachial index (< 0.90) were enrolled in this prospective cohort study. The study was

TABLE 1 Levels of coagulation factor complexes for PAD patients with events during follow-up and PAD patients without events during follow-up

Coagulation complex	Event	No event	p-value
FIXa:AT (pM)	120 (102-139)	103 (93-118)	0.066
FXa:AT (pM)	127 (120-148)	124 (116-139)	0.456
FXIa:α1AT (pM)	188 (155-256)	187 (144-230)	0.774
FXIa:AT (pM)	22.1 (19.2-28.3)	21.5 (18.9-24.1)	0.381
FXIIa:C1inh (U)	$181x10^3$ ±$46x10^3$	$151x10^3$ ±$47x10^3$	***0.039****
TAT (pM)	2.43 (1.65-5.95)	2.33 (1.88-3.27)	0.848

TABLE 2 Thrombin generation with and without tissue factor for PAD patients with events during follow-up and PAD patients without events during follow-up

TG	Parameter	Event	No event	p-value
0 pM TF	Lag time (min)	15.2 (12.4-21)	14.3 (12-19.1)	0.600
	ETP (nM/min)	1238±266	1236±190	0.761
	Peak (nM)	234±72	222±63	0.538
1 pM TF	Lag time (min)	6.8 (6-9.4)	6.3 (5.7-7.3)	0.119
	ETP (nM/min)	1262 (1046-1415)	1217 (1095-1344)	0.873
	Peak (nM)	190±58	172±51	0.271

approved by the recognized medical ethical committee of MUMC+ and all participants gave written informed consent. Blood samples and patient data were collected at baseline. During a one-year follow-up period, CV events (myocardial infarction, cerebrovascular accident, acute limb ischemia) and (CV) mortality were recorded. Citrated platelet-poor plasma was used to perform thrombin generation (with and without tissue factor) and to measure coagulation factor complexes Factor IXa:Antithrombin (FIXa:AT), FXa:AT, FXIa:a1-antitrypsin, FXIa:AT, FXIIa:C1inhibitor and thrombin:AT (TAT).

Results: The investigated population was 68±10 years old, predominantly male (60%) and was adequately treated by antiplatelet drugs (100%), antihypertensive drugs (75%) and lipid-lowering drugs (97%). In twelve patients (10%) a CV event was recorded during follow-up. FXIIa:C1inhibitor complexes were significantly elevated in patients with a CV event versus patients without event ($181x10^3 \pm 46x10^3$ vs $151x10^3 \pm 47x10^3$, p 0.039). Other coagulation factor complexes (Table 1) and thrombin generation (Table 2) were not different between the two groups.

Conclusions: Our findings show increased contact activation in PAD patients with increased risk of CV events despite contemporary medical treatment. Further studies are needed to confirm FXIIa as a potential biomarker and druggable target.

PB0117 | The Prevalence of Peripheral Artery Disease in Hospitalized Patients with Heart Failure

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Background: Peripheral artery disease (PAD) and heart failure (HF) share the same risk factors and pathophysiological process. Patients with PAD have 2-fold higher risk of develop HF and the prevalence of PAD in these patients varies from 12-19%. However, most of these data are based on the prevalence of symptoms suggestive for PAD with scarce evidence from diagnostic specific tests.

Aims: To describe the prevalence of PAD diagnosed with ankle-brachial index (ABI) in hospitalized patients with acute HF and to evaluate the prevalence of symptomatic PAD assessed with the "San Diego Claudication Questionnaire" (SDCQ).

Methods: We conducted a multicentre prospective study in 5 Italian Internal Medicine Units. Hospitalized patients with acute HF were evaluated with the ABI test and the SDCQ. According to the International Guidelines, PAD was confirmed when ABI was ≤ 0.9. Patients with ABI >1.4 were excluded from the analysis. We also collected information about cardiovascular risk factors, comorbidities, concomitant medications and the last transthoracic echocardiogram. For every patient enrolled informed consent was obtained and the study was approved by the local medical ethic committees.

Results: From January 2018 and July 2019 we consecutively enrolled 206 patients admitted for acute HF. In our analysis 90 patients (43.7%) had ABI index ≤ 0.9 (95% CI 37-51%); in this group, 12 patients only were aware of the disease. The results of the SDCQ showed that 53.3% of the population with ABI ≤ 0.9 had no symptoms suggestive for PAD. Compared with patients with normal or borderline ABI index, patients with ABI ≤ 0.9 more frequently suffer from chronic renal impairment (51.1% and 32.8%, p 0.007) and presented with cardiovascular or cerebrovascular events in the past (55.6% and 37.1%, p 0.008).

Conclusions: PAD is a frequent comorbidity in patients with HF, however this condition is widely under-diagnosed.

PB0118 | Poorly Compressible Lower Extremity Arteries Are Associated with Venous Thromboembolism

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Background: Peripheral artery disease (PAD) impacts millions of people around the globe and is commonly diagnosed using lower extremity physiologic studies. PAD is associated with endothelial

dysfunction and inflammation and shares several common risk factors with venous thrombosis. Patients with PAD are at increased risk for vascular complications however, there is little evidence for its association with thrombosis.

Aims: To understand the relationship between arterial occlusive disease and venous thrombosis.

Methods: We reviewed vascular studies between 1996 and August 2018. Based on the ankle brachial index (ABI) recordings, patients were classified into 3 categories: patients with PAD (ABI< 1.0), non-compressible (NC) arteries (ABI>1.4), and patients with normal ABI (1.0-1.4). The main outcome analyzed was new venous thromboembolism. Multivariate Cox proportional regression was used to calculate hazard ratios (HR) with 95% confidence intervals after adjusting for age, gender, and prior history of cancer, or incidence of VTE.

Results: There were 38,016 unique patients (mean age 66.1 ± 14.8 years, female 42.3%) who underwent ABI measurement. Compared to normal ABI, patients with abnormal ABI were more likely to be older and male. Compared to those without PAD, low ABI patients did not have a significantly increased risk of VTE (adjusted HR: 1.06, 95% CI: 1.00-1.11, p=0.04). However, patients with NC vessels had a statistically significant risk of developing VTE compared to patients with normal ABI (adjusted HR: 1.21, 95% CI: 1.12-1.30, p-value< 0.001). Interestingly, NC vessel patients compared to PAD patients had a statistically significant risk of a VTE event (adjusted HR: 1.24, 95% CI: 1.15-1.33, p-value< 0.001).

Conclusions: Compared to patients without PAD those with poorly compressible calcified lower extremity arteries are more prone to develop venous thromboembolic disease. Aggressive treatment of this cohort with anticoagulants might be beneficial.

PB0119 | Development of a Clinical Cohort of HIV-infected Patients with Peripheral Vascular Disease

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Background: Human immunodeficiency viral (HIV) infection is prevalent in South Africa with over 7 million patients estimated to be living with HIV. HIV increases the risk of arterial and venous thrombosis. This has been attributed to various factors including chronic inflammation (associated with microbial translocation, HIV viral replication and the presence of opportunistic infections). Peripheral vascular disease with critical limb ischaemia, common in black South Africans, is associated with traditional risk factors (smoking, hypertension, diabetes and dyslipidaemia). The impact of HIV-infection on peripheral vascular disease development has not been fully assessed. As part of a larger study looking at HIV-associated endothelial dysfunction, a cohort of patients was established at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) to assess the impact of HIV-infection on the clinical presentation of this disease.

Aims: To assess clinical features in a cohort of black African patients with peripheral vascular disease.

Methods: This study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (M130372). All black African patients scheduled for arterial bypass grafting presenting to the vascular ward at CMJAH were invited to participate. Age, gender, presence of traditional cardiovascular disease risk factors and CD4+ T cell count and HIV Viral load in infected patients were collected. The clinical profile of patients with and without comorbid HIV infection was compared.

Results: HIV-infected patients presented at a significantly younger age than HIV-uninfected patients (median 46 yrs and 64.5 yrs respectively, p< 0.001), were predominantly male (90% vs 50%) and were less likely to have underlying diabetes (20% vs 42%) or hypertension (30% vs 58.3%, Table 1). Importantly, HIV-infected patients presented with more extensive vascular disease. All HIV-infected patients were treated with antiretroviral therapy.

Conclusions: Although this represents a small cohort of patients, HIV infection appears to influence the clinical presentation of peripheral vascular disease.

TABLE 1 Characteristics of patients presenting with peripheral vascular disease at Charlotte Maxeke Johannesburg Academic Hospital

	HIV-infected patients (n=10)	HIV-uninfected patients (n=12)
Age (Median, years)	46 (range: 37-67)	64.5 (range: 43-78)
Male gender (%)	90	50
HIV viral load (median, copies/ml)	Lower than detectable limit (range: LDL - 283000)	N/A
Smoking history (%)	60	66
Diabetes mellitus (%)	20	42
Hypertension (%)	30	58.3
Dyslipidaemia (%)	10	10
Femoral/iliofemoral disease (assessed angiographically, %)	50	72
Aorto-iliac disease (assessed angiographically, %)	50	28

PB0120 | Rationale and Design for the Study Apixaban versus ClopidoGRel on a Background of Aspirin in Patients Undergoing InfraPoPliteal Angioplasty for Critical Limb Ischemia - AGRIPPA Trial

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Background: Proper new antithrombotic strategies that reduce primary thrombosis and restenosis might improve vascular outcomes in patients with peripheral artery disease (PAD) undergoing arterial angioplasty.
Aims: The study objective is to evaluate the potential benefit of apixaban on top of aspirin in reducing thrombotic restenosis and artery re-occlusion in patients undergoing endovascular Infrapopliteal revascularization as compared to standard of care dual antiplatelet therapy.
Methods: It is a multicenter, parallel-group, prospective, randomized, open-label, blinded-endpoint adjudication, proof-of-concept, exploratory trial. Two hundred patients will be randomized in a 1:1 ratio, 72 hours after successful infrapopliteal angioplasty for critical limb ischemia. In the study group, patients will receive oral apixaban 2.5mg twice daily plus aspirin 100 mg once daily for 12 months. In the control group, aspirin 100 mg once daily will be taken for 1 year on a background of clopidogrel (75mg daily) for the first three months. The primary endpoint is the composite of target lesion revascularization (TLR), major amputation or restenosis/occlusion (RAS) plus MACE (myocardial infarction, stroke, and cardiovascular death) at 12 months. The primary safety endpoint is the composite of major bleeding and clinically relevant non-major bleeding at 12 months.
Results: It was enrolled 5 patients until now.
Conclusions: This study will evaluate the efficacy and safety of apixaban 2.5 mg twice daily plus aspirin compared to the standard treatment (clopidogrel plus aspirin) in patients with critical limb ischemia undergoing infrapopliteal arterial endovascular intervention and might prove the concept of an alternative antithrombotic regimen for these patients to be tested in a future large randomized clinical trial.

COAGULATION AND NATURAL ANTICOAGULANTS

ANIMAL MODELS IN THROMBOSIS AND HEMOSTASIS

PB0121 | Small-Molecule Factor XIa Inhibitor, BMS-986177/JNJ-70033093, Prevents and Treats Arterial Thrombosis in Rabbits at Doses that Preserve Hemostasis

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Background: Factor XIa (FXIa) is a promising antithrombotic target. BMS-986177/JNJ-70033093 (BMS-177) is an orally-bioavailable, reversible and direct inhibitor of human and rabbit FXIa with Ki of 0.11, and 0.38 nM, respectively.
Aims: The antithrombotic and bleeding time (BT) effects of BMS-177 were characterized in rabbits.
Methods: Efficacy of BMS-177 was evaluated in the prevention- and treatment-mode in the rabbit model of electrically-mediated-carotid-arterial-thrombosis. In the prevention-mode, BMS-177 or vehicle was administered as IV bolus plus infusion from 30 min before thrombosis initiation until the end of the experiment. In the treatment-mode, growth of thrombus was initiated first for 15 min before administration of BMS-177 or vehicle. Carotid blood flow (CBF) and thrombus weight reduction (TWR) were used as markers of efficacy. In addition, effects of BMS-177 alone and in combination of aspirin on hemostasis were assessed in a provoked cuticle BT model.
Results: In the prevention-mode, BMS-177 given at 0.063+0.04, 0.25+0.17 and 1+0.67 mg/kg+mg/kg/h produced a preservation of CBF by 32±6*, 54±10* and 76±6* and TWR by 15±10*, 45±2* and 70±4%*, respectively (*P< 0.05, n=6 per dose). In the treatment-mode, after initiation of thrombosis for 15 min, CBF was gradually decreased to 40% of control levels. BMS-177 was then administered to arrest growth of preexisting thrombus. CBF at 90 min averaged 1±0.3, 39±10 and 66±2%* in the groups treated with vehicle and with BMS-177 (mg/kg±mg/kg/h) at 0.25+0.17 and 1+0.67, respectively (*P < 0.05 vs. vehicle, n=6 per group). BMS-177 increased ex vivo activated partial thromboplastin time without changing prothrombin time. The combination of BMS-177 at 1+0.67 mg/kg+mg/kg/h and aspirin at 4 mg/kg/h IV resulted in no additional increases in BT (vs. monotherapy options).
Conclusions: BMS-177 is an effective antithrombotic agent with limited impact on hemostasis even when combined with aspirin in rabbits. BMS-177 is currently in Phase 2 clinical trials.

PB0122 | Developing of a New Animal Artery Model of Atherothrombotic Stenosis using Perivascular Monopolar High-Frequency Electrical Current-Mediated Endothelial Injury Followed by a Cholesterol-Rich Diet Injury and Finally Perivascular Severe Cold Injury using Liquid Nitrogen

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Background: A plaque may rupture with high risk of subsequent thrombus- mediated acute clinical events such as myocardial infarction and stroke.
Aims: The aim of this study was to generate a reproducible experimental rabbit femoral artery model of atherothrombotic stenosis with morphological similarities to the human disease.

Methods: Briefly, New Zealand white rabbits were submitted to atherothrombotic stenosis by primary perivascular monopolar high-frequency electrical current (I_{avg}= 150 mA, F= 250 KHz)- mediated endothelial injury followed by a 1.5% cholesterol- rich diet injury and finally perivascular severe cold injury using liquid nitrogen (–196°C) at the femoral artery.

Results: Histopathology results showed the formation of advanced atherosclerosis with lipid-laden cells, atheroma, neovessel- rich plaque and severe stenosis (> 70%) in all of the rabbits′ arteries after 12 weeks. Four weeks after perivascular severe cold injury, results from histopathology and ultrasonography showed the formation of atherothrombotic stenosis in all of the rabbits′ arteries. Results from color Doppler ultrasonography at the stenotic region showed a significant increase in the mean value for blood mean velocity, wall mean thickness, percentage of luminal cross- sectional area of stenosis and formation of intraplaque hemorrhage and significant reduction in the mean value for blood volume flow in the atherothrombotic group compared with the control group
($p < 0.05$).

Conclusions: In conclusion, we successfully produced thrombosis on ruptured atherosclerotic plaque in the rabbits′ arteries, that is similar to the condition seen in patients. This condition in rabbits can be properly assessed by color Doppler ultrasonography and histopathology.

PB0123 | Liquorice and Sumac Attenuate Brain Damage Caused by Ischemic Stroke in Rat

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Background: Much evidence suggests that brain ischemia inducts an unrestrained inflammation that starts within a few minutes and remains for a few days after an ischemic stroke. Hence, the antioxidant and anti-inflammatory agents may be practical providing protection from neurological damage caused by stroke. Currently, there is a lack of approved therapies that are able to reduce infarct size or neurological disability following a stroke. One of the approaches that may limit the damage after a stroke is prophylactic treatment. It is worth mentioning that thromboembolism is the second leading cause of death in patients with cancer.

Aims: This study was designed to investigate whether liquorice or sumac are effective in a thromboembolic model of stroke in rats.

Methods: Stroke was induced in rats by middle cerebral artery occlusion using an autologous pre-formed blood clot. Liquorice solution (150 mg/kg) or sumac extract solution (250 mg/kg) was injected intraperitoneally to each rat of the seven-member groups for 7 subsequent days prior to stroke induction. Behavioral scores, infarct size, and brain edema, as well as the level of tumor necrosis factor-alpha, malondialdehyde, glutathione, and catalase activity, and also superoxide dismutase activity were determined in the ipsilateral cortex 24 hours following the stroke induction. The control group received normal saline and a similar protocol was carried out.

Results: Liquorice (150 mg/kg) and sumac extract (250 mg/kg) for 7 days, statistically similar to each other, but different to normal saline, curtailed brain infarction and edema, improved behavioral scores and prevented enhanced oxidative stress and tumor necrosis factor-alpha level in the ischemic brain tissue.

Conclusions: Since liquorice and sumac have been documented as two powerful antioxidant, anti-inflammatory, and antithrombotic agents, the observed effects may be justifiable. Future studies of possible mechanisms could provide novel therapeutic strategies in clinical stroke, especially in patient with cancer receiving chemotherapy medications.

PB0124 | Anti-Aggregant Effect of Butanolic Extract of Rubia Tinctorum on Platelets in vitro and ex vivo

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Background: : It is well recognized that blood platelets play crucial role in the pathologies linked to cardiovascular diseases (CVD). In this regard, the search for strong and safer antiplatelet agents is of high interest.

Aims: The aim of this study is to examine the anti-aggregant activity induced by collagen of butanolic extract of *Rubia tinctorum* L (BERT) *in vitro* and *ex vivo*.

Methods: by measuring the bleeding time and platelets count. Phytochemical analysis was assessed by measuring DPPH free radical-scavenging method. Identification and quantification of flavonoids compounds were determinate by *HPLC* analysis.

Results: Our finding demonstrated that B-RT extract showed a significant *($P< 0.001$)* inhibition of collagen induced platelets aggregation (1.48 ± 0.76, *n=3)*. These results correlate well with data of bleeding time and platelet count. Phytochemical analysis revealed that B-RT extract contain a high amount of total flavonoids 0.7± 0.055mg CAE/100g DM,*HPLC* revealed that the main polyphenols identified and quantified were vanillin (22.61%) in highest concentration followed by Rosmarinic acid (13.21%) and Cinnamic acid (13.04%) respectively, finally Quercetin (12.39%).

Conclusions: Rubia tinctorum butanolic extract exhibited a high antioxidant activity. The present investigation has shown that BERT

extract has an anti-aggregant effect in which flavonoids could be involved, in particular vanillin.

PB0127 | Hemolysis Triggers Platelet-Dependent Pulmonary Arteriole Thrombosis

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Background: Intravascular hemolysis promotes platelet activation, endothelial dysfunction and sterile inflammation, leading to a chronic prothrombotic state in hemolytic disorders. Although, pulmonary thrombosis is one of the most common pulmonary complication associated with hemolytic disorders, the *in vivo* pathogenesis and the role of platelets in promoting this hemolysis-driven morbidity remains unclear.
Aims: To elucidate the cellular, molecular and biophysical pathways contributing to the pathogenesis of hemolysis-induced pulmonary thrombosis in mice.
Methods: C57BL/6J (WT) mice were intravascularly (IV) administered distilled water (dH_2O) to trigger acute hemolysis or IV administered with pathologically relevant agonists known to be upregulated in hemolytic disorders. Mice were IV administered with fluorescent Abs against lineage markers for platelets and a fluorescent dextran. The pulmonary microcirculation was visualized using multi-photon-excitation enabled quantitative intravital fluorescence lung microscopy (qFILM).
Results: Acute hemolysis triggered transient obstruction of pulmonary arteriolar bottle-necks located at the junction of pulmonary arterioles and capillaries by platelet aggregates, resulting in loss of blood flow in the down-stream pulmonary capillaries. Identical to acute hemolysis, IV adenosine diphosphate (ADP), collagen, thrombin and thromboplastin (TF) also triggered dose-dependent pulmonary arteriole thrombosis in mice. Although heparin prevented TF-induced pulmonary arteriole thrombosis, it failed to prevent ADP-triggered pulmonary thrombosis in mice. Unlike heparin, eptifibatide completely abrogated both TF- and ADP-dependent pulmonary thrombosis.
Conclusions: We provide the first ever real-time *in vivo* characterization of the pathogenesis of acute hemolysis induced pulmonary thrombosis. Our data suggest that hemolysis-triggered pulmonary thrombosis involves entrapment of platelet-rich thrombi in the pre-capillary pulmonary arterioles. Our results also suggest that platelet procoagulant activity contributes to pathogenesis of hemolysis-associated pulmonary arteriole thrombosis.

PB0128 | Increased Levels of von Willebrand Factor Associated with Acute Inflammation Contribute to Enhanced Venous Thrombosis

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Background: Sepsis has been correlated with increased risk for deep vein thrombosis (DVT), concurrently with increased levels of plasma von Willebrand factor (VWF:Ag), a crucial component of DVT.
Aims: To determine the role of VWF in the murine stenosis model of DVT concurrent with lipopolysaccharide (LPS)-induced sepsis.
Methods: DVTs were induced in wildtype mice by stenosis of the inferior vena cava, followed by intraperitoneal administrations of 0.5mg/kg LPS or vehicle control. Mice were sacrificed at either 1.5h or 24h post-stenosis. Thrombosis incidence was noted after 24h. VWF:Ag, cell-free DNA, and VWF activity were quantified by ELISA, PicoGreen, and collagen-binding assays, respectively. To further elucidate the role of VWF in the thrombogenic process, inhibitory anti-VWF or isotype control antibodies were intravenously administered prior to stenosis. We also employed the Hyperion Mass Imaging System to simultaneously quantify and spatially localize nine thrombus constituents at 1μm resolution.
Results: LPS-treated mice had a significantly higher thrombosis incidence compared to controls (P=0.0453), but without a difference in thrombus weight. Compared to pre-stenosis levels, only the LPS-treated mice showed significantly increased VWF:Ag after 1.5h (P=0.0029). At 24h, both the control and LPS cohorts had increased VWF:Ag (P< 0.0001) and collagen-binding (P< 0.0001). When the

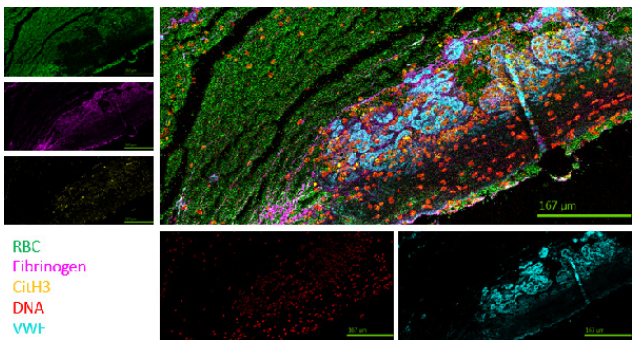

FIGURE 1 Multiplexed labelling of five murine venous thrombus constituents using Hyperion Mass Imaging System. Single channel images are also shown

consumptive effects of thrombosis were considered, the increased VWF:Ag was only significant in the non-thrombosed LPS cohort compared to the non-thrombosed controls (P=0.0108). No increases in ultra-large VWF multimers were observed during the early time points. Furthermore, the LPS-treated mice had significantly higher plasma levels of cell-free DNA at 24h (P=0.0009). Further analysis using inhibitory antibodies showed that VWF inhibition significantly decreased thrombosis incidence in the LPS cohort (P=0.0198). Finally, Hyperion-derived images demonstrate discrete spatial interactions between thrombus constituents (Figure 1).

Conclusions: The increased levels of VWF:Ag (but not activity nor large multimers) associated with LPS-induced acute inflammation significantly contributes to an enhanced tendency for venous thrombosis.

PB0130 | Non-Invasive Optical in vivo Imaging Reveals Tumor-Derived Prothrombin with a Functional Role in Hemostasis and Tumor Progression

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Background: The anatomical and temporal bioavailability of hemostatic components may control distinct (cellular) processes. However, tools to non-invasively visualize the expression dynamics and spatiotemporal distribution of such proteins at a systems level are scarce.

Aims: Here we set out (1) to generate a reporter-mouse model to interrogate the spatiotemporal dynamics of prothrombin (F2) expression, and (2) to explore the suitability of this model to identify cellular programs executed by extrahepatic expression of this central hemostatic component.

Methods: Utilizing a multimodal tagging strategy, a novel constitutive knock-in reporter-mouse model was created. This model is based on fluorescence and luminescence reporters separated by P2A peptides for tailored multicistronic expression. It enables simultaneous monitoring of the expression and secretion dynamics of prothrombin in its physiological context in a living animal with non-invasive optical imaging. Septicemia and tumor induction were used to validate the model. Complementary syngenic (primary) cell lines derived from this mouse model were used to deconvolute regulatory mechanisms in high resolution. The functional dimension of extrahepatic prothrombin was probed by targeted depletion in vivo and in vitro, coagulation assays and RNAseq.

Results: In a proof-of-concept, we confirm that this newly generated reporter-mouse model faithfully recapitulates established modifiers of F2 expression. We further discover extrahepatic F2 expression in emerging fibrosarcomas, resulting in significant amounts of functional tumor-derived prothrombin in the bloodstream. By selective ablation, we identify that tumor-derived prothrombin functionally contributes to a procoagulant and protumorigenic state. RNA expression analysis identifies central cellular programs perturbed by tumor-intrinsic prothrombin.

Conclusions: Using a newly generated reporter system, we uncover previously unknown sources of harmful F2 expression. Beyond discovering new functions of the hemostatic system, this model is widely applicable to identify rheostats involved in the cross-talk between gene expression and bioavailability of a secretory protein. It may also represent a resource to uncover novel (tissue-specific) therapeutic vulnerabilities.

PB0131 | Heart Failure, Induced by Excess Neurohormonal Activation, Results in Significant but Non-Critical Telomere Shortening in Cardiomyocytes and Peripheral Blood Mononuclear Cells

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Background: Extreme telomere shortening has been shown to impair cardiac function. Also first evidence suggests that heart failure (HF) might in turn result in telomere attrition. However, the responsible pathomechanism in the affected tissues remains completely obscure.

Aims: We investigated what degree of telomere shortening is elicited by myocardial injury via excess neurohormonal activation (NHA), the key factor driving HF, and what sequelae result. NHA is known to induce elevated levels of reactive oxygen species (ROS), which due to the susceptible telomeric ultrastructure seems a plausible candidate causing the shortening process.

Methods: By subjecting C57BL/6J (B6) mice to infusion of angiotensin II (AngII), unilateral nephrectomy and high-salt diet we induced excess NHA. Cardiac function was determined by ultrasound. H9c2-cardiomyocytes (CMs) were incubated with AngII and H_2O_2 to assess the effect of NHA and ROS directly on telomere length (TL) in vitro. Oxidative stress levels were detected by the MitoSOX assay. TL was measured by quantitative fluorescence in situ hybridization (Q-FISH) in H9c2, isolated mouse CMs and peripheral blood mononuclear cells (PBMCs).

Results: NHA resulted in significant hypertrophy and impaired systolic function after 5 weeks paralleled by significant telomere shortening in CMs and PBMCs compared to controls (length CMs: B6 1.0, NHA 0.82; PBMCs: B6 1.0, NHA 0.8). Stimulation of H9c2 cells with AngII for 4h resulted in 20% shortened telomeres and MitoSOX measurements revealed an increase of oxidative stress of 11% compared to untreated cells.

Conclusions: Excess NHA-induced heart failure is associated with increased ROS production and significant telomere shortening. Further, the degree of telomere shortening appears to correlate with the pathological status of the affected myocardium. The PBMCs seem to mimic these myocardial patterns. These data strongly suggest a causative role of telomere shortening-induced signalling pathways in the pathogenesis of HF, even after moderate telomere shortening, and further points towards ROS as the culprit.

PB0132 | The Initial Formation of a Transient Sub-Occlusive Mural Thrombus Appears to Protect Coagulation to Leave a Thin Platelet- and Fibrin-Rich Patch Covering the Site of Injury

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Background: A large thrombus forms during initial stages in various experimental models of localized arterial injury. This is followed by embolization events which leave a small clot covering the injury. The reason why a sub-occlusive mural thrombus forms during early stages while a small clot is required to seal the breach is puzzling.

Aims: To determine the physiological relevance of the formation of a large thrombus during the initial stages following an injury of healthy arteries.

Methods: Intravital microscopy was used to image fibrin, P-selectin and platelet staining in two models based on forceps-injury of the aorta and $FeCl_3$-injury of the carotid. The remaining patch was characterized using scanning electron microscopy. A computational model was used to predict thrombin generation inside thrombi of different heights and under different rheological conditions.

Results: Thrombus formation in the two in vivo models progressed in three phases with:

i) a growth phase forming a sub-occlusive thrombus,

ii) a rapid disaggregation,

iii) a stabilization of a remaining patch.

Using intravital and electron microscopy, we observed that the patch is composed of highly activated and degranulated platelets and is rich in fibrin. Combining *in silico* and *in vivo* approaches, we showed that a large transient thrombus provides a protective shell to facilitate intra-thrombus coagulation, even under high shear rates. The numerical model indicates that the initial formation of a sub-occlusive thrombus is essential to generate sufficient amounts of thrombin to assemble the underlying fibrin-rich patch. $P2Y_{12}$ or αIIbβ3 blockers injected at the end of the growth phase to remove the sub-occlusive thrombus prevents fibrin formation in the remaining patch, but not of the patch itself.

Conclusions: The initial formation of a transient large thrombus appears to provide a protective shell allowing coagulation to proceed and the formation of a residual patch that seals the breach.

PB0133 | High Salt Intake Increases Platelet Counts and Plasma Fibrinogen Levels but Has No Effect on Thrombus Formation or Resolution in a Murine Model of Venous Thrombosis

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Background: Conditions with dehydration are associated with an increased risk for thrombosis. It has been shown that water-restriction in mice increases plasma von Willebrand factor (vWF) levels. Furthermore, there is evidence that dehydration increases the secretion of vWF by endothelial cells in-vitro.

Aims: As vWF is critical for the development of venous thrombosis in murine models, we tested the hypothesis that a high-salt diet, which induces renal water loss, also leads to a pro-thrombotic state in a murine model of venous thrombosis.

Methods: Starting at the age of 6 weeks, C57BL/6J mice were fed a high-salt diet (4% NaCl chow and 0,9% NaCl water). After four weeks, we performed subtotal vena cava inferior ligation and studied development of deep vein thrombosis via ultrasound and histology. We performed a short-time experiment over 3 days and a longer study over 21 days to depict thrombus formation and resolution. Blood counts and coagulation parameters were investigated after the experiments.

Results: After 3 days, we did not see differences in thrombus formation in mice on a high-salt diet. However, histological studies revealed a significant decrease of the fibrotic area of the thrombi in high salt fed mice. The long-term experiment confirmed these findings and showed that thrombus resolution was not altered by the high-salt diet as well. After 21 days of subtotal IVC-ligation, mice on high-salt showed a significant increase in platelet counts and fibrinogen levels. Furthermore, circulating neutrophils were increased, whereas erythrocyte counts were not affected.

Conclusions: Our findings indicate that a high-salt diet has effects on blood cells and fibrinogen, which could contribute to a pro-thrombotic state. However, these changes were not paralleled by increased thrombus formation in our model of subtotal vena cava inferior ligation. Further studies are required to clarify the influence of high salt diet on venous thrombosis.

PB0134 | Platelet Analysis of Diet-Induced Obesity Rat Model: Elucidating Platelet Dysfunction in Obesity

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Background: Obesity is one of the main health problems in industrialized countries. The contribution of multiple factors developed in obesity can hardly be modeled in vitro. In this context, the development of animal models mimicking human obesity could be essential.
Aims: The main goal of the present study was to standardize a blood collection and platelet isolation methodology to study a diet-induced obesity (DIO) rat model both at biochemical and functional level. Moreover, the results obtained were compared between DIO and control groups in order to understand platelet dysfunction in obesity.
Methods: According to the methodology, optimal blood collection was obtained from *vena cava* using a 19G needle and a 10mL syringe obtaining up to 8 mL. Platelet isolation was based on a serial of centrifugations avoiding platelet activation.
Results: Our results show that the DIO model simulate perfectly the pathological mechanistic of obesity since weight gain and fasting glucose are increased in obese rats. Moreover, the methodology showed a greater grade of reproducibility between groups, and pointed towards an increment in platelet counts in the DIO group, in line with human studies. To further understand platelet dysfunction in obesity, we demonstrated that platelet adhesion to collagen and the platelet levels of the active form of Src ($pTyr^{419}$) were increased in the DIO rat model. These results pointed towards a potential dysfunction on GPVI and Src family kinases (SFK)-related signalling pathways in obesity.
Conclusions: These results confirm that obesity, in line with human studies, present an atherothrombotic risk that should be considered, and highlight the relevance of considering novel antithrombotic drug targets in these patients, such as GPVI.

PB0135 | Fibrin Deposits on Peritoneal Carcinomatosis Serves as a Niche for Cancer Cell Implantation and Dissemination. A Scanning Electron Microscopy Analysis

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Background: Peritoneal carcinomatosis (PC) is a very serious complication of gastrointestinal and gynecological malignancies which is poorly documented.
Aims: to analyse the role of mesothelial cell layer that can favor fibrin deposition for cancer cell adhesion.
Methods: Scanning and transmission electron microscopy of peritoneal surface and cancer cell clusters from cancer patients as well as mouse induced carcinomatosis was done. For *in vitro* studies, several post-operative peritonea from digestive and ovarian cancer were collected. In mouse models, carcinomatosis is induced by CT26 colon cancer and D8, a mouse ovarian cancer cell lines. The samples were destined for immunohistochemistry (IHC) and scanning electron microscopy analysis. For the first method, tissues were fixed with paraformaldehyde (PFA) and paraffin embedded. For the second method, tissues were fixed with PFA and then with glutaraldehyde. Anti-fibrin F1E1 monoclonal antibody was used for fibrin detection in IHC. For *in vivo* studies, mice carcinomatosis induced by pilling of peritoneal surface before intraperitoneal CT26 cells injection, the presence of fibrin on the peritoneum surface was identified by bioluminescence apparatus. The fibrin deposit detected using an anti plasmin peptide that binds to fibrin: F13P peptide 1mg/ml (GNQEQVSPLTLLKC) labelled with either Alexa 488 or 560 fluorochromes (Thermofisher- France).
Results: Our results indicate that;
i) in carcinomatosis peritoneum, mesothelial cell shape were modified and the cells were detached from basal membrane,
ii) fibrin deposit was identified in the intercellular space,
iii) cancer cells interact with fibrin network on the peritoneum.
iv) by immunohistochemistry using F1E1, fibrin deposit was detected in carcinomatous peritoneum.
v) in animal model, the co-localization of fibrin deposit with the cancer nodules was demonstrated on the peritoneum using fluorescent F13P.
Conclusions: Fibrin deposition in the peritoneal cavity can serves as a niche for cancer cell implantation and dissemination.

PB0136 | Novel Mouse Model for Studying Hemostatic Function of Human Platelets

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Background: Platelets are critical for the formation of hemostatic plugs and the pathogenesis of atherothrombosis. Preclinical animal models, especially the mouse, provide an important platform to assess the efficacy and safety of antiplatelet drugs. However, these studies are limited by inherent differences between human and mouse platelets and the species-selectivity of many drugs. Previous mouse models of *in vivo* human platelet function have proved inadequate due to the presence of confounding mouse platelets.
Aims: We report a new protocol for the adoptive transfer of human platelets into thrombocytopenic NOD/SCID mice; a model where all

endogenous platelets are replaced by human platelets in mice accepting xenogeneic tissues.

Methods: Thrombocytopenia in NOD/SCID mice was achieved by injection of anti-GPIbα antibodies, in the presence of a platelet activating factor receptor inhibitor. Human platelets were transfused to a final concentration of 150-400 K/µl followed by infusion of human-vWF. Visualization and quantification of hemostatic plug formation at saphenous vein laser ablation injury sites was done using intravital spinning disk confocal microscopy.

Results: Integrin $\alpha_{IIb}\beta_3$-dependent hemostatic platelet plug formation was achieved ~30 seconds after laser ablation injury in humanized platelet mice. Pre-treatment of mice with standard dual antiplatelet therapy (DAPT, Aspirin + Ticagrelor) or PAR1 inhibitor, L-003959712 (vorapaxar analog), mildly prolonged bleeding time (BT) and significantly reduced platelet adhesion at injury sites. Consistent with clinical trials, inhibition of PAR1 in combination with DAPT markedly prolonged BT in our model.

Conclusions: Studies on the anti-hemostatic effect of PAR1-inhibition in the absence and presence of DAPT validate our humanized platelet mouse model as a robust platform to test and predict the safety and efficacy of experimental antiplatelet drugs. We propose that this model will also be of great value for the characterization of *in vivo* hemostatic/thrombotic potential of synthetic platelets, stored platelets or platelets from patients with a platelet function disorder.

PB0137 | Cardiac Myosin Promotes Thrombin Generation and Coagulation in vitro and in vivo

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Background: Skeletal muscle myosin (SkM) has procoagulant activities and is structurally very similar to cardiac myosin (CM) which is exposed and released during myocardial infarction.

Aims: We evaluated CM's procoagulant activities in vitro and in vivo.

Methods: Standard assays included: thrombin generation assays in purified clotting factor mixtures and in human plasma; factor Xa binding to immobilized CM; tail-cut-induced bleeding in acquired hemophilia-A mice; and murine myocardial ischemia-reperfusion injury. The ability of a myosin-based peptide mimicking SkM residues 816-837 (P-816/837) of myosin's neck region to inhibit CM's procoagulant activities was studied in vitro and in vivo.

Results: CM, similar to SkM, enhanced thrombin generation in mixtures of purified clotting factors Xa and Va, calcium ions, and prothrombin, and in human platelet-rich and platelet-poor plasmas. Immobilized CM, like SkM, directly bound FXa. These data support the concept that CM's surface is a site for prothrombinase assembly and thrombin generation. In vivo testing for CM's procoagulant activities in acquired hemophilia-A mice subjected to tail cutting showed that intravenously-administered CM significantly reduced tail-cut-initiated bleeding. In vivo testing for CM's activities in a murine myocardial ischemia-reperfusion injury model showed that intravenously-administered CM augmented myocardial injury because CM significantly increased infarct size from 25% to 45% and serum troponin-I level from 54 to 179 ng/mL. Notably, the myosin sequence-based peptide, P-816/837 which binds FXa, inhibited CM's procoagulant activities in vitro and in vivo.

Conclusions: CM exhibits procoagulant activities in vitro and in vivo, and CM can either promote hemostasis in the context of bleeding or exacerbate thrombosis-related myocardial injury in the context of myocardial ischemia/reperfusion. Remarkably, peptide P-816/837 targets and inhibits CM's procoagulant activities in vitro and in vivo, and such myosin based peptides may provide myosin-specific anticoagulants for translational research. Research is needed to clarify CM's pathophysiology and its mechanistic influences on hemostasis or thrombosis.

PB0138 | Thrombin Generation Following *Vipera berus* Envenomation: A Canine Model

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Background: Envenomation by the European adder (*Vipera berus)* is an important medical emergency. *V.berus* venom contains both pro- and anticoagulant toxins, the overall *in-vivo* effects of which are unclear. Dogs are more commonly bitten than humans, making them an accessible natural model for investigation of the clinical effects of envenomation.

Aims: To describe the haemostatic status of dogs after *v.berus* envenomation, compared to a group of healthy control dogs. And, to investigate any differences between dogs treated with and without antivenom.

Methods: Citrated platelet-poor plasma was collected from 26 dogs envenomated by *v.berus* at presentation, 12 hours (h), 24 h, 36 h and 14 days post bite, and from 28 healthy controls at a single time point. Thrombin generation (TG) was initiated with three different triggers (no trigger, tissue factor (TF) 0.5 pM, or TF 0.5 pM and phospholipids (PLs) 4 µM). At each time point, comparisons were made between cases and controls as well as between antivenom and

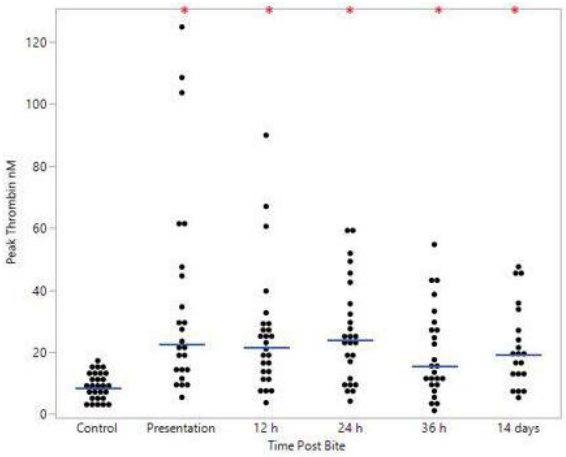

*Figure 1: Thrombin generation was initiated after addition of only calcium (no trigger) in plasma obtained from dogs after vipera berus envenomation, and in plasma from healthy control dogs. Peak thrombin (nM) is presented at five timepoints post envenomation, compared to a single control group. * indicates significant difference (p< 0.01) between cases and control group (Wilcoxon each pair with Bonferroni correction).*

FIGURE 1 Peak Thrombin

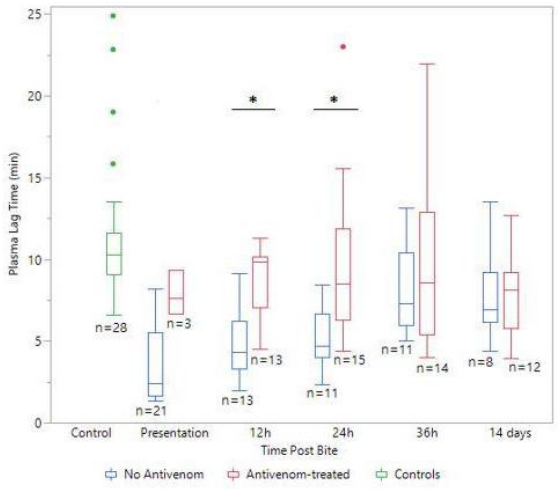

*Figure 2: Thrombin generation was initiated after addition of only calcium (no trigger) in plasma obtained from dogs after vipera berus envenomation, and in plasma from healthy control dogs. Lag time (min) is presented at five timepoints post envenomation, compared to a single control group (coloured green). Envenomated dogs treated with and without antivenom are coloured red and blue. * indicates significant difference between antivenom and non-antivenom treated dogs at a given timepoint p < 0.01 (Wilcoxon with Bonferroni correction for multiple comparisons)*

FIGURE 2 Lag Time

non-antivenom treated dogs. P values of < 0.05 were considered significant. Bonferroni correction was applied for multiple comparisons.

Results: When TG was initiated without trigger, or with TF alone, lag time (LT) was shorter and peak higher at all time points in envenomated dogs compared with controls (figure 1). After initiation with both TF and PLs, LT and peak were only changed at presentation and at 36 hours post bite. Endogenous thrombin potential was higher at all time points in envenomated dogs compared to controls, with all three triggers. LT was shorter in non-antivenom treated dogs, compared with antivenom treated dogs, at 12- and 24-hours post envenomation, with all three triggers (figure 2).

Conclusions: Dogs in this study were hypercoagulable following *v.berus* envenomation. Venom-induced consumptive coagulopathy was not observed. Dogs treated with antivenom may be less hypercoagulable than their non-antivenom treated counterparts.

PB0139 | Multiple Gene Knockdowns Simultaneously to Study Hemostasis in Zebrafish

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Background: The piggyback knockdown (PK) method was used to study hemostasis by performing genome-wide screens using zebrafish. Here, we intravenously inject a vivo morpholino (VMO) that piggybacks an antisense deoxyoligonucleotide into the somatic cells and somehow reduces mRNA levels. The application of the PK method requires enhancing efficiency and understanding the mechanism.

Aims: To increase efficiency and understand the mechanism of the PK method.

Methods: PKs, kPT, kPTT assays, qRT-PCRs, and thrombocyte aggregation assay were performed according to published methods. Student's t-test was used in statistical analysis.

Results: To increase the efficiency of the PK method, we tested whether we can piggyback more than one deoxyoligonucleotide with one VMO. We designed hybrids that had more than one deoxyoligonucleotide that could be piggybacked with VMO. Since we have only 25 bases in the VMO, tested 10, 7, and 5 bp lengths to accommodate pairing of 2, 3, and 4 different deoxyoligonucleotides. We used *f7*, *f8*, and *αIIb* and tested the efficiency of knockdown by kPT, kPTT, and thrombocyte aggregation assay, respectively. The PK using four deoxyoligonucleotides did not yield acceptable efficiency, whereas the knockdown with two and three deoxyoligonucleotides yielded excellent results. We used qRT-PCR to check the efficiency of knockdown and found that all three genes were knocked down by >85%. As expected, we also noted bleeding spots in 20-30% of these fish. We also performed knockdown of RNaseH and argonautes along with *f7* and found the knockdown occurs with argonautes and not with RNaseH suggesting that RNaseH is involved in mRNA degradation.

Conclusions: We developed a method where we could knockdown three genes at one time. We established that knockdown is due to RNaseH-mediated mRNA degradation. Multiple gene knockdowns (up to 6 genes) will not only increase the efficiency of the method in genome-wide knockdowns but will also be useful to study multifactorial disorders.

PB0140 | Sex Bias in Animal Models of Thrombosis Research

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Background: The incidence, risk factors, and prognosis of thrombotic disorders differ among men and women. However, if the use of sex-matched animal models is not enforced in preclinical stages of research, it might lead to sex bias in proceeding clinical trials.

Aims: We aimed to systematically review the gender of animal models used in thrombosis research in the past 2 years.

Methods: We searched PubMed using search terms "thrombosis" AND "animal". Articles published in the past 2 years (between January 2018 and December 2019) and reporting original data from in-vivo experiments were included in our analysis. Each article was independently reviewed for types and sexes of the animal models used and, if study samples were sex-matched.

Results: Of 1052 articles screened, our review included 308 studies for analysis. The kappa interrater agreement for study inclusion before resolution was 0.92. Among included studies, sex of the animals used was not reported in 30.5%; males were exclusively used in 49.7%; females were exclusively used in 9.1%; and both sexes were used in 10.7%. Sex matching of animals was reported in 54.5% of studies that included both sexes. A total of 11 animal models were employed; mice were used in 54.9% of studies, followed by rats (25.1%) and pigs (7%).

Conclusions: Although there are different risk factors and mechanisms involving thrombus formation between males and females, a predominant number of studies in the past 2 years employed only male animal models. In order to ensure gender balance and improve the quality of in-vivo thrombosis research, journals and funding bodies need to emphasize the report of gender in animal models used.

PB0141 | CRISPR/Cas9-Mediated Generation of a RUNX1 Knock-In Mice Model and Platelet Characterization

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Background: Inherited mono-allelic *RUNX1* variants are associated with Familial Platelet Disorder with predisposition to Acute Myeloid Leukemia (FPD/AML), characterized by abnormal platelet function, and/or thrombocytopenia and up to 40% predisposition to neoplasia. Animal/cellular models would help to elucidate the pathogenicity and biological consequences of *RUNX1* variants.

TABLE 1 Results of functional platelet assay. All comparisons were made between two experimental groups; p value is referenced to WT mice. Mean ± SEM

Platelet Assay	WT	$RUNX1^{WT/L43S}$	$RUNX1^{L43S/L43S}$
Bleeding time (s)	94.24 ± 14.16	154.4 ± 26.03; p = 0.048	170.08 ± 26.8; p = 0.016
Platelet count (x1000/μL)	587.16 ± 29.22	563.26 ± 28.17; p = 0.559	480.94 ± 28.19; p = 0.012
% α-granule secretion (thrombin 1U/mL)	31.88 ± 1.69	25.65 ± 1.76; p = 0.014	25.17 ± 0.78; p = 0.001
% α-granule secretion (PMA 3μM)	15.24 ± 0.8	12.53 ± 0.93; p = 0.032	11.77 ± 0.71; p = 0.002
% δ-granule secretion (T1U/mL)	5.19 ± 0.32	5.55 ± 0.26; p = 0.376	5.02 ± 0.5; p = 0.778
% δ-granule secretion (PMA 3μM)	2.08 ± 0.14	2.24 ± 0.27; p = 0.611	2 ± 0.18; p = 0.739
% αIIbβ3 integrin activation (T1U/mL)	36.11 ± 2.03	25.44 ± 2.17; p = 0.001	26.71 ± 1.06; p < 0.001
% αIIbβ3 integrin activation (PMA 3μM)	16.75 ± 0.9	12.03 ± 1.19; p = 0.003	10.6 ± 0.61; p < 0.001
% Active αIIbβ3 - fibrinogen binding (ADP 30μM)	9.51 ± 0.85	7.4 ± 0.53; p = 0.041	4.78 ± 0.38; p < 0.001

Aims: Generate a RUNX1 knock-in murine model by CRISPR/Cas9 technology, and assess its platelet and hemostatic phenotype.

Methods: Two gRNA against exon 2 of *Runx1*, Cas9 nickase protein, and DNA template carrying the mutation c.127–128>TC were microinjected into zygotes of C57BL/6J strain. Platelet dysfunction was evaluated by tail-bleeding time and flow cytometry assays: 1) CD41+ platelet count, 2) α and δ granules secretion (anti-CD62P, anti-CD63), 3) $\alpha_{IIb}\beta_3$ integrin activation (JON/A, fibrinogen-AF488), 4) platelet aggregation (anti-CD9). Adhesion and spreading were performed by confocal microscopy with phalloidin-514. Thrombin (0.5–1U/mL), PMA (3μM), and ADP (30μM) were used as agonists.

Results: We generate a murine model carrying the mutation c.127–128>TC, which leads to mice RUNX1 L43S variant, mimicking human L56S alteration. Twenty-five mice for genotype were investigated (WT, heterozygous: WT/L43S; homozygous: L43S/L43S).

$RUNX1^{WT/L43S}$ and $RUNX1^{L43S/L43S}$ animals showed statistically significant increase of bleeding-tail times compared to control animals. Homozygous mice displayed thrombocytopenia (Table1). $RUNX1^{WT/L43S}$ and $RUNX1^{L43S/L43S}$ vs. $RUNX1^{WT/WT}$ displayed impaired α-granule release, while there were no differences in δ-granule secretion. Moreover, agonist-induced $\alpha_{IIb}\beta_3$ integrin activation, fibrinogen-binding, and aggregation were significant impaired in platelets from $RUNX1^{WT/L43S}$ and $RUNX1^{L43S/L43S}$ (Table1, Figure1A,B,C). No differences in adhesion were found, while spreading was significantly reduced after thrombin stimulation in platelets from $RUNX1^{WT/L43S}$ and $RUNX1^{L43S/L43S}$ vs. control mice (Figure1D).

Conclusions: A knock-in RUNX1 murine model was successfully generated by CRISPR/Cas9, which allowed assessing the deleterious effect of the relatively common RUNX1 L56S variant. Noteworthy, both heterozygosis and homozygosis status associates with defective $\alpha_{IIb}\beta_3$ integrin activation, α-granule secretion and platelet spreading in the mice model.

Funding: PI17/01966,GRS2061A/19,FMM AP172142019,Premio López Borrasca 2019

PB0142 | Maternal Deficiency of Endothelial Protein C Receptor Causes Severe Pregnancy Complications Prevented by Inactivation of Protease Activated Receptor 4, but not Clotting Factor VIII

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Background: Placenta-mediated pregnancy complications are a major challenge in the management of maternal-fetal health. Maternal thrombophilia is a suspected risk factor but the role of thrombotic processes in these complications and the potential for antithrombotic treatment have remained unclear. Endothelial Protein C Receptor (EPCR; gene name *Procr*) is an anticoagulant protein highly expressed in the placenta. EPCR autoantibodies and gene variants are associated with poor pregnancy outcomes. In mice, fetal EPCR deficiency results in placental failure and *in utero* death. Adult EPCR-deficient mice generated by maintaining placental expression exhibit plasma markers of thrombophilia without overt thrombosis.

Aims: The goals of this study were to determine if maternal thrombophilia due to EPCR deficiency is associated with pregnancy complications and to test the potential for anti-platelet and anti-thrombotic therapy.

Methods: EPCR-deficient dams generated by maintaining EPCR expression in the placenta ($Meox2^{+/cre}Procr^{lox/lox}$; abbreviated as $Procr^{\delta/\delta}$) were mated to wild type C57Bl6 males and pregnancy outcomes were evaluated. We also evaluated pregnancy outcome of $Par4^{-/-}Procr^{-/-}$ and $FVIII^{-/-}Procr^{-/-}$ dams generated by intercrossing $Par4^{-/-}Procr^{+/-}$ and $FVIII^{-/-}Procr^{+/-}$ mice, respectively. Outcome studies were combined with surgical and histological evaluations.

Results: We analyzed 18 pregnancies of 12 $Procr^{\delta/\delta}$ dams. Of these, 44% resulted in maternal death or moribund mother. Mid-gestational vaginal bleeding, pregnancy loss and stillbirth were frequently observed. Surgical evaluation revealed retroplacental hemorrhaging and blanched appearance of maternal organs. The placentae appeared normally developed but blood clots were observed in the decidua basalis with infiltration of neutrophils and release of myeloperoxidase. All pregnancies of $FVIII^{-/-}Procr^{-/-}$ dams resulted in maternal death (4 evaluated). In contrast, pregnancies of $Par4^{-/-}Procr^{-/-}$ dams were uneventful with no observable signs of distress
(5 evaluated).

Conclusions: The murine model of EPCR deficiency establishes a cause-effect relationship between maternal thrombophilia, retroplacental hemorrhage and severe pregnancy complications. It highlights the potential for therapeutic inhibition of Par4 in a subset of pregnancy complications.

PB0143 | Immunization with Recombinant Atherogenic Antigen (AHC) Does Not Increase Susceptibility to Venous Thrombosis

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Background: Venous thromboembolism (VTE) refers to formation of blood clot in the veins. This include deep vein thrombosis (DVT), and pulmonary embolism (PE). Although recombinant atherogenic antigen (AHC) containing epitopes derived from ApoB, heat shock protein (HSP) 60, and *Chlamydia pneumoniae* outer membrane protein would represent the most successful vaccine candidate to reduce autoantigen (such as ApoB and HSP)-associated inflammation towards atherosclerosis, little is known about the effect of the immune response induced by AHC construct containing autoantigen epitopes on VTE.

Aims: To assess whether immunization of C57BL/6 mice with the recombinant atherosclerotic antigen AHC has an effect in a carrageenan-induced mouse tail thrombosis model.

Methods: 6-week old C57BL/6 mice (N=6–8 per group) were immunized with AHC and a non-immunized group of mice were used as controls. Two weeks after the first immunization, VTE was induced by injection of carrageenan into the tail vein of the mice. Blood samples were collected and the plasma was tested for cytokine levels by ELISA.

Results: Similar plasma levels of thrombosis markers (TCK-1, IL-6, IL-10, fibrinogen, and TIMP-1) were observed in AHC-immunized mice and non-immunized controls.

Conclusions: Immune response to AHC did not contribute to promotion of VTE in tested mice.

PB0144 | Antithrombotic Properties of Carvedilol Shown in Two Independent Intravital Real Time Models of Thrombosis

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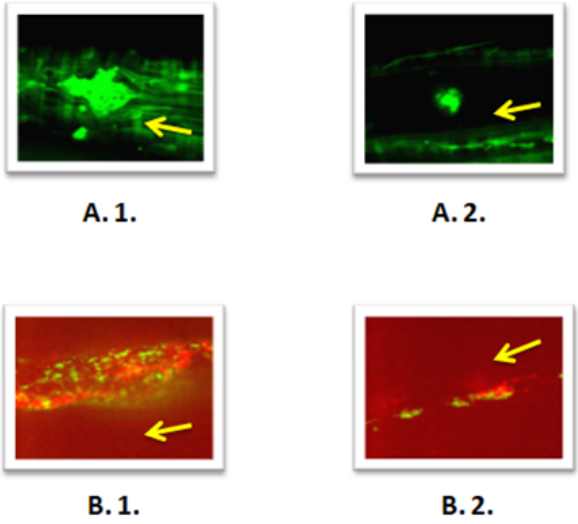
Background: Carvedilol is a β-adrenoceptor antagonist of β1 and β2 receptors and a competitive antagonist of α1-adrenoreceptor. There are data that carvedilol exerts antithrombotic activity in extracorporeal circulation, probably due to its metabolites, which act as a β2-adrenoreceptor agonists.

Aims: The aim of the study was to evaluate antithrombotic properties of carvedilol and its metabolites by the use of two different intravital real time thrombosis models: laser induced thrombosis and ferric chloride induced thrombosis.

Methods: The mesenteric vascular bed of C57BL/6-Tg(CAG-EGFP)1Osb/J transgenic mice was exposed after making a midline laparotomy incision. For intravital fluorescence, confocal microscopy of the microcirculation of a living mouse, carvedilol was administered *p.o.* at doses of 3 and 10 mg/kg. To assess possible involvement of β-receptors in antithrombotic effect of carvedilol, atenolol (10mg/kg,.) and labetalol (30mg/kg) was used. We inhibited metabolism of carvedilol with ketoconazole (50mg/kg) or 1-aminobenzotriazole (1-ABT,200 mg/kg). Platelet accumulation in the blood vessel at the site of thermal laser injury or chemical ferric chloride injury of endothelium was measured as an area of fluorescence which is corresponding to the thrombus volume.

Results: Carvedilol significantly decreases platelet accumulation at the site of endothelial injury. Atenolol, labetalol, ketokonazole and 1-ABT does not exert antiplatelet effect when administered alone. Furthermore, platelet accumulation was reversed by labetalol and ketoconazole.

Conclusions: Carvedilol exerts antithrombotic effect due to inhibition of platelet accumulation by various mechanisms. On the one hand, this effect is not-dependent to β-1 adrenoceptor (atenolol study) and not-dependent to CYP2D6 (1-ABT study). On the other hand, study with ketoconazole revealed that this effect is dependent on CYP3A4 metabolites of carvedilol and labetalol (receptor study).



A. - laser induced thrombosis; B. - ferric chloride induced thrombosis; A.1. and B.1. - pictures taken after induction of thrombosis in control mouse; A.2. and B.2. - pictures taken after induction of thrombosis in mouse that was treated with substances that show antiplatelet and antithrombotic effect. The arrow (yellow) indicates the direction of blood flow.

FIGURE 1 Representative pictures and comparison of two independent confocal methods

Summing up, carvedilol seems to be very attractive drug alternative in cardiovascular states complicated with thrombosis. Further experiments under individual carvedilol metabolites are planned.

PB0145 | Reported Values and Methodologies for Detecting Plasma D-dimer in Rat Models: A Systematic Review and Meta-analysis

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Background: Plasma D-Dimer (D2D) is a degradation product of cross-linked fibrin and represents the activation of the fibrinolytic and coagulation system. Clinically, D2D tests have a high negative prediction value for thrombotic events and can be used to rule-out venous thromboembolism (VTE)[1]. The D2D cut-off value for VTE in humans is 500ng/mL; however, the baseline and cut-off values for rats are unknown.

Aims: To systematically evaluate the reported results and methodology of D2D tests on rat models.

Methods: A systematic literature search was conducted using MEDLINE, EMBASE and Web of Science to include relevant

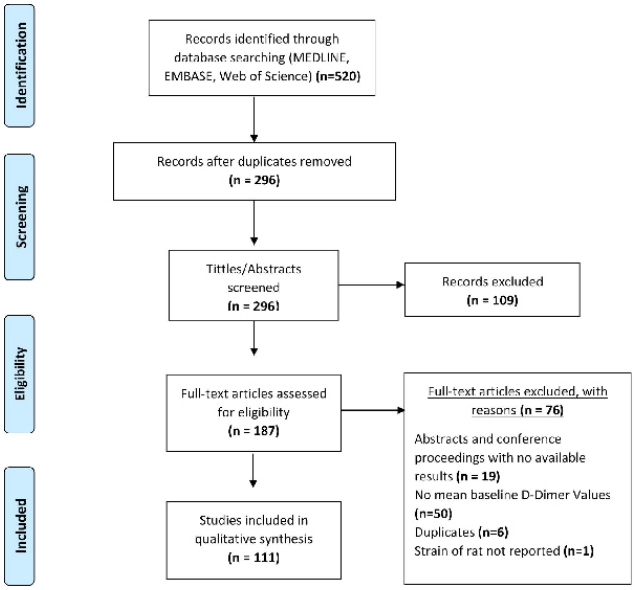

FIGURE 1 PRISMA flow diagram of study selection

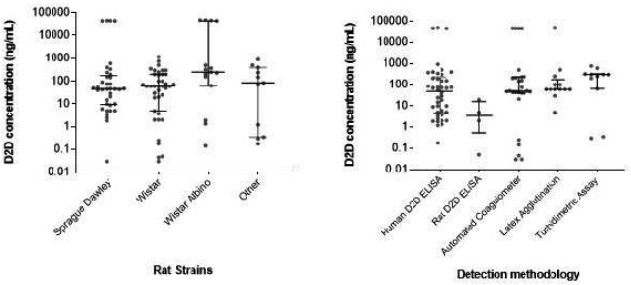

FIGURE 2 Baseline rat plasma D2D stratified by detection methodology and strain. Horizontal bars and error bars represent median and IQR

full-length publications, published abstracts and conference proceedings from Jan-01-2000 to Dec-20-2019 that reported rat D2D values. The search strategy used categorical search terms: "rat" AND "D-dimer" OR "fibrin degradation product". Eligible articles were independently reviewed for strain, age, sex, baseline D2D and measurement methodology.

Results: Of the 520 identified records 111 studies were included for final analysis. The kappa interrater agreement for study inclusion before resolution was 0.94. Twelve strains with a body mass ranging from 70–410 grams and of both sexes were included in the analysis. Figure 2 illustrates baseline D2D levels stratified by strain and methodology. There was a significant difference in reported baseline D2D between rat strains (P= 0.005). Despite large variances in reported baseline D2D between measurement methodologies, the differences were not statistically significant.

Conclusions: Large discrepancies in reported rat plasma D2D values suggest that factors such as species and detection methods can lead to the variation of results and should be considered when designing a rat model that measures D2D. We recommend using related negative control models as a baseline D2D reference range for each study aiming to measure D2D level in rats. Further research is required to establish a standardized reference range for baseline D2D levels to help scientists better interpret rat D2D test results.

PB0146 | Von Willebrand Factor (VWF) Deficiency Leads to Altered Angiogenesis in the Gastrointestinal Tract

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Background: Gastrointestinal bleeding in Von Willebrand Disease (VWD) is a challenging complication. Recently, impaired angiogenesis has been discussed as a possible reason. In vitro studies have shown, that the ANG-TIE-system regulates the activation of endothelial cells (EC) to guarantee proper angiogenesis but is altered by VWF.

Aims: We, therefore, investigated the impact of VWF deficiency on angiogenesis and angiogenic modifiers in the stomach in porcine VWD type 1 (T1) and type 3 (T3).

Methods: A total of 11 pigs (3 WT, 5 T1, 3 T3) were used for immunohistochemistry (IHC) and 13 pigs (6 WT, 5 T1, 2 T3) for RT PCR to investigate the expression and distribution of Ang2 and TIE1. Pigs with a heterozygous mutation in the VWF gene represented T1 and homozygous animals represented T3. Healthy pigs were used as controls (WT, n=3). Hematoxylin-eosin (HE) staining was performed to evaluate the blood vessel architecture.

Results: HE-staining showed angiodysplasia in T3 pigs. In IHC, mean expression levels of ANG2 had a tendency to increase in both VWD types relative to the WT in endothelial cells, while mean expression levels of TIE1 were reduced in the same groups in vascular smooth muscle cells. In RT PCR, *ANG2* and *TIE1* showed the same tendencies in T1 pigs compared to the WT. Regarding the ratio of ANG2/TIE1, a shift towards ANG2 was visible in IHC in all affected pigs and in qPCR in the T1 pigs.

Conclusions: Our results indicate a shift away from TIE1, an inhibitor of EC, towards ANG2, an activator of EC in VWD type 1 and 3. This might lead to excessively upregulated angiogenesis, thus resulting in angiodysplasia and gastrointestinal bleeding as a consequence.

PB0148 | Real-time in vivo Visualization of Infused FVIIa in Hemophilia A Mice Following Injury

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Background: Localization of infused activated FVII (FVIIa) and its procoagulant reactions in vivo in hemophilia remain unclear.

However, the prothrombinase complex was demonstrated to form on injured endothelium, at sites distinct from platelets, suggesting that activated endothelium can support fibrin-forming reactions. It is possible that FVIIa may also localize at sites distinct from platelets on the injured endothelium.

Aims: The aim of this study is to develop reagents and visualize FVIIa participation in clot formation in vivo under hemophilic conditions.

Methods: We generated biotinylated recombinant mouse FVIIa (mFVIIa) by adding a C-terminal, single biotin acceptor peptide tag (Avitag). Purified mFVIIa-Avitag was tested in vitro in a prothrombin time (PT) clotting assay and a thrombin generation assay (TGA) to determine protease activity. In vivo, mFVIIa-Avitag was visualized in a cremaster laser injury in hemophilia A (HA) mice. Prior to injury, HA mice were infused with fluorescently-labeled anti-CD41, anti-Fibrin, and streptavidin. Following injury, mice were infused with PBS or biotinylated mFVIIa-Avitag.

Results: We found that biotinylated mFVIIa-Avitag had 60% activity compared to mFVIIa in a PT assay, but reduction of activity was not observed in a TGA. Following infusion of mFVIIa-Avitag into HA mice that had undergone a laser injury, visualized mFVIIa-Avitag localized to the injury site. Three-dimensional reconstructions of confocal z-stack images revealed primarily extraluminal localization of mFVIIa-Avitag, and suggested additional binding to damaged endothelial cells adjacent to the injury site. The mFVIIa-Avitag did not substantially colocalize with platelets, it modestly increased platelet accumulation following injury, and had no effect on the extent of fibrin generation.

Conclusions: We generated site-specifically-labeled, biologically active mFVIIa. When infused in injured hemophilia A mice, it was localized at sites distinct from the growing platelet mass. These data suggest that, in this system, infused mFVIIa primarily localizes on the injured endothelium and extraluminally.

PB0149 | Novel Oral Anticoagulants Do Not Expand Haematoma Volume in a Intracerebral Hemorrhage Model in Rats

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Background: Intracerebral hemorrhage (ICH) is the most feared complication of anticoagulant therapy, and haematoma expansion during new oral anticoagulants treatments is still under debate. To study this event in more detail and to test therapeutic strategies, experimental models of ICH have been developed.

Aims: In this work, we studied the effects of Warfarin and new oral anticoagulants (NOACs) (Rivaroxaban, Apixaban and Dabigatran) in haematoma expansion using a ICH model in Rats.

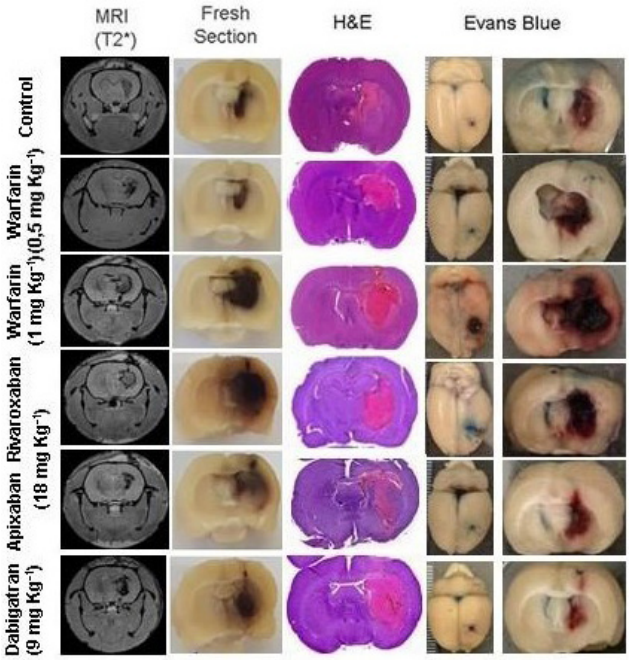

FIGURE 1 Effect of oral anticoagulants on haematoma expansion in a intracerebral hemorrhage model induced by collagenase VII-S in rats

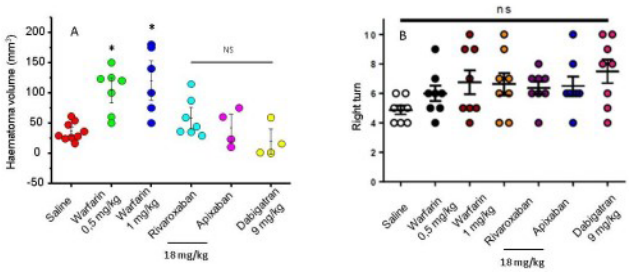

FIGURE 2 (A) Volume of hemorrhagic lesions using MRI and (B) Evaluation of motor dysfunction using the Elevated Body Swing Test

Methods: Oral anticoagulants were given by gavage at different doses and, at the peak of their anticoagulant and antithrombotic activities, ICH was induced by injecting collagenase VII-S into the left striatum. Haematoma lesion volume was determined 24h later using MRI and H&E-stained brain sections. We also estimated Blood Brain Barrier breakdown by Evans Blue staining and quantified the amount of hemoglobin in the brain using Drabkin's reagent. Neurological deficits were evaluated using Body Elevated Swing Test. Differences between groups were analyzed using analysis of variance followed by ANOVA test with Origin 9.0 software.

Results: We observed that Rivaroxaban and Apixaban (18 mg/kg) or Dabigatran (9 mg/kg) did not expand lesion volume as Warfarin (0.5 and 1 mg/kg) when compared to control group, although Rivaroxaban showed a higher bleeding tendency than Apixaban and Dabigatran (Figures 1 and 2A). We observed a clear correlation

between the images obtained by MRI and histological staining. These findings were confirmed by Evans blue staining and hemoglobin content using Drabkin's reagent, which showed an increase in rats treated with warfarin but not with NOACs. We did not observe any difference in neurological test (Figure 2B).

Conclusions: Unlike Warfarin, NOACs do not promote larger haematoma volumes in a collagenase model of ICH in Rats.

PB0150 | Effects of Hibernation on the Platelet Proteome of 13-lined Ground Squirrels

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Background: Hibernating 13-lined ground squirrels (*Ictidomys tridecemlineatus*) undergo a dramatic drop in temperature and blood flow during torpor. Remarkably, platelets stored in the ground squirrel's body at temperatures of 4–8°C remain functional when the animal arouses. To avoid stasis clots, both primary and secondary hemostasis are suppressed through mechanisms that remain unclear.

Aims: We aimed to determine how the proteomes of platelets from animals in summer, fall entrance, and winter hibernation differ to in a manner that may contribute to hemostatic adaptation.

Methods: Platelets were isolated from blood collected from five animals in June, October and January. Platelet lysates from each animal were digested and purified to prepare peptides for tandem mass tag (TMT) labeling and quantitative, multiplex mass spectrometry analysis.

Results: Relative to entrance and summer animals, platelets from ground squirrels in hibernation showed increases in 86 proteins, including coagulation regulators, platelet degranulation factors, and apolipoproteins. Platelets from summer non-hibernating animals showed increased levels of 79 proteins enriched in chaperone and transport pathways, and decreases in 48 proteins associated with translation. Platelets from fall animals entering into hibernation showed an increase 27 proteins with few function associations, as well as decreases in 24 proteins involved in complement and coagulation cascades.

Conclusions: Our results suggest that during hibernation, platelets may bind or take up plasma proteins and apolipoproteins. Increases in transport and chaperone proteins in non-hibernator animals may reflect more active and dynamic platelets, while translation is suppressed in the cold, and an increase in translation proteins in platelets from animals either entering or in hibernation could compensate. In fall entrance platelets, a decrease in bound or internal stores of complement and coagulation proteins may protect platelets from becoming activated through repeated rounds of warming and cooling, or ischemic events.

PB0151 | Identification of Thrombosis Predictors in an ex vivo Shunt Model through Retrospective Analysis

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Background: A unique non-human primate (NHP) *ex vivo* shunt model has long been used to robustly assess material thrombogenicity and anticoagulant efficacy by measuring platelet deposition from whole, flowing, uncitrated blood in real time. A retrospective analysis of control trials was undertaken to identify biological predictors of thrombus dynamics.

Aims: Determine biological parameters predictive of platelet deposition dynamics in *ex vivo* NHP studies.

Methods: Expanded polytetrafluoroethylene (ePTFE) tubes (4 mm ID) were placed in the blood flow path of a chronically implanted femoral artery to vein loop in male juvenile *Papio anubis* baboons. Autologous ^{111}In-labeled platelets and homologous ^{125}I-labeled fibrinogen were infused into NHPs prior to experiments. Blood flow was controlled at 100 ml/min, and the platelet deposition signal from ^{111}In was measured for 1 hr by a gamma camera once every one, three or five minutes. For the current analysis, data from 20 experiments with ePTFE controls over seven animals is used. Study files are manually segmented using FIJI (SciJava) and exported as 4D matrices to R (R foundation for statistical computing) for analysis.

Results:

As illustrated in Figure 1A, the final twenty minutes of platelet deposition grouped by animal reveal a trend consistent within animals. Thrombus tends to either continue growing at a linear rate, level off, or decrease in size. The rate of platelet deposition in these final twenty minutes inversely correlates with the average activated partial thromboplastin time (aPTT) of each animal, as shown in Figure 1B.

Conclusions: The platelet deposition data from ePTFE shunts demonstrate similarity among experiments from the same animals and is inversely related to aPTT, suggesting that aPTT may be a predictor of platelet deposition dynamics on biomaterials. Further analysis of

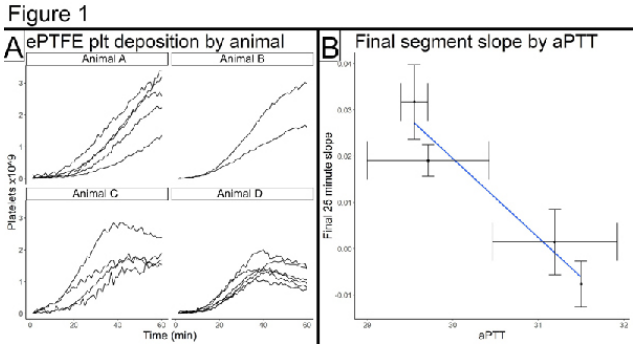

FIGURE 1 Platelet deposition by animals and its co-relation with aPTT

an expanded dataset using linear mixed effects modeling could identify more predictors of thrombus growth dynamics.

PB0152 | Early Indicators of Thromboembolic Events Caused by Prolonged Tourniquet Application: A Rat Model

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Background: Tourniquets are commonly used to control intraoperative blood loss in major orthopedic surgeries, such as total knee arthroplasty (TKA). Despite the widespread use, the research evaluating the risk of developing postoperative complications and effectiveness of intraoperative tourniquet use remains inconclusive. With a symptomatic incidence of ~10%, thromboembolic events are a major postoperative complication for TKA patients and can result in associated morbidity and mortality[1].
Aims: To evaluate the association between tourniquet application and early indicators of thromboembolic events in a rat model.
Methods: Male Sprague Dawley rats weighing ~325g were anesthetized under isoflurane. For experimental animals, a tourniquet standardized to 38 newtons was applied for 3-hours around the hind-leg, at the proximal region of the femur. Control animals did not receive tourniquet application. All subjects were placed on a heating pad, had vitals regularly monitored and placed in a supine position. After 3 hours, the tourniquet was removed on experimental animals and blood was collected from the tail vein of all subjects. Plasma D-Dimer was measured using *Asserachrom D-Di (Diagnostic Stago)*, an enzyme immunoassay of D-Dimer by ELISA method.
Results: Four experimental and four control animals were included in the study. The experimental animals demonstrated significantly elevated levels of plasma D-Dimer. The mean plasma D-Dimer values for experimental and control animals was 289.3 ng/mL and 67.37

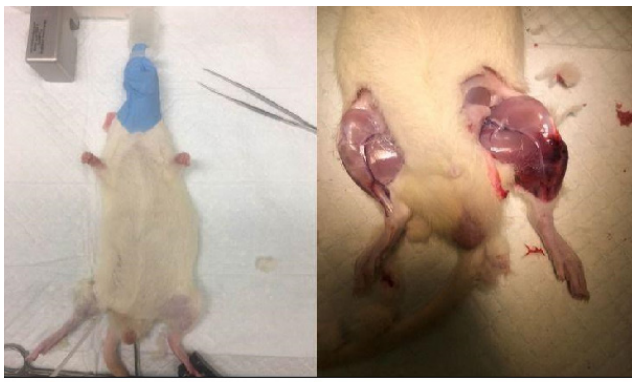

Figure 1. Left: 3-Hour tourniquet application on anesthetized, experimental animal. Right: Vascular & muscular tissue damage on hind limb. 3-hours after tourniquet application.

FIGURE 1

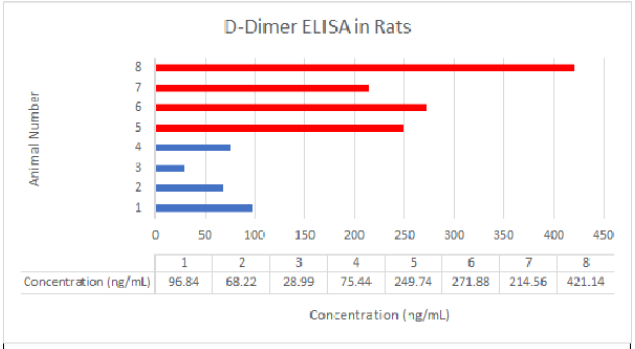

Figure 2: D-Dimer ELISA results of **control** (blue) and **experimental** (red) animals. P-Value: **0.0035.** Difference between means ± SEM: **222 ± 47.65**. R^2: **0.7834.**

FIGURE 2

ng/mL, respectively (*P*=0.0035). Three hour tourniquet application caused visible tissue damage in the vasculature and musculature of the rat's hind limb (Figure 1).
Conclusions: The results suggest greater blood clot formation and an increased risk for venous thromboembolism in animals with tourniquet application. This animal model highlights the potential thromboembolic risks and complications associated with tourniquet application in major orthopedic surgeries. Further research is required to identify thromboembolic events in rats, such as deep vein thrombosis and pulmonary embolism.

COAGULATION FACTORS AND INHIBITORS

PB0153 | Inhibitor against Congenital Coagulation Factor XIII Deficiency: A Case Report

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Background: Coagulation factor XIII deficiency (FXIIID) is one of the rare bleeding disorders (1:2,000,000 births). Umbilical cord bleeding, delay of wound healing, intracerebral hemorrhage, ecchymosis and hematomas are common clinical manifestations.
Aims: This study describes a case of congenital FXIIID who developed an autoantibody after repeated exposures to plasma derived FXIII concentrate.
Methods: A 3-year-old female was diagnosed with severe congenital FXIIID (c.1262–1263ins (ACGC)(p.H422Rfs*8) in F13A1 gene) following bleeding from the tongue. The patient was assigned to get prophylaxis

TABLE 1 Investigation on inhibitor against FXIII

Tests/ sample	Clot solubility test	Clot solubility test after mixing with NPP (30 min 37°c)	1/2 serial dilution	1/4 serial dilution	1/8 serial dilution	Interpretation
patient	No clot after 24 hours	No clot after 24 hours	partial sufficient (+)	Stable clot (++)	Stable clot (+++)	Presence of inhibitor against FXIII
Control (normal)	Stable clot (++++)	Stable clot (++++)	Stable clot (+++)	Stable clot (+++)	Stable clot (+++)	Normal
Control (abnormal)	No clot after 24 hours	Stable clot (+++)	Stable clot (+++)	Stable clot (+++)	Stable clot (+++)	FXIII deficiency

with plasma derived FXIII concentrate (10–15 U/Kg/ month). She was referred to hemophilia treatment center with posterior left leg hematoma one-year later. The dose of prophylaxis was increased to 25–30 U/kg monthly with no improvement. Mixing study with normal pooled plasma (NPP) was performed and 5M Urea was used to evaluate the stability of formed clot which was abnormal after 24 hours.

Therefore, evaluation of inhibitor against FXIII was performed. A serial dilutions of patient plasma was mixed with NPP. Then, clot solubility was done for each dilution.

Results: The clot was stable in serial dilutions after 24 hours. The clot of 1/8 dilution had a denser structure in comparison to 1/4 and 1/2 dilutions (table 1). The laboratory investigation revealed the presence of inhibitor but due to lack of commercial FXIII activity assay kit, titration of inhibitor was not possible. New prophylaxis with higher dose of FXIII concentrate (37–40 U/Kg/ month) has been scheduled which was promising with no sign of significant bleeds following six months follow up.

Conclusions: It seems higher doses of FXIII concentrate can control bleedings in patients with inhibitor and should be applied as a first optional treatment as we have seen in our case report. Also, availability of commercial kit plays an essential role to evaluate the titer of inhibitor and follow up of these patients.

PB0154 | Activated recombinant Factor VII (rFVIIa) Inhibitor in Iran: Immunogenicity is a Major Challenge through Treated Bleeds Covered with rFVIIa

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Background: The development of FVII inhibitor is a rare phenomenon in patients with congenital FVII deficiency (2.6%) rendering replacement therapies ineffective. The condition appears as a sudden aggravation in symptoms unresponsive to rFVIIa.

Aims: We evaluated the immunogenicity of Iranian patients with congenital FVII deficiency using rFVIIa.

Methods: A retrospective analysis of four hemophilia treatment center was conducted for a period of five years to evaluate the immunogenicity of reference rFVIIa product compared to biosimilar rFVIIa product. A total of 91 severe congenital FVII deficiency (less than 1%) were included. Inhibitor was measured via Modified Nijmegen method of Bethesda assay.

Results: Age range of the patients was 3 months to 8 years old at the time of antibody formation (6 M / 2 F). The prevalence of inhibitor development was 8.8% (8 of 91 subjects) and the inhibitor titers ranged from 7 to 217 BU at the time of initial detection. The patients showed resistance to rFVIIa between 30 to 500 exposure days. Six patients managed by higher doses of local product (40 to 270 ug/kg) but two of them were unresponsive and one patient to reference product. They treated with FEIBA (80–100 IU/kg) but they were expired due to recurrent intra cranial hemorrhage. Our results showed 5.5% (5/91) of patients who received local biosimilar product developed inhibitor as compared to 2.2% (2/91) who received reference product.

Conclusions: Our study demonstrates long-term immunogenicity of a biosimilar must always be deeply investigated since alloantibody can increase the risk of morbidity and even mortality. Recent study in Iran showed 2 out of 50 patients with FVII deficiency developed inhibitor (4%) against rVIIa. The pharmaceutical market in Iran shifted from reference product to biosimilar product around 2011-2012, prompting consideration of whether there is a difference in long-term immunogenicity between the two brands.

PB0155 | Efficacy and Safety of Prothrombin Complex Concentrate in Obstetric Hemorrhage

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Background: The use of FFP is associated with an increased incidence of complications such as acute respiratory distress and infections, and the rate of complications increased with the quantities of FFP transfused (1). PCC contain several important coagulation

factors and it has been suggested that they could replace FFP. This has been shown mainly in case reports or series in which coagulation factor deficit was detected by using POC viscoelastic tests in trauma (2) or traditional hemostatic tests in obstetric patients (3).

Aims: To assess the effectiveness and safety of the prothrombin complex concentrate in obstetric bleeding

Methods: Multicenter observational study of the safety and efficacy of the prothrombin complex concentrate. A survey of anesthetists was conducted in 19 maternity hospitals at various levels of care in the Russian Federation. Data has been collected and processed. As a result, 251 patients were analyzed. PPH was determined as a volume of blood loss more than 500 ml during vaginal delivery or CS.

Results: The most significant risk factors for PPH were: preeclampsia or arterial hypertension and a history of postpartum hemorrhage. 32.3% had no risk factors for PPH. It was determined that the use of Prothromplex 600 IU decreased the number of patients with transfusion FFP 12–15 ml/kg by 27.8% and increased the number of patients without transfusion by 25.9%, compared with patients without use of Prothromplex 600 IU. No complications were detected.

Conclusions: The use of PCC safety and efficacy reduce use of FFP during PPH.

PB0156 | Protein C Activity and Fibrinogen Levels in Nigerians Receiving Renal Replacement Therapy for Chronic Kidney Disease

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Background: Chronic kidney disease (CKD) is now recognised as public health problem. It is a major cause of morbidity and mortalility in Nigeria where it constitutes about 1.6–10% of hospital admissions. Both thromboembolic and bleeding manifestations occasioned by the uraemic state have been reported in affected patients. Fibrinogen and protein C have been reported to be deranged in individuals with CKD.

Aims: To assess the plasma fibrinogen levels and protein C activity in patients with chronic kidney disease on renal replacement therapy.

Methods: Chronic renal disease patients on regular haemodialysis at the renal unit of the University of Benin Teaching Hospital,Benin City, Nigeria were recruited into the study. Age and sex matched apparently healthy individuals were also recruited as the control group. Plasma fibrinogen was determined using Clauss method (Technoclone Fibrinogen reagent, REF: 5138080); protein C activity was assayed using standard chromogenic method (Technochrom Protein C, REF: 5341013).

Results: A total of 148 subjects were recruited into the study comprising, 74 CKD subjects on renal replacement therapy (RRT), and 74 apparently healthy individuals as control subjects. The mean age was 49.5±15.73 for CKD subjects on haemodialysis, and, 49.3±12.5 years for the controls. The mean plasma fibrinogen levels were 241.8±54.87 for patients on RRT, and 360.9±69.5mg/dl for the controls. The difference in mean was statistically significant. The mean protein C activity was 63.6 ± 28.89 in the RRT group and 86.7±2.6% for the controls. Protein C activity was significantly reduced in the CKD subjects and the mean difference was statistically significant.

Conclusions: In this study, Protein C activity was low in patients with CKD and those with cardiovascular or thromboembolic events. Contrary to existing literature, plasma fibrinogen levels were not significantly elevated in patients with CKD, and there was no association with thromboembolic events.

PB0157 | von Willebrand Factor Levels and Expression Pattern Are Altered in Response to Aging

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Background: Von Willebrand factor (VWF) is a prothrombotic endothelial specific protein and unregulated elevated VWF levels present a risk factor for thrombus formation. Aging process has been associated with increased circulating levels of VWF.

Aims:

1) To Explore the regulation of VWF expression during aging.

2) To determine association of increased VWF expression with thrombogenicity during aging.

3) To determine the molecular mechanism of aged-related upregulation of VWF expression.

Methods: Elisa, Western blot and RT-PCR analyses were used to determine circulating plasma, cellular protein, and mRNA levels of VWF in young and aged mice. Immunofluorescent analyses of major organs were performed to establish vascular pattern of VWF and the presence of platelet aggregates. Cultured endothelial cells were used as an in vitro model of aging to explore the mechanism of increased VWF levels.

Results: Increased plasma levels of VWF were observed in aged mice. VWF mRNA and protein levels were significantly increased in the brains, lungs, and livers, but not in kidneys and hearts of aged mice. In organs of aged mice that demonstrated increased VWF, the distribution pattern of expression was altered from primarily large vessels to include VWF detection in small vessels as well. Increased platelets aggregate formation in vessels of aged organs were concomitant with increased VWF expression. Aspirin treatment notably reduced platelets aggregates formation in the brain vasculature of aged mice. The prolonged culture of endothelial cells exhibited cell senescence that correlated with a significant increase in VWF mRNA. Increased VWF expression was specifically detected in senescent cell population.

Conclusions: VWF levels and expression patterns are significantly increased in response to aging in an organ-specific manner, which is concomitant with increased platelet aggregates formation as a risk factor for age-associated cardiovascular disorders. A potential mechanism of age-associated increase in VWF expression may be through cell senescence.

PB0158 | A Novel Rare C. - 39C>T Mutation in the PROS1 5′UTR Causes Protein S Deficiency by Creating a New Upstream Translation Initiation Codon and an Overlapping Open Reading frame

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Background: Inherited Protein S deficiency (PSD) (MIM176880) is a rare automosal dominant disorder caused by rare mutations, mainly in the coding sequence of the structural PROS1 gene, and associated with an increased risk of venous thromboembolism (VT).
Aims: We report the study of an extended French pedigree originated from Bordeaux area, South West of France, in which 7 relatives were affected with PSD with free Protein levels ranging between 18% and 38%, and in who no candidate deleterious PROS1 mutation was detected by the direct deep sequencing of the 15 PROS1 exons and of their flanking intronic regions. Of note, 6 of the 7 PSD patients had experienced VT events.
Methods: To identify the molecular defect underlying this familial PSD, we performed whole genome sequencing in 5 PSD patients and 2 unaffected relatives whose main finding was validated by Sanger sequencing. RT-qPCR and Western blot analyses were conducted using different molecular constructs to investigate the impact of the identified PSD causing mutation on the expression and translation of PROS1 in HeLa cells.
Results: We identified a never reported C to T substitution at c.-39 from the natural ATG codon of the PROS1 gene that completely segregates with PSD in the whole family.. This substitution ACG->ATG creates a new start codon upstream of the main ATG. We experimentally demonstrated that the variant generates a novel overlapping ORF and inhibits the translation of the wild type protein from the main ORF.
Conclusions: This work describes the first example of 5′UTR PROS1 mutation causing PSD through the creation of an upstream ORF, a mutation that is not predicted to be deleterious by standard annotation software. This work further illustrates the importance of exploring regulatory regions when searching for a molecular diagnostics of PSD and other Mendelian disorders.

PB0159 | G-Quadruplex Related Aptamers to Thrombin Exosite I with Additional Duplex Region. Relationships between their Structure, Stability and Antihtrombin Activity

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Background: Basic DNA aptamer containing G-quadruplex and interacting with thrombin exosite I consists of 15 nucleotides (5′-GGTTGGTG TGGTTGG-3) (15TBA, Thrombin Binding Aptamer). 15TBA inhibits exosite I binding with fibrinogen and platelet thrombin receptors and suppressed thrombin-induced fibrin formation and platelet aggregation.
Aims: We compared properties of G-quadruplex related aptamers (GQRAs), containing in addition to 15TBA structure hinge region (4 nucleotides) and duplex region with different number of complementary nucleotides. Earlier we have shown that aptamer RE31 (31 nucleotides) with 6 nucleotide pairs in the duplex region is more stable and has higher antithrombin activity than 15TBA [Dobrovolsky et al, 2011; Spiridonova et al, 2015].
Methods: GQRAs with 2–8 nucleotide pairs in the duplex region (RE23-RE35 series) and RE31 analogues with nucleotide insertions in the hinge region were synthesized. Stability of aptamers was assessed by measuring melting temperature at which 50% of G-quadruplexes are unfolded. Antithrombin activity of aptamers was evaluated by prolongation of thrombin, prothrombin and activated partial thromboplastin clotting times. RE31 effects on fibrinolysis were tested in *in vitro* model and its antithrombotic effects in *in vivo* FeCl3 thrombosis model in rats.
Results: Increasing the number of nucleotide pairs in the duplex region of GQRAs enhanced their stability and antithrombin activity. Stability curves reached saturation at 6 and clotting time curves at 5 nucleotide pairs (RE31 and RE29 aptamers respectively). Thus according to combined results of both tests RE31 aptamer has an optimal length of the duplex region. Nucleotide insertions in RE31 hinge region decreased its antithrombin activity. RE31 potentiate fibrinolysis in *in vitro* model by inhibiting activation of thrombin activated fibrinolysis inhibitor and prevents thrombosis in *in vivo* model.
Conclusions:
(1) Hinge and duplex regions increase stability and antithrombin activity of GQRAs.
(2) Minimal optimal structure was achieved in RE31 aptamer containing 6 nucleotide pairs in the duplex region.

PB0160 | Prophylaxis in Adult Population. Experience and Results in a 5-Year Follow-up Cohort

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Background: Prophylaxis is considered a standard of care in the population with severe or moderate haemophilia A and B with pattern of severe.
Aims: Describe the demographic and clinical characteristics of adult patients in factor prophylaxis at a Haemophilia Center in Colombia.
Methods: Descriptive cohorts over a 5-year period, in patients with haemophilia over 18 years of age in factor prophylaxis, patients with inhibitors and with prophylaxis less than 1 year were excluded. Qualitative variables were described by absolute and relative frequencies and quantitative variables by central trend and dispersion measures by distribution.
Results: In 2015 the population in prophylaxis was 8 patients, median age 22 years, 87% with haemophilia A and 13% haemophilia B. Median factor dose per weight was 23U / Kg. 87% of patients received recombinant products and 13% plasmatic.
By 2019 the population increased from 8 to 19 patients, 73% haemophilia A and 27% haemophilia B. Median age 24 years. The median dose of factor by weight 26 U/ Kg, 3 times per week for hemophilia A and for haemophilia B 31 U/Kg, 2 times per week. 26% of patients received plasma derived factor and 74% standard Half-Life recombinant products. 84% of the patients are in the program of self-infusion. The median number of compromised joints was 3. Median Gilbert scale 10 in 13 patients. Median scale of FISH 32. Half of the patients had zero bleeding. Most of the bleeds were handled on an outpatient basis.
Conclusions: Severe haemophilia can lead to important bleeds that limit the quality of life and joint health, prophylaxis is considered in Colombia a standard of treatment. There is evidence of an increase in the population in the follow-up years, corresponding to patients passing from paediatric to adult age.

PB0161 | Operational Feasibility of a Pharmacist-Guided Personalized Medicine Consult Service for Warfarin in Mandatory and Optional Testing Settings

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Background: The pharmacist-guided personalized medicine consult service at the University of Illinois Hospital and Health Sciences System (UI-Health) provides warfarin-dosing recommendations to providers and patients newly initiated on warfarin therapy. Starting in August 2012, *CYP2C19, VKORC1, and CYP4F2* genotyping was made mandatory to guide warfarin-dosing recommendations and subsequently became optional starting in April 2014.
Aims: To evaluate the operational feasibility of a pharmacist-guided personalized medicine consult service at an urban academic health center for improving warfarin dosing following transition from mandatory (mPGx) to optional (oPGx) genotyping.
Methods: This was a retrospective observational cohort study consisting of patients newly initiated on warfarin therapy at UI-Health during the mPGx phase (cohort 1) and oPGx phase (cohort 2). Service utilization and feasibility of oPGx compared to mPGx were summarized using quantitative outcomes including frequency of genotype order, acceptance of recommended dose, genotype result turnaround time, and time to pharmacist dose recommendation documentation in the medical record. Multiple bivariate analyses including student t-test, chi-square test, and non-parametric approaches were conducted.
Results: A total of 577 and 528 patients started warfarin during the mPGx and oPGx periods, respectively. Genotyping was ordered for 52.08% of patients during the oPGx phase. The acceptance rate of pharmacist-recommended doses by medical staff improved during the oPGx phase compared to mPGx (from 83.81 to 86.57%, $P< 0.01$). Significant decreases were observed in median genotype result turnaround time (25.82 to 24.27 hours, $p< 0.01$) and time to pharmacist dose recommendation documentation during the oPGx phase (5.46 to 2.38 hours, $p< 0.01$). Over 95% of dose recommendations were available within 24 hours in both phases (96.41% mPGx versus 98.61% oPGx, $p< 0.05$).
Conclusions: Warfarin dosing recommendations provided by a pharmacist-guided personalized medicine consult service were well accepted by medical staff despite transitioning from mandatory to optional genotyping. Additionally, operational feasibility of the service improved.

PB0162 | Acquired Haemophilia Spanish Registry, First Five Years of Activity

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Background: Acquired hemophilia (HA) is a rare bleeding disorder caused by autoantibodies against coagulation factors, especially factor VIII (FVIII). These inhibitors may induce severe bleeding episodes. It is necessary to establish hemostatic treatment and to eliminate the inhibitor using immunosuppressent drugs.

Aims: To describe the clinical characteristics and response to the treatment of the case series of HA of the Spanish registry to define new lines of knowledge on this topic.

Methods: In 2014 the Spanish registry of acquired hemophilia was activated. It is located on the SETH (Spanish Society of Thrombosis and Haemostasis) website. This registry has been implemented according to all national requirements and the Helsinki Declaration.

Results: 110 patients are registered (estimated incidence 200 cases), 53.6% of the subjects are male. Median age 74 years old (IQR, 63–84 years). Mean inhibitor titer is 17.6UB (IQR, 6.5–44.6UB). 55.5% of patients have an underlying disease (40% autoimmune, 17% neoplasms, 13% postpartum). 98% of patients present bleeding at diagnosis: 43% mucocutaneous. 3 or 4 WHO grade bleedings are described in 16% of patients. Regarding to inhibitor eradication, 38% were treated with steroids and cyclophosphamide, 32% steroids, 22.7% rituximab associated with immunosuppressants and 7.3% steroids and cyclosporine.

Median follow-up of 9.5 months (IQR, 2–31 months), median time for inhibitor eradication is 1.5 months (IQR, 1–3 months). We do not find differences in time to eradication between treated or not with rituximab. 86% got response with the first line of treatment. Four patients relapsed, all of them within 2 months after treatment discontinuation. Mortality has been 17.5%: 47% due to infection/sepsis, 26% underlying disease, 11% hemorrhage and 11% angor.

Conclusions: Acquired hemophilia is underreported. The first cause of death is infectious, so initiatives to optimaze immunosuppressive treatment or infectious prophylaxis are necessary. We did not find differences in response rates between different eradication schemes.

PB0163 | Impact of DOACs on Hemostasis Diagnostic Tests. Practical Recommendations and Guidance for the Laboratory

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Background: The knowledge of the impact of direct oral anticoagulants (DOACs) on hemostasis diagnostic tests is a clinical need. The regulatory documents provide some information but are generally limited to the most common hemostasis assays and guidance are not available to explain how to deal with all these interferences.

Aims: To provide an in-depth overview on the impact of DOACs on a series of hemostasis diagnostic tests. The final goal is to provide

Laboratory testing of factor Xa and IIa inhibitors and their impacts on diagnostic assays. Red and blue lines represent plasma concentrations at peak and through in NVAF and VTE, respectively. Green boxes represent ranges of concentrations that can impact diagnostic assays and lead to misdiagnosis while grey boxes represent unaffected assays. For clotting factors measurement, boxes represent the dynamic range of quantitation (the dynamic range of quantitation is defined as range covering from the lowest observed limit of quantification to the maximal concentration tested or supposed) of PT and aPTT for sensitive reagents. $C_{through}$: Minimum plasma concentration during the dosing interval; C_{max}: maximum plasma concentration during the dosing interval; VTE: Venous thromboembolism; NVAF: Non-valvular atrial fibrillation; OD: once daily; bid: twice daily; APCR: Activated Protein C Resistance. These DOAC concentrations may not be reflective of all reagents or methods. Notes: Data on plasma concentrations were extracted from Douxfils et al. Gosselin et al. for apixaban, edoxaban, rivaroxaban, from Siriez et al. and SmPC for betrixaban and from Douxfils et al., Favaloro et al. Gessoni et al. Bonar et al. and Gosselin et al for dabigatran.

FIGURE 1 Impact of factor Xa inhibitors related to indications on diagnostic assays

Tests		Apixaban	Betrixaban	Dabigatran	Edoxaban	Rivaroxaban	Comments
Prothrombin time (sec)		+	+	+	+	+	All clotting assays are impacted by DOAC with an inter-molecule and inter-reagent variability. Interpretation of such tests should be done with caution in presence of DOACs. Nevertheless, some reagents (e.g. Dade® Innovin® until 100ng/mL) are less sensitive to DOACs and could be proposed in presence of DOACs.
Activated partial thromboplastin time (sec)		+	+	+	+	+	
Fibrinogen	Clauss method	=	-	=	=	=	Clauss-method seems less impacted by DOAC unlike PT-derived measurement. Some reagents such as Fibrinogen-C® and Multifibren U® should be avoided in presence of dabigatran.
	PT-derived	=	-	-	-	-	
Coagulation factor activity assays		-	-	-	-	-	PT and aPTT reference curves being impacted by DOAC, coagulation factors' levels are underestimate. The use of unsensitive reagent with adequate dilution of plasma sample is recommended.
Antithrombin activity	Antigenic assays	=	=	=	=	=	The presence of anti-IIa influence thrombin-based antithrombin assays while anti-Xa influence FXa-based antithrombin assays leading to an overestimation of antithrombin activity. Thrombin-based assays should be preferred for antithrombin activity measurement in presence of FXa inhibitors and conversely for FIIa inhibitor. Immunological assays for antithrombin are not impacted by DOACs and could be used for a quantitative assessment.
	Anti-Xa based	+	+	=	+	+	
	Anti-IIa based	=	=	+	=	=	
Protein C activity	Antigenic assays	=	=	=	=	=	Clot-based assays for evaluation of protein C activity should be avoided in presence of DOAC. Chromogenic assays are recommended. Immunological assays for protein C are not impacted by DOACs and could be used for a quantitative assessment.
	Clot-based test	+	+	+	+	+	
	Chromogenic test	=	=	=	=	=	
Protein S activity	Clot-based test	+	+	+	+	+	Clot-based assays for evaluation of protein S activity should be avoided in presence of DOAC. Immunological assays for protein S are not impacted by DOACs and could be used for a quantitative assessment.
	Protein S free Ag	=	=	=	=	=	
Lupus anticoagulant testing: LA-sensitive aPTT and DRVVT (screening, mixing, confirmatory)		-	+	+	+	+	Antiphospholipid syndrome cannot be evaluated in presence of DOAC. DRVV screen, DRVV confirm and LA-sensitive aPTT should be avoided in presence of DOAC. The use of Taipan snake venom time and ECT time could be an alternative. Furthermore, the use of DOAC removals could avoid the influence of FXa et FIIa inhibitors.
Activated protein C resistance		+	+	+	+	+	DOAC interfere with APTT-based assay for aPCR assessment. Pefakit aPCR could be an alternative except for edoxaban and dabigatran. DRVV-based aPCR may be affected by DOACs and should be avoided.

+: Overestimated tests, should be avoided or used with caution in presence of DOAC; -: Underestimated tests, should be avoided or used with caution in presence of DOAC; =: Unimpacted tests, could be used in presence of DOAC.

FIGURE 2 Summary of recommended assays for the measurement of direct oral anticoagulants in plasma

biologists and clinicians with practical recommendations for thrombophilia testing.

Methods: Results were obtained from the compilation of our series of investigation addressing the impact of DOACs (i.e. apixaban, betrixaban, dabigatran, edoxaban, rivaroxaban) on routine and thrombophilia testing. This was completed by a review of the literature.

Results: Overall, factors Xa and factors IIa inhibitors affect clot-based hemostasis diagnostic tests resulting in false-positive or false-negative results, even at low concentrations obtained with a 24-hours wash-out (►*Figure 1 & 2*).

An effect on thrombin-based hemostasis diagnostic tests is observed with dabigatran but not with anti-Xa and vice-versa. No impact was observed with antigenic or chromogenic methods for the assessment of protein S and C activity.

Conclusions: Current routine coagulation tests as well as hemostasis diagnostic tests are in most cases impaired by DOACs potentially leading to incorrect clinical decision making. Overall, chronometric and chromogenic tests requiring factor Xa and/or factor IIa are impacted by DOACs with an inter-reagent variability and concentration-dependently. Indeed, some reagents (e.g. Dade®Innovin® until 100ng/mL) are less sensitive to DOACs and could be proposed in their presence. The use of a devices or chemical compounds able to remove DOACs is still under-investigation but seems to be a cheaper promising solution, even for high concentration of DOACs. Antagonizing the anticoagulant could be interesting but with a higher cost. The development of new diagnostic tests insensitive to DOACs should also be considered.

PB0164 | Drug Interactions in Edoxaban Treated Patients: A Pilot Study Revealing the Changes of the Metabolite to Parent Ratio and its Potential Clinical Importance

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Background: Among the factor Xa inhibitors, edoxaban is the only compound for which some of the metabolites (edoxaban-M4, -M6 and -M8 metabolites) are reported to be pharmacologically actives. These metabolites could potentially interfere with chromogenic assays usually used for the estimation of edoxaban concentrations. Edoxaban has usually low concentrations of active metabolites, which do not impact the anticoagulant activity. However, in presence of drug interactions, the levels of edoxaban-M4 may contribute significantly to factor Xa inhibition.

Aims: To evaluate the impact of rifampicin and carbamazepine on edoxaban-M4/edoxaban ratio measurements in patients.

Methods: Measurements of edoxaban were performed with chromogenic assay (STA® Liquid Anti-Xa) and compared with a validated ultra-high-performance-liquid-chromatography coupled with a tandem mass spectrometry (UHPLC-MS/MS) method assessing concentrations of edoxaban and edoxaban-M4. Patients with drug-drug interactions (n=14) were compared with a group without interaction (n=79).

Results: Mann-Whitney test reports significative difference of edoxaban-M4/edoxaban ratio (p=0.0152) between patients' group with drugs interactions and without interaction (►*Figure 1*).

This difference is more important at $C_{through}$ (p=0.0017) and not significant at C_{max}. Wilcoxon test showed a significant difference from the theoretical edoxaban-M4/edoxaban ratio of 10% at $C_{through}$ (p=0.0156) but not at C_{max}. However, one patient with renal insufficiency accumulates edoxaban-M4 to higher levels. Finally, Spearman correlations showed significant difference for correlation between chromogenic assay and UHPLC-MS/MS edoxaban measurements for interactions group (r=0.87) than for group without interaction (r=0.98). Bland-Altman and Spearman correlations for the interaction group is improved by taking into account edoxaban-M4 (r=0.96) (►*Figure 2*).

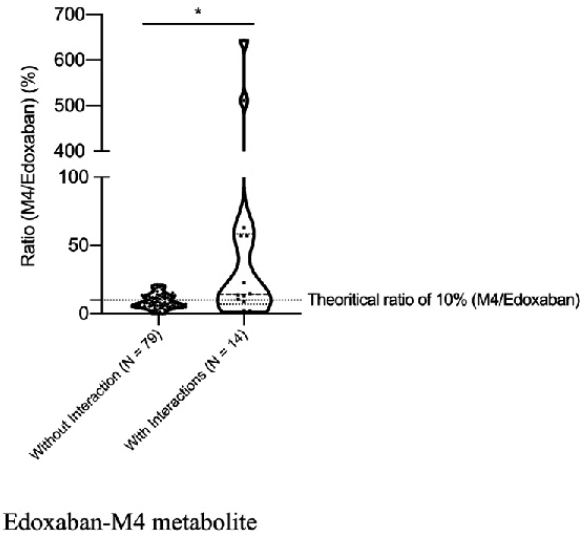

FIGURE 1 Density distribution of edoxaban-M4/edoxaban ratios for patients with and without interaction

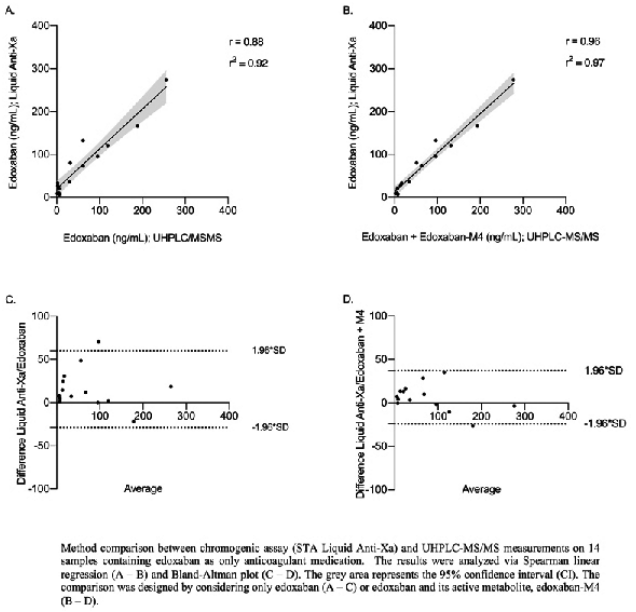

FIGURE 2 Method comparison between chromogenic anti-Xa assay and UHPLC-MS/MS measurements

Induction of other metabolites, in particular edoxaban-M6, may explain this discrepancy.

Conclusions: Drug-drug interactions may increase edoxaban-M4/edoxaban ratio leading to a higher risk of inadequate control of coagulation. These results support the need to measure metabolites concomitantly to edoxaban. Further investigations are needed to assess the pharmacokinetic impact of interaction on a larger population.

PB0165 | Comparison of a Validated UHPLC-MS/MS Method and Chromogenic Anti-Xa Assays from Different Manufacturer for the Assessment of Edoxaban and its M4 Metabolite Levels in Plasma

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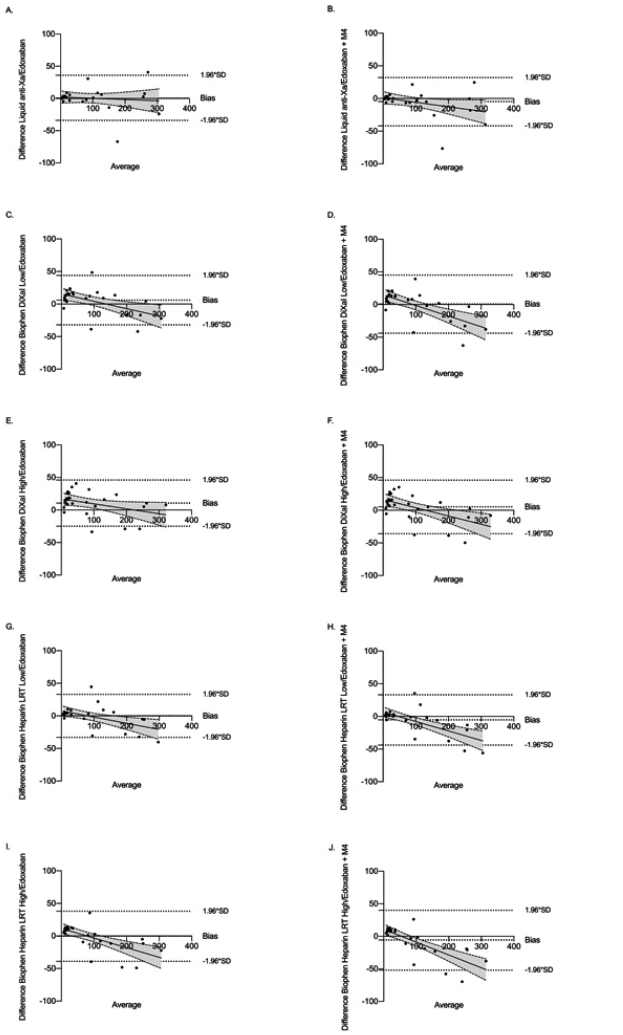
Background: Currently, chromogenic anti-Xa assays are considered as the most appropriate assays to estimate the concentration of factor Xa inhibitors. Among them, edoxaban is the only compound for which some of the metabolites are reported to be pharmacologically actives. Although generally found at low concentrations, these metabolites could potentially interfere with chromogenic assays.

Aims: To assess the performance of several commercial chromogenic anti-Xa assays with a validated ultra-high-performance-liquid chromatography coupled with a tandem mass spectrometry (UHPLC-MS/MS) method measuring edoxaban and its major active metabolite (edoxaban-M4).

Methods: Measurements of edoxaban were performed on patient samples (n=27) with three chromogenic assays (STA®Liquid Anti-Xa, Biophen Heparin LRT® (High and Low) and Biophen DiXaI® (High and Low)) and compared with a validated UHPLC-MS/MS method assessing concentrations of edoxaban and edoxaban-M4. Spearman's rank correlation coefficient and Bland-Altman plot were used to compare methodologies.

Results: Spearman correlations showed high correlations between chromogenic assays and UHPLC-MS/MS edoxaban measurements (r ranging from 0.94 (Biophen DiXaI®Low) to 0.98 (STA®Liquid Anti-Xa and Biophen Heparin LRT®). Correlations are improved by adding edoxaban-M4 measures to edoxaban. The participation of M6- and M8-metabolite may partly explain this discrepancy. Bland-Altman plots show that chromogenic assays calibrated with edoxaban underestimate edoxaban concentrations when compared with UHPLC-MS/MS measurement. Linear regressions show a tendency to underestimate edoxaban concentrations, especially at higher concentrations (►*Figure 1*).

Conclusions: Calibrations involving concomitantly edoxaban and active metabolites should be preferred. This will to avoid, especially



Difference plot between chromogenic assays: STA®Liquid Anti-Xa (A – B), Biophen DiXal® Low (C – D), Biophen DiXal® High (E – F), Biophen Heparin LRT® Low (G – H), Biophen Heparin LRT® High (I – J) and UHPLC-MS/MS measurements on 27 samples containing edoxaban as only anticoagulant medication. The analysis was designed by considering only edoxaban (A – C – E – G – I) or edoxaban and its active metabolite, edoxaban-M4 (B – D – F – H – J). The grey area represents the 95% confidence interval (CI).

FIGURE 1 Bland-Altman plot for chromogenic assays and UHPLC-MS/MS: Involvement of edoxaban-M4 metabolite

in case of drug-drug interactions (e.g. P450 cytochrome or transporters inducers), a risk of inadequate control of coagulation, due to different contribution of the metabolites to the total anti-Xa activity which serves for the estimation of edoxaban concentration. Further investigations are needed to evaluate the impact of edoxaban and its active metabolite, edoxaban-M4, on chromogenic assays on a larger sampling.

PB0166 | Anticoagulation with the Novel, Small-molecule Factor XIa (fXIa) Antagonist, EP-7041, Prevents Oxygenator Clotting but Conserves Hemostasis in a Canine Extracorporeal Circulation (ECMO) Model

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Background: Bleeding and thrombosis are the most common complications of veno-venous (V-V) ECMO support for patients with respiratory failure. ECMO requires anticoagulation to prevent clotting in the circuit, but heparin and other thrombin inhibitors significantly increase bleeding risk at therapeutic concentrations. Recent evidence suggests fXIa inhibition might prevent thrombosis with less bleeding risk.

Aims: We aimed to show that intravenous administration of EP-7041 prevents clotting in the ECMO membrane oxygenator while causing less bleeding than heparin.

Methods: The hound model of V-V ECMO employs standard tubing, pumps and oxygenator components (Medtronic). Animals were anesthetized and 14F cannulae were placed via cutdown in the jugular and femoral veins for venous drainage and return. Heparin and EP-7041 were dosed to target aPTT of 2× baseline (BL); diluent was administered to match active agent fluid volume as a negative control. Study drug boluses and infusions were started 30 min prior to initiation of ECMO to allow anticoagulation to reach steady-state. Low-flow (20 mL/kg/min) ECMO circulation was maintained for 2.5 hours or until pressure proximal to the oxygenator reached 350 mmHg due to clotting. Bleeding rates were determined by placing preweighed sponges on incision sites for 15 minutes at 30-minute intervals.

Results: Pressure increased in all groups during ECMO, but only the diluent control reached the system limit of 350 mmHg, requiring termination within 1 hour. Anticoagulation with heparin and EP-7041 maintained aPTT in the range of 1.5×–2.5× BL and prevented

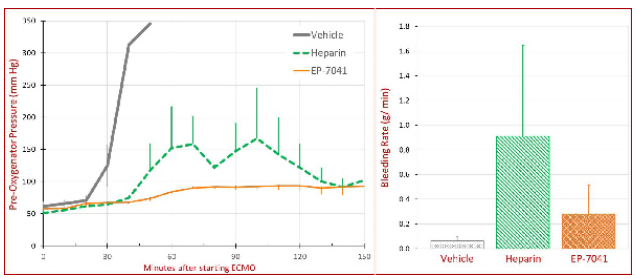

FIGURE 1 Pressure Proximal to Membrane Oxygenator During ECMO; **FIGURE 2** Bleeding Rate During ECMO

the circuit from clotting during the 2.5-hour ECMO run (Figure 1). Bleeding rates (Figure 2) and total measured blood loss during ECMO were >3× greater with heparin treatment vs. EP-7041-treatment. Data will be updated prior to presentation.

Conclusions: Compared with heparin, anticoagulation with EP-7041, a short-acting, potent and selective, small-molecule Factor XIa antagonist, prevents oxygenator clotting but maintains hemostasis in a canine ECMO model.

PB0167 | Rivaroxaban Reduces Arterial Thrombosis by Inhibition of FXa Driven Platelet Activation via Protease Activated Receptor-1

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Background: A reduced rate of myocardial infarction has been reported in patients with atrial fibrillation (AF) treated with factor Xa (FXa) inhibitors including rivaroxaban (RIVA) compared to vitamin K antagonists. At the same time, low-dose RIVA has been shown to reduce mortality and atherothrombotic events in patients with coronary artery disease (CAD). Yet, the mechanisms underlying this reduction remain unknown.

Aims: In this study we hypothesized, that RIVA's antithrombotic potential is linked to a hitherto unknown RIVA effect that impacts on platelet reactivity and arterial thrombosis.

Methods: In this study, we identified FXa as potent, direct agonist of the protease activated receptor-1 (PAR-1), leading to platelet activation and thrombus formation which can be inhibited by RIVA. We found that RIVA reduced arterial thrombus stability in a mouse model of arterial thrombosis using intravital microscopy. For *in vitro* studies, atrial fibrillation (AF) patients on permanent RIVA treatment for stroke prevention, respective controls and patients with new onset AF prior to and after first intake of RIVA (time-series analysis) were recruited.

Results: Platelet aggregation responses as well as thrombus formation under arterial flow conditions on collagen and atherosclerotic plaque material were attenuated by RIVA. We show that RIVA's antiplatelet effect is plasma dependent but independent of thrombin and RIVA's anticoagulatory capacity.

Conclusions: Here we identified FXa as potent platelet agonist that acts through PAR1. Therefore, RIVA exerts an antiplatelet effect that together with its well-known potent anticoagulatory capacity might lead to reduced frequency of atherothrombotic events and improved outcome in patients.

PB0168 | Validation of a Non- Sensitive aPTT Reagent for the Presence of Anti-Phospholipid Abs (Lupus Anti-Coagulant)

S. Nakav[1]; Y. Sulayev[2]; T. Mendez[2]; A. Bragg[1]; A. Ben-Dor[2]; I. Pickholtz[1]; I. Kat[2]

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Background: The aPTT (activated partial thromboplastin time) test is designed to evaluate the activity of the intrinsic factors. Elongated aPTT may be due to heparin treatment, lack of one of the intrinsic factors, or presence of antibodies against phospholipids, such as Lupus Anti-Coagulant (LAC).

Until now, Instrumentation Laboratory (IL) had a single reagent available for aPTT on the ACL TOP system. This reagent, SynthASil, contains low levels of phospholipids, and is therefore sensitive to the presence of LAC. In cases of prolonged aPTT, we cannot distinguish between the presence of LAC or factor/factors deficiency. Recently, IL adapted an additional reagent for aPTT, SynthAFax, which is rich in phospholipids and therefore is not sensitive to LAC.

Aims: To perform a validation of the SynthAFax reagent, in comparison to SynthASil and Actin FS (Siemens).

Methods: Blood was drawn from 350 subjects in a 3.2% sodium citrate vacuum tube. An aPTT test was done using platelet-poor plasma within four hours, with SynthASil (sensitive to LAC), Actin FS (not sensitive to LAC), and SynthAFax (not sensitive to LAC) on the ACL TOP 750/500.

Results: The reference range of 120 healthy subjects (60 men, 60 women) was 21.2–29 sec. (Manufacturer′s reference range is 22.4–32.9 sec.). The repeatability was found to be CV< 1.3% for controls and samples.

aPTT results of subjects lacking LAC were in high correlation between all reagents (SynthAFax vs. Actin FS, R^2 = 0.886, SynthAFax vs. SynthASil, R^2 = 0.977). 43% (16 out of 37) of subjects who were LAC positive and had prolonged aPTT with SynthASil were corrected to the normal range by Actin FS or SynthAFax.

Conclusions: SynthAFax is highly correlated with the other reagents in subjects negative to LAC. SynthAFax corrects aPTT in some of the LAC positive patients with prolonged aPTT using SynthASil reagent.

PB0169 | A Novel Prediction Platform to Enhance the Design of Therapeutic SERPINs

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Background: Serine protease inhibitors (SERPINs) are a superfamily of inhibitors, which act as molecular mousetraps. SERPINs only interact with activated serine proteases. This enables physiological protease activity, but limits it to prevent pathology. Interestingly, the specificity of SERPINs can be altered by changing their reactive center loop

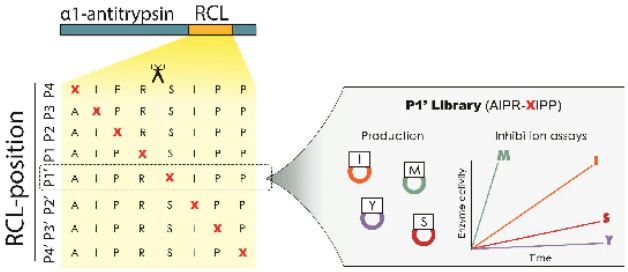

FIGURE 1 α1-antitrypsin reactive center loop libraries. Libraries were created based upon the α1AT-Pittsburgh reactive center loop (RCL)

(RCL), which has substantial therapeutic potential. Recently, an engineered α1-antitrypsin (α1AT) variant (^{357}KRK^{359}) that is specific for activated protein C (APC), showed great promise for the treatment of hemophilia B (Polderdijk et al, 2017). Currently, there is limited insight into how RCL variation affects SERPIN specificity, mostly because design is based on synthetic tripeptide substrate studies. This hampers effective redesign of therapeutic SERPINs.

Aims: To develop a SERPIN specificity prediction platform and to apply this in the design of novel therapeutic SERPINs.

Methods: Insight into RCL specificity was obtained by creating eight libraries for each of the key positions within the RCL (P4-P4′; Figure 1) based upon α1AT-Pittsburgh backbone (P1=arginine; M358R). Mutants were screened against 7 coagulation enzymes and marked for their inhibitory capacity.

Results: The inhibitory capacity from the eight libraries (160 variants) were combined into a linear prediction model. We subsequently tested its predictive power in separate test-cohorts (P3 and P2 mutations only) where it showed strong correlation against 4 out of 7 targeted enzymes. In comparison, no correlation was observed against the predictive value of peptide-substrate libraries. To validate our platform, we designed novel APC-specific inhibitors for therapeutic use in hemophilia. Compared to the published ^{357}KRK^{359} variant, our SERPINs showed a 3 to 5-fold increase in APC inhibition, while maintaining the required specificity. Furthermore, in the presence of thrombomodulin, our SERPINs restored the coagulation potential at 10 to 20-fold lower concentrations compared to the ^{357}KRK^{359} variant.

Conclusions: The library based approach offers unique insight into SERPIN RCL-specificity, allowing for the improved design of therapeutic SERPINs.

PB0170 | Natural Compounds as Anticoagulant Drug: A New Avenue for Improving Thrombosis Outcome

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Background: The limitations of present established allopathic formulations for antithrombotic therapies has resulted in surge of natural or herbal based medications to find out potential treatment and cure for the ailment. The on-going studies reveal that traditional natural compounds possess remarkable antithrombotic properties both in clinical and experimental phases. However, their crude extract from plants may act on multiple targets exerting pleiotropic and synergistic effect making the mechanism of action more complex

Aims: The aim of present study is to explore the potential of natural compounds as oral anticoagulants and revealing the under lying molecular targets and mechanisms.

Methods: Pertaining above hypothesis, a natural compound library was prepared. The library consisted of 158 distinct compounds after screening 90,000 compounds. Each compound was docked with identified molecular target i.e. FXa, which is a FDA approved molecular target for thrombosis. The blind docking study gave 11 natural compounds as probable leads that could inhibit FXa. These leads were further analysed by SWISS-ADME and FXa activity assay. The four compounds, pravastatin, wogonin, hyperoside and diosmetin qualified above assays. They were further investigated by FES and CD for the binding. Wogonin and pravastatin based on FES and CD profile were tested in IVC ligated murine model.

Results: Pravastatin, an already reported drug for atherosclerosis is found to inhibit FXa and decrease platelet activation. It also showed decrease in thrombus size in IVC (inferior vena cava) ligated animals injected with pravastatin as compared to IVC controls.

Conclusions: In our study we tried to explore natural compounds as new anticoagulants or antithrombotic drugs. Our study based on in silico and in vivo animal work reveals for the first that pravastatin is a promising advancement as pharmacological agents available for thrombosis

PB0171 | Factor Xa Stimulates PAR-Independent Pro-Inflammatory Responses in Primary Adult Rat Cardiac Fibroblasts

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Background: Atrial fibrillation (AF) is associated with a hypercoagulable state. Activation of protease-activated receptors (PAR) by thrombin mediates pro-fibrotic and pro-inflammatory responses in adult atrial fibroblasts which may support AF progression. Although factor (F) Xa is also known to activate PARs, its contribution to cardiac remodeling during AF remains unclear.

Aims: To elucidate the effect of thrombin and FXa on cardiac fibroblasts and clarify the role of PAR activation in cardiac remodeling during AF.

Methods: Primary adult rat cardiac fibroblasts were incubated with thrombin (0.01 U/mL) and FXa (100 nM). Gene expression of α-smooth muscle actin (α-SMA), Transforming growth factor-β1 (TGF-β1), Interleukin-6 (IL-6), Monocyte chemoattractant protein 1 (MCP-1) and PAR were determined after 4, and 24 hours. The expression of PAR-1 and PAR-2 was inhibited by siRNA.

Results: Thrombin and FXa increased cardiac fibroblast mRNA expression of α-SMA (24h: 3 and 2.5-fold respectively, p< 0.05; n=8) and TGF-β1 (4h: 2-fold, p< 0.05; n=8). PAR-1 silencing tended to inhibit this upregulation.

Thrombin and FXa induced a fast upregulation of IL-6 (4h: 2 and 6-fold respectively, 24h: 3-fold by FXa, p< 0.05; n=8) and MCP-1 (4h: 7 and > 10-fold respectively, 24: > 10-fold by FXa, p< 0.05; n=8).

PAR-1 silencing prevented the upregulation of MCP-1 induced by thrombin at 4h. However, neither PAR-1 nor PAR-2 knockdown reduced the upregulation of MCP-1 induced by FXa (n=8).

Conclusions: The upregulation of α-SMA and TGF-β1 triggered by thrombin and FXa in cardiac fibroblasts, suggests that these proteases may play a role in cardiac fibrosis during AF.

Both stimuli increased IL-6 and MCP-1 expression highlighting a pro-inflammatory stimulation in this cell type. Surprisingly, PAR-silencing downregulated the effect of thrombin but not the effect of FXa on MCP-1 expression, suggesting that FXa might induce pro-inflammatory responses through a PAR-independent pathway.

PB0172 | Evaluation of the CEPHEN™ Reagent for the Determination of Activated Partial Thromboplastin Time in Current Practice

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Background: The CEPHEN™ reagent is a liquid, ready to use reagent for the determination of Activated Partial Thromboplastin Time (APTT) on citrated human plasma. Data on its use in current practice are scarce.

Aims: To compare the CEPHEN™ with other APTT reagents.

Methods: Blood samples from 20 healthy volunteers were collected to generate reference-values. APTT was determined in plasma samples of 42 patients undergoing a preoperative hemostatic evaluation, 30 patients with known factors VIII, IX, XI, XII low levels, 39 patients receiving unfractionated heparin (UFH), and 21 patients already diagnosed with lupus anticoagulants (LA) using the CEPHEN™, STA®-CK Prest® and STA®-PTT Automate® reagents. All analyses were performed on STA-R Max or CS-5100 analyzers. Patient APTT /Normal plasma APTT (P/N) ratios of >1.2 were considered as abnormal values.

Results:: Significant correlations were observed between the CEPHEN™ and the STA®-CK Prest® APTT in patients undergoing a preoperative hemostatic evaluation (r=0.89, p< 0.0001, Figure 1 A) and in those with known factors VIII, IX, XI, XII low levels (r=0.91, p< 0.0001, Figure 1 B). All patients with known bleeding disorders had a P/N ratio >1.2. The inter-agreement rates between the results obtained with the CEPHEN™ and the STA®-CK Prest® reagents was 100% (weighted kappa=1;p< 0.0001). In patients receiving UFH, a significant correlation was observed between CEPHEN™ and STA®-PTT Automate® P/N ratios (r=0.93, p< 0.0001, Figure 1 C). The CEPHEN™ P/N ratio was >1.2 in only 8 out of 21 (38%) patients already diagnosed with LA.

Conclusions: CEPHEN™ reagent performances are very close to those of the STA®-CK Prest® reagent for the detection of bleeding disorders and to those of the STA®-PTT Automate® in patients receiving UFH, indicating that it may be an interesting alternative for the detection of bleeding disorders and the follow-up of patients receiving UFH. The CEPHEN™ reagent demonstrates a very poor sensitivity towards LA.

PB0173 | Natural Anticoagulant from Peony

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Background: Many plants have an effect on blood clotting and vascular-endothelial function. Previously, the presence of anticoagulant activity in extracts from the roots of Mlokosevich peony was proved

Aims: To show the effect of extracts from the roots of peony Lutea on vascular endothelial function, anticoagulant and fibrinolytic activity of the blood during their intranasal administration to animals.

Methods: The experiments were carried out on healthy animals-rats weighing 200–220 g in accordance with the ethical principles and documents recommended by the Helsinki Declaration. Study design: a 5% extract from peony roots was administered to rats intranasally daily for 5 days at a dose of 0.1 ml. 20 hours after the last injection, blood was taken from vena jugularis and analyzed. Used koagulological standard methods for determination of anticoagulant activity by APTT test and fibrinolytic activity (FA). Vascular-endothelial function of the organism was studied by its markers - the activity of the tissue activator of plasminogen (t-PA) and the level of final metabolites of nitric oxide (NO) - the total content of nitrates and nitrites in blood plasma.

Results: 20 hours after the fifth intranasal administration of the extract from the roots of lutea peony, hypocoagulation was observed in the blood plasma of rats: t-PA activity increased by 22%, anticoagulant activity-by 49%, and FA-by 37% compared to the baseline level (Control). At the same time, the blood levels of total metabolites of NO increased by 110% compared to the Control.

Conclusions: Anticoagulant extracts from the roots of lutea peony when administered repeatedly (within 5 days) to animals stimulate vascular-endothelial function of the organism, increasing markers

of endothelial function-t-PA and total metabolites of nitric oxide, as well as anticlotting properties of blood.

PB0174 | Identification of Novel Proteins Binding to Coagulation Factor XIII Free B-Subunit

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Background: Coagulation factor XIII (FXIII) is the main stabilizer of the fibrin clot and is therefore an important player not only in haemostasis but also in cardiovascular and thrombotic diseases. It circulates in plasma as a tetramer of two A- and two B-subunits. About half of FXIII-B subunits are wrapped around FXIII-A_2 at an average concentration of 21.6 µg/ml. Half of FXIII-B subunits (free FXIII-B) circulate in plasma without binding to FXIII-A. The function of this free FXIII-B is unknown. Recent studies show that some proteins could bind to the B-subunit. It indicates that the free FXIII-B-subunit may have unknown binding partners and functions.
Aims: To identify proteins that could bind to free FXIII-B-subunit, and further investigate the physiological functions of these interactions.
Methods: Monoclonal antibodies (IgG) specific for free FXIII-B (kind gift from L. Muszbek) were coupled to magnetic Dynabeads™ covalently conjugated with protein G. These magnetic beads were then incubated with human plasma to isolate free FXIII-B and its binding partners. The proteins that may bind to free FXIII-B are currently investigated using mass spectrometry.
Results: Free FXIII-B was successfully bound to the Dynabeads coated with monoclonal antibody specific for free FXIII-B. No non-specific binding of FXIII-B on Dynabeads without coating of antibody was observed. Preliminary data of SDS-PAGE revealed several proteins that bound to free FXIII-B subunit and pulled down with FXIII-B in the elution from the beads. Those proteins did not appear when control beads without antibody were used. The potential binding partners for free FXIII-B are currently analysed by mass spectrometry.
Conclusions: FXIII has been studied for many years, but little is known about the function of free FXIII-B. Our study investigates the proteins that could bind to free FXIII-B, and characterizes these interactions. This could contribute to understanding the role of free FXIII-B in vivo.

PB0175 | Higher Protection against Inhibitors with pdFVIII/VWF Complex Concentrate Compared to Complex Formed in the Circulation between Isolated FVIII and VWF of HA Patients: An ex vivo Study

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Background: The development of inhibitors to FVIII is currently the most common and challenging complication of haemophilia A (HA) treatment. Our previous *in vitro* studies suggested that protection against inhibitors was higher in plasma-derived (pd) FVIII/VWF complexes, than in concentrates of isolated FVIII pre-mixed in vitro with VWF.
Aims: To compare the protective role of VWF against inhibitors in a pdFVIII/VWF complex concentrate with respect to the complex formed subsequently in the plasma of the patient between patient's VWF and FVIII concentrates.
Methods: Bethesda Units (BU) of a commercial pool of plasma from HA patients with inhibitors (HAp-i; Technoclone) were determined by the regular method (i.e., using as a source of FVIII a normal pool of plasma), or using plasma obtained from severe HA patients without inhibitors (n=15, Hospital La Paz, Madrid, Spain), 15–30 min after administration of FVIII concentrates of different origin (pdFVIII/VWF; rFVIII and BDD-rFVIII from CHO cells; BDD-rhFVIII and BDD-rhFVIII-Fc from HEK cells). Bethesda-Nijmegen assay was performed using the chromogenic method for the determination of residual FVIII activity (Coamatic®, Chromogenix).
Results: HA plasma containing pdFVIII/VWF consistently showed less inhibitor reactivity (higher residual FVIII activity) compared to the complex formed in the circulation between isolated FVIII concentrate and patient's VWF, regardless of the type of FVIII product evaluated. Hence, the BU ratios of the HAp-i evaluated with the different sources of FVIII compared with that obtained with a normal plasma were: pdFVIII/VWF=0.9; rFVIII=1.3; BDD-rFVIII=1.6; BDD-rhFVIII=1.6; and BDD-rhFVIII-Fc=1.5.
Conclusions: For the first time, the immunologic fate of therapeutic FVIII after being infused to HA patients has been investigated *ex vivo*. Results suggest that the complex FVIII+VWF formed in the circulation is not equivalent to the natural complex FVIII/VWF. Therefore, isolated FVIII infused into HA patients with inhibitors would be less protected by VWF than the FVIII in pdFVIII/VWF complex concentrates.

PB0176 | Rare Coagulation Disorders in Children - Is it Like to Buy a Pig in a Poke or Nothing Special? Experience of Saint Petersburg, Russia

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Background: In 21st century hematological diagnostic tools are just amazing. But what should we do when we receive abnormal clotting tests in healthy child? There many guidelines about II,VII,X clotting factors deficiencies, but not so much about XII and V.
Aims: We want to find out the clinical significance of laboratory changes, management of pre-and post surgical treatment in children with a deficiency of XII and V clotting factors.
Methods: We have reviewed 22 child case histories with rare coagulation disorders. Study was carried out on the basis of the hematology department of Sant- Petersburg State Children Hospital in 2014–2019 years.
Results: All children had significant changes in basic coagulation tests, that were made because of planned surgery. They were sent to hematologist, who made more tests to clarify clotting deficiency. 19 children had mild and severe deficiency of XII factor (less 1–4%) and 3 children had lower level of V factor (21–35%). No history of bleeding was seen in any of the patients. Parents of that children were scared and wanted to have hemostatic agents. All children were successfully surgical treated, without any bleeding episodes and use of any hemostatic medication. In all the cases good venous access and fresh frozen plasma were prepared in case of bleeding. The trust to hematologist after the procedures was 100%.
Conclusions: Despite the knowledge that deficiency of XII factor is asymptomatic there are difficulties in pre and post operative management in many hospitals in Russia. Parents are very scared when receive abnormal basic clotting tests. Children with "light" deficiency of V clotting factor do not require any hemostatic agents, but for safety of the surgeon good venous access and preparation of fresh frozen plasma is made in our hospital. More information of rare coagulation disorders must be provide to other specialties like pediatricians and surgeons.

PB0177 | Transplacental Delivery of Maternal Factor VIII for Induction of Immune Tolerance to Therapeutic Factor VIII

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Background: The major complication of factor VIII (FVIII)-based therapy in hemophilia A patients is the development of inhibitory anti-FVIII antibodies. Using FVIII-deficient mice, we have shown that the transplacental delivery of the Fcγ1-fused A2 and C2 domains of FVIII during gestation induces immune tolerance to therapeutic FVIII in the progeny. Babies from A2-Fc/C2-Fc-treated mothers developed regulatory T cells and were partially protected, albeit not completely, from the development of FVIII inhibitors.
Aims: To determine whether the transplacental delivery of whole FVIII induces complete FVIII tolerance, we developed molecules able to capture circulating maternal FVIII and deliver it to the fetuses.
Methods: We cloned Fc-fused molecules that crosslink human FVIII and the neonatal Fc receptor (FcRn): two monovalent FabFcs (V_LC_k/V_HCH1CH2CH3-linker-CH2CH3) derived from the specific human anti-C2 BO2C11 and anti-A2 BOIIB2 IgG4k monoclonal antibodies, and a D'D3-Fc derived from human von Willebrand factor. The molecules produced in CHO cells were validated for binding to FVIII and FcRn by ELISA and surface plasmon resonance. Their capacity to cross the placenta and deliver B domain-deleted FVIII (BDD) to fetuses was tested in pregnant FVIII-KO mice.
Results: BO2C11-FabFc, BOIIB2-FabFc and D'D3-Fc bound to FVIII and to FcRn with affinities of 0.4, 0.3 and 5 nM, and 11.6, 5.22, and 0.69 nM, respectively. The molecules were transplacentally delivered. The delivery of BDD-FVIII remains to be confirmed.
Conclusions: We generated 3 recombinant proteins able to bind FVIII and FcRn, and demonstrated FcRn-dependent transcytosis. Confirmation of the transplacental delivery of exogenously administered BDD-FVIII to fetuses is pending. We are developing a model of transgenic mice expressing human FVIII. This model will be instrumental to bring the proof-of-concept that maternal FVIII can be delivered through the placenta to the offspring. The consequences on the induction of FVIII-specific immune tolerance will be investigated.

PB0178 | Endocytosis of Factor VIII by Human Dendritic Cells in the Presence of Anti-Factor VIII Antibodies from Patients with Hemophilia A

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Background: The development of immune responses to therapeutic factor VIII (fVIII) implies fVIII internalization by antigen presenting cells. The study of the endocytosis of fVIII complexed to anti-fVIII IgG by dendritic cells (DCs) *in vitro* has yielded contradictory results with either increased, decreased or unchanged uptake. The differences could be due to the murine/human origin of the IgG and DCs, or to the nature of the anti-fVIII IgG: subclass and epitope specificity.
Aims: To understand how the nature of fVIII-IgG immune complexes (fVIII-IC) might influence fVIII uptake by DCs.
Methods: Four human monoclonal inhibitory anti-A2, anti-A3, anti-C1 and anti-C2 IgG were produced as IgG1κ or IgG4κ. Binding and inhibition of fVIII was validated by ELISA and Bethesda assay.

Endocytosis of fVIII alone or complexed with single or pooled IgG was studied *in vitro* using human monocyte-derived DCs (MODCs) and blood DCs (bDCs).

Results: Binding to fVIII was not affected by the change in IgG subclass. The inhibitory activity was greater for IgG1κ than IgG4κ. Both MODCs and bDCs expressed surface FcgRIIA. With the exception of the anti-A2 IgG1κ, all single and pooled fVIII-IC reduced fVIII endocytosis by MODCs and bDCs, irrespective of the IgG subclass. The maturation profile of MODCs was not affected by the incubation of the cells with fVIII alone or fVIII-IC.

Conclusions: Changes in subclass did not drastically alter the recognition of fVIII by the monoclonal anti-fVIII IgG. Most anti-fVIII IgG blocked fVIII uptake by DCs, with the exception of the anti-A2 IgG1κ. Co-incubation of DCs with fVIII-IC did not impact their maturation status. Future work will determine whether fVIII-IC alter fVIII uptake under pro-inflammatory conditions, and the potential consequences on DC maturation and on the polarization of CD4+ T-cell responses. At term, our findings will foster the rational design of fVIII-IC with tolerogenic properties for the treatment of inhibitor-positive patients.

PB0179 | Antithrombotic Effects of a Novel Small Molecular Fxia Inhibitor BMS-986177/JNJ-70033093 in a Rabbit AV-Shunt Model of Thrombosis

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Background: FXIa inhibition is a promising drug target with the potential for an improved therapeutic index over the currently approved oral anticoagulants. BMS-986177/JNJ-70033093 (BMS-177/JNJ-3093) is a novel small molecular inhibitor of FXIa currently in Phase II clinical trials.

Aims: The current study characterized the antithrombotic efficacy of BMS-177/JNJ-3093 in a rabbit arterial-venous (AV)- shunt model of venous thrombosis and compared it to the FXa inhibitor apixaban and the direct thrombin inhibitor dabigatran.

Methods: The AV-shunt model of thrombosis was conducted in anesthetized rabbits where a shunt was placed between the femoral artery and femoral vein for 40 mins. A thread placed in the shunt served as the thrombogenic surface. Vehicle or drugs were administered as i.v. bolus plus infusion. Thrombus weights were served as the primary efficacy end-point. Blood was collected to measure activated partial thromboplastin time (aPTT), prothrombin time (PT), thrombin time (TT), and drug levels.

Results: BMS-177/JNJ-3093 significantly reduced thrombus weight in the rabbit AV-shunt model of thrombosis, with 34.3 ± 7.9, 51.6 ± 6.8 ($p< 0.01$, n=5) and 66.9 ± 4.8% ($p< 0.001$, n=6) inhibition vs. vehicle, respectively, at 0.52 + 0.06, 1.70 + 0.06 and 5.47 + 0.53 μM BMS-177/JNJ-3093 plasma levels, with 50% inhibition of thrombus weight (ED_{50}) at 1.60 ± 0.05 μM. Ex vivo clotting data supported a dose-dependent prolongation of aPTT (with 1.54-, 2.23- and 3.12-fold increases respectively from the baseline), but no changes in PT and TT. Dose-dependent inhibition in thrombus weight was also demonstrated for both apixaban and dabigatran, with ED_{50} at 0.11 ± 0.02 μM and 0.20 ± 0.02 μM, respectively.

Conclusions: These results demonstrate that BMS-177/JNJ-3093 is an effective antithrombotic agent for the prevention of venous thrombosis supporting the clinical testing in a Phase II total knee arthroplasty study.

PB0181 | Effects of Anticoagulants on Fibrin Clot Structure: A Comparison between Vitamin K Antagonists and FXa inhibitors

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Background: Anticoagulant therapy is known to reduce blood clot formation, nevertheless, there is limited data on how different anticoagulants influence fibrin clot structure. Effects on clot structure could be important to understand the risk of bleeding associated with different anticoagulants.

Aims: To investigate how fibrin clot structure is impacted by vitamin K antagonists and DOACs.

Methods: Clotting of normal-pooled plasma (NPP), NPP+rivaroxaban, NPP+apixaban, NPP+enoxaparin, and warfarinized plasma (INR 2.22 and 4.11) was initiated with tissue factor or thrombin. The kinetic profile of polymerizing clots was analyzed by turbidity. Fiber density was analyzed by confocal microscopy, fiber size by SEM, and clot pore size (Ks) by permeation.

Results: Following clotting with tissue factor, all anticoagulated clots showed significantly ($p\leq0.0001$) increased lag-time (2.3-, 1.8-, 2-, and 2.9-fold for rivaroxaban, apixaban, and warfarin INR 2.22 and 4.11, respectively) when compared to NPP, with the exception of enoxaparin. Rivaroxaban clots were also less dense (71±4, $p\leq0.0001$) and more permeable (Ks $3.7\times10^{-8} \pm 0.3\times10^{-8}cm^2$, $p\leq0.01$), with thicker fibres (108±10 nm, $p\leq0.01$) relative to NPP control (fibre count 89±7, Ks $1.8\times10^{-8} \pm 0.6\times10^{-8}cm^2$, fibre size 93±9 nm). In contrast, clotting with thrombin showed no effects of FXa-inhibitors, as expected, with only enoxaparin prolonging the lag-time (3-fold, $p\leq0.0001$). Warfarinized clots with thrombin were denser (INR 2.22 75 ± 2, $p\leq0.01$ and INR 4.11 77 ± 9, $p\leq0.05$), with thinner fibers (INR 2.22 83±3 nm, $p\leq0.05$ and INR 4.11 85±9, $p\leq0.05$), while enoxaparin clots made with thrombin were less dense (45±15, $p\leq0.01$) and more permeable (Ks $5.6\times10^{-8} \pm 0.9\times10^{-8}cm^2$, $p\leq0.01$), relative to NPP control (fibre count 64±6, fibre size 88±5 nm, pore size $3.5\times10^{-8} \pm 0.9\times10^{-8}cm^2$).

Conclusions: Our results show that anticoagulants alter fibrin clot structure following clotting with tissue factor. We propose that clot structure measurements may be used to test for anticoagulant efficacy and safety.

PB0182 | Anticoagulant Effects of Parenteral Sulodexide as Measured by Activated Clotting Time. Relevance to Dosing in Various Indications

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Background: Sulodexide is a mucosal tissue derived glycosaminoglycan of mammalian origin which is clinically used in oral (capsule) and parenteral (ampules) forms for various clinical indications. USP referenced potency of sulodexide is reported to be 95-105 U/mg by anti-Xa assay and 30-40 U/mg by anti-IIa assay.

Aims: The purpose of this study is to determine the whole blood anticoagulant effect of parenteral preparations of sulodexide and its neutralization by protamine sulfate.

Methods: Parenteral formulations (ampules) containing 600 LSU/2 ml were diluted with saline to make 10 and 100 LSU/ml working solutions. Protamine sulfate was obtained from the Loyola hospital pharmacy and diluted to 1.00-0.12 mg/ml. Anticoagulant effects of sulodexide over the concentration range of 1.0-0.25 LSU/ml were measured on the Hemachrom ACT instrument in freshly drawn normal human blood (n = 5-10). Protamine neutralization studies were carried out at a fixed sulodexide level (1.0 LSU/ml) and variable protamine sulfate levels (50-12.5 μg/ml).

Results: Sulodexide produced a concentration-dependent anticoagulant effect in normal human whole blood over a concentration range of 0.25-1.0 LSU/ml in the range of (238 ± 24 to 401 ± 30 seconds) in comparison to saline control (136 ± 12 seconds). Protamine sulfate supplementation at a concentration range of 50-12.5 μg/ml produced varying levels of neutralization of the anticoagulant effects of sulodexide (1 LSU/ml).

Conclusions: Parenteral (ampules) sulodexide can be used for anticoagulation in dose dependent fashion and protamine sulfate can be used as an antidote for this agent thus in the event of a shortage of unfractionated heparin.

PB0183 | Structures in the Presence and Absence of an Anti-Factor VIII C1 Domain Antibody Bound to a Bioengineered Factor VIII Construct Indicates Multiple C2 Domain Conformations

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Background: Hemophilia A is a congenital bleeding disorder that affects 1 in 5,000 males worldwide and is caused by dysfunction or absence of blood coagulation factor VIII (fVIII). The most common treatment for hemophilia A is repeated therapeutic infusions of recombinant and/or plasma-derived fVIII, which often results in the development of inhibitory antibodies.

Aims: Recent evidence indicates that the C1 domain contributes to the inhibitor response, potentially disrupting the ability of fVIII to bind PS and/or vWF, blocking the endocytosis of fVIII by antigen presenting cells, or serving as a weak inhibitor that rapidly accelerates fVIII clearance. Here, we aim to structurally characterize the epitopes of the anti-C1 domain immune response.

Methods: This study employs X-ray crystallography to determine the structures of a bioengineered construct of fVIII (termed ET3i) to 2.8 Å resolution alone and in complex with an anti-C1 domain pathogenic inhibitor (2A9) to 3.9 Å resolution.

Results: The 2A9 inhibitor overlaps with anti-C1 antibodies present in hemophilia A patients, and the structure shows that the epitope directly interacts with F2068 in the C1 domain, as well as makes specific contacts with the adjacent A3 domain. Further conformational changes are observed for the C2 domain within the ET3i/2A9 complex. Here, the C2 domain swings outward from the remaining ET3i structure by over 17 Å. In the newly refined model of ET3i alone, we observe another conformation of the fVIII C2 domain that is different from that observed for the ET3i/2A9 complex.

Conclusions: This study represents the first antibody complex with an intact construct of B domain-deleted fVIII and lends structural evidence to the anti-C1 domain pathogenic immune response. Additional data presented illustrates the highest resolution structure of fVIII, which may be critical for future bioengineering efforts that will yield better performing fVIII replacement therapeutics.

PB0184 | Pharmacopeial Potency Equated Sulodexide Produces Comparable Anticoagulation as Studied in Whole Blood

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Background: Sulodexide is a widely used glycosaminoglycan derived drug for oral (antithrombotic) and parenteral (anticoagulant) indications and is composed of fast moving heparin (80%) and dermatan sulfate (20%).

Aims: The purpose of this study is to compare the anticoagulant activities of sulodexide with heparin in simulated studies to mimic surgical and interventional anticoagulation.

Methods: Powder versions of sulodexide were obtained from Alfasigma (Bologna, Italy). Powder versions of porcine mucosal heparin were obtained from Medefil (Glendale Heights, IL, USA). Stock solution of both heparin and sulodexide were prepared in sterile saline at 10 mg/ml. Sterile solutions were made at 1.0 and 0.1 mg/ml. USP potency for both drugs were measured, cross referenced against US pharmacopiel standard and expressed as units per milligram. Whole blood activated clotting time was measured in healthy

human volunteers at graded concentrations of both drugs. Plasma based anticoagulant assays such as activated partial thromboplastin time (APTT) and thrombin time (TT) were measured. Protamine neutralization studies were also carried out in the different assays used.
Results: The USP potency of sulodexide range from 90-105 U/mg whereas the porcine heparin exhibited a potency range of 180-200 U/mg as measured by the anti-Xa activity. In the ACT assays USP potency adjusted sulodexide and heparin produced concentration dependent prolongation of this test which were comparable to heparin. Both the sulodexide and heparin produce concentration dependent anticoagulant effects in the APTT and TT assays. Protamine sulfate produced effective neutralization of the anticoagulant effects of both the sulodexide and heparin.
Conclusions: These results suggest that at USP potency equated levels sulodexide is capable of producing similar anticoagulant effects as unfractionated heparin. The USP potency of sulodexide can be adjusted at a comparable potency to porcine heparin to obtain comparable anticoagulant responses in the in vitro setting.

PB0185 | Factor XII Deficiency - A Very Rare Coagulation Disorder: Case Report

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Background: Factor XII is believed to play a role tissue repair and the formation of blood vessels Its definite role is still unclear, because many cases of FXII deficit experience thrombotic events, instead of a bleeding problem. Moreover most of them are asymptomatic.
Aims:
To show the role of Factor xII in the coagulation:
Methods: Case report.
Results: A 25 year primigravida presented with normal pregnancy and delivery at 39 weeks.A healthy baby was born by normal vaginal delivery. Seven days after mother came with swollen hot and sick leg. I have indicated Doppler ultrasonography of the lower limbs Dimer and checkup of coagulation. The results - deep vein thrombosis in the right lower leg, elevated DD values correlated with Doppler finding and recent delivery, but the surprise was the abnormal coagulation status - prolonged aPTT 45, 43,48 sec (three samples were taken, three different days, the plasma sample operated manual and automatic) Any past history of abnormal spontaneous bleeding episodes was not found, without prolonged and profuse bleeding even after childbirth 7 days ago. Mixing probe was - with complete correction of aPTT, suggesting factor deficiency. Deficit was suspected, but who and how much? And why in such a deficit, thrombosis is presenting,is there a correlation between the deficit and the thrombosis, and why there was no postpartum hemorrhage, as expected with coagulation deficiency? Our conclusion was confirmed in a reference center and a coagulation factor deficiency was determined. It was a factor XII deficit 27% activity.
Conclusions: The therapy was aimed at treating deep vein thrombosis while treatment for this disorder is usually not necessary since bleeding abnormalities only mild or most often nonexistent. Factor XII deficiency should be a risk factor for bleeding episodes as result of reduced coagulation factors, or thrombosis risk- in our case-as a consequence of reduced fibrinolysis activity.

PB0186 | Suppression of Factor VIII-Specific Memory B Cells by Murine and Human BAR-Tregs

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Background: Inhibitor formation is a major obstacle in the therapeutic treatment of hemophilia A (HA). Herein, we utilized a chimeric receptor-modified natural regulatory T cells (Tregs) approach to target FVIII-specific memory B cells, the cells responsible for persistent anti-FVIII neutralizing antibodies (inhibitors).
Aims: To develop and test a chimeric receptor-modified natural regulatory T cell (Treg) approach to target FVIII-specific memory B cells, the cells responsible for persistent anti-FVIII neutralizing antibodies (inhibitors).
Methods: $CD4^{+}CD25^{hi}CD304^{+}$ natural Tregs were obtained from naïve C57BL/6 mice and retrovirally transduced to express a chimeric B-cell antibody receptor (BAR) containing the immunodominant A2 domain of FVIII. Plasmablast-depleted ($CD138^{neg}$) splenocytes from FVIII/IFA immunized FVIII-knockout HA mice served as the source of FVIII-specific memory B cells, which were then stimulated *in vitro* with FVIII-specific and enumerated for B-cell ELISPOT after six days *in vitro*.
Results: A2-BAR Tregs (ratio 1:150 spleen cells), but not conventional T cells, to the $CD138^{-}$ splenocytes significantly suppressed the formation of anti-FVIII producing cells, compared to control OVA-BAR Tregs controls. Suppression was found to contact-dependent based on its failure in transwell experiments. $CD4^{+}CD25^{hi}CD127^{low}$ A2-BAR *human* natural Tregs completely suppressed polyclonal anti-FVIII APC formation, as well. Moreover, this suppression occurred in the presence of specific antibody to FVIII.

Conclusions: Thus, we have demonstrated *in vitro* that FVIII domain expressing BAR Tregs could be used to efficiently target and suppress FVIII-specific memory B cells.

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PB0187 | Preclinical Pharmacology of BAY 2433334, a Small Molecule Inhibitor of Coagulation Factor XIa

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Background: Coagulation factor XIa (FXIa) contributes to the development of thrombosis but seems to play only a minor role in hemostasis. In contrast to current anticoagulants, which are limited in their clinical efficacy by a dose-dependent increase in bleeding risk, inhibition of FXIa may therefore offer the potential to reduce the risk of thrombosis without increasing the risk of bleeding.

Aims: To evaluate the *in vitro* pharmacology of BAY 2433334 and to assess its *in vivo* antithrombotic properties and bleeding profile in rabbits.

Methods: The *in vitro* properties of BAY 2433334 were analyzed by using FXIa assays in buffer and human plasma, standard clotting assays (aPTT and PT) and thrombin generation assays in human and rabbit plasma. Antithrombotic efficacy of BAY 2433334 was assessed in rabbits in an arterial thrombosis model after ferrous chloride-injury of the carotid artery and in an arterio-venous shunt model after i.v. and p.o. administration. The impact of BAY 2433334 on hemostasis was evaluated in the same animals by measuring ear and gum bleeding.

Results: BAY 2433334 inhibited human FXIa activity with an IC_{50} of 1 nM in buffer and an IC_{50} of 0.14 μM in human plasma. It prolonged the aPTT 1.5-fold at 0.6 μM while having no significant effect on PT. Thrombin formation induced by low tissue factor concentrations was inhibited with an IC_{50} (peak) of 0.3 μM. BAY 2433334 reduced thrombus formation in the ferrous chloride model and in the AV-shunt model correlating with aPTT prolongation, and reduction of FXIa activity closely following plasma concentrations. Simultaneously determined ear and gum bleeding times were not prolonged.

Conclusions: BAY 2433334 is a potent, direct and reversible inhibitor of FXIa showing strong antithrombotic efficacy without increasing the bleeding risk. Its potential as anticoagulant with a broad therapeutic window is currently being evaluated in a phase 2 development program.

PB0188 | Role of Kunitz Domain 3 of Tissue Factor Pathway Inhibitor in Zebrafish

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Background: Tissue Factor Pathway Inhibitor (tfpi) is an anticoagulant that inhibits both VIIa and Xa in the blood coagulation pathways. There are three Kunitz domains, K1, K2, and K3 in tfpi. K1 and K2 mainly inhibit factor VIIa and Xa, respectively. The role of K3 *in vivo* is not studied.

Aims: To establish the role of K3 using the zebrafish model.

Methods: Cas9 DNA was made by a plasmid DNA preparation method. gDNA template was amplified by PCR with primers containing a T7 promoter. kPT, kPTT assays, qRT-PCRs, *in vitro* transcriptions, laser-induced thrombosis, and piggyback knockdowns were performed according to published methods. Student's t-test was used in statistical analysis.

Results: To address the role of K3, we transcribed both Cas9 DNA and gDNA template corresponding to K3, and this mixture was injected into single cell-stage zebrafish embryos. Genomic DNA from the tail clips of adult fish resulting from the injections was subjected to PCR across the K3 domain coding region. From these, a founder line with a deletion in the K3 region was identified. We then reared homozygous mutants and tested for kPT, kPTT, and laser-induced thrombosis using control siblings, and found that there were increased kPT and kPTT activities and decreased venous TTO but not arterial TTO. We performed piggyback knockdowns of tfpi, confirmed the knockdowns by qRT-PCRs, and found similar coagulation activities. In both tfpi and factor VIII knockdowns, factor VIII deficiency was rescued. tfpi RNA level of knockout fish was lower compared to the control. Since the results between knockout and knockdown fish are similar, we believe that K3 is not playing a role *in vivo*.

Conclusions: We generated a knockout tfpi zebrafish where the K3 domain is missing, a *tfpi* knockdown zebrafish, and assayed for clotting. The results suggest that the K3 domain does not have any *in vivo* function.

PB0189 | A Double Heterozygous Factor VII (FVII) Deficiency: Exon 9 Missense Mutation (Arg364Gln - FVII Padua) and a Novel Exon 3 Missense Mutation (Ala87Gly)

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Background: Hereditary Factor VII deficiency is a rare autosomal recessive bleeding disorder. FVII Padua is a variant form that was first described in 1978. It characteristically displays varying levels of FVII activity depending on the origin of the tissue factor used in the thromboplastin assay.

TABLE 1 Prothrombin results among different assays

	Rabbit Brain Thromboplastin (Neoplastine CI Plus, Stago Diagnostica)	Recombinant Human Tissue Factor Thromboplastin (Dade Innovin, Siemens)	Human Placental Thromboplastin (Thromborel S, Siemens)	FVII Antigen (VisuLize FVII antigen - Affinity Biologicals)
Patient's Prothrombin	27.9	14	18.4	
Reference range	11.7-14.0	9.9-11.8	10.4-12.6	
50% Correction	Patient: 14.9, Control: 13.7	Patient: 9.8, Control: 9.2	Patient: 13.5, Control: 12.3	
FVII Activity	4%	28%	28%	
FVII Antigen				87%

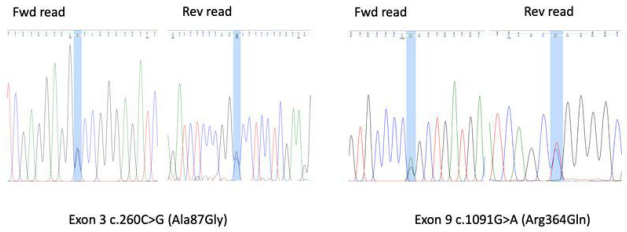

FIGURE 1 Double Heterozygous Mutation in FVII Deficiency

Aims: Here we report a case of a 60-year-old Chinese male presenting incidentally with a prolonged PT identified during a pre-operative consultation; he had no bleeding tendencies.

Methods: Initial evaluation using rabbit brain thromboplastin assay showed 4% FVII activity. Assays using recombinant human tissue factor and human placental thromboplastin showed FVII activity levels of 28% and 21% respectively. FVII antigen levels were at 87%.

Results: Genotype analysis revealed our patient to be a double heterozygote. The Arg364Gln mutation in exon 9 was observed, and this was the mutation originally described in the initial reports of FVII Padua. The other mutation seen was a novel missense mutation in exon 3 (c.260 C>G; Ala87Gly) which corresponds to the gamma-carboxyglutamic (Gla) domain of the protein.

Conclusions: While the clinical significance of the latter mutation is yet to be elucidated, previously reported variants in the Gla domain have been classified as pathogenic. Based on guidelines provided by the American College of Medical Genetics, this mutation can be classified as likely pathogenic. To our knowledge, this is the first report of such a mutation, whether alone or in combination, in patients with FVII deficiency. The use of genotype-phenotype association studies or *in silicon* analysis may be helpful in predicting the consequences of this mutation.

PB0190 | Congenital Factor X Deficiency with Isolated Prothrombin Time Prolongation

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Background: Congenital Factor X (FX) deficiency is a rare bleeding disorder with autosomal recessive inheritance. It may present with bleeding from umbilical cord, gastrointestinal system, mucosa, and central nervous system (CNS). As for the types of genetic mutation, missense and nonsense mutations caused by single-base substitutions were the most prevalent. FX deficiency results prolonged partial thromboplastin time (PTT) and prothrombin time (PT) with normal thrombin time (TT).

Aims: We herein present a girl with factor X deficiency and isolated PT prolongation.

Methods: FX activity and FX antigen levels were assessed. Genomic DNA was extracted from peripheral blood leukocytes of the proband for sequencing.

Results: A full-term infant with umblical bleeding and cephal hematoma was admitted to hospital on the 7th day of life. Her family had consanginous marriage. Coagulation tests showed prolonged PT (>170 s) and normal PTT. Her factor analyses showed that factor X level was 0.3%, Factor VII was 80%, and other factors levels were normal. She was treated with prothrombin complex concentrate (PCC) until discharge. On the 50th day of life, she was readmitted to the hospital with gastrointestinal bleeding. PCC was immediately given, then she had a right sided focal convulsion. Cranial tomography showed parenchymal hematoma (24x20 mm) in left frontal lobe and left subdural hematoma. Anticonvulsant therapy and daily PCC (20 U/kg) and FFP replacement every 12 hours were given for 15 days. Cranial imaging showed organization of the hematoma and PCC was given every 36 hours from 3rd week till discharge. Molecular analysis of the factor X gene revealed a homozygous mutation in Exon 2 (Gly51Arg), and her mother and father was heterozygous for this mutation.

Conclusions: This variant in Exon 2 (Gly51Arg) was reported before by Peyvandi et al. with prolonged PT and PTT which was associated with intracranial hemorrhage.

PB0191 | Molecular Analysis in 12 factor XI Deficiency Patients from China: Identification of Three Novel Splicing Mutations

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Background: Coagulation factor XI (FXI) deficiency is an autosomal bleeding disorder characterized by variable bleeding tendency. To date, many allele variants have been shown to cause this rare bleeding disorder.
Aims: The aim of this study is to characterizing the clinical manifestations and mutation spectrum of 12 unrelated patients with FXI deficiency in China.
Methods: Clinical data were collected and mutations were identified by next generation sequencing (NGS) and verified using Sanger sequencing. The potential interference of exon-intron boundary mutations with normal splicing efficiency was predicted by using the NetGene2 program and the BDGP splicing predictor program.
Results: Bleeding histories were mentioned in 7 patients (7/12, 58.3%) with easy bruising 3/12 (25%), post-traumatic hemorrhage 1/12 (8%), intra-articular bleeding 1/12 (8%), epistaxis 1/12 (8%), and menorrhagia 1/7 (14% of female subjects). Other bleeding phenotypes were not found in these patients. A total number of 11 mutations were identified including 3 novel splicing mutations (c.55G>A, c.1717-1G>A and c.1575_1576delAG), and in silico analysis revealed that these mutations might abolish the native splice sites.
Conclusions: NGS is an effective technique to detect the *F11* gene mutations, and our results further enrich the variation spectrum of the *F11* gene in Chinese population.

PB0192 | Point-of-Care and Storage-Stable, Single-Use 8-Channel Microfluidic Chip for Rapid Testing of DOACs and DOAC Reversal Agents under Whole Blood Flow

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Background: Point-of-Care and storage-stable, single-use 8-channel microfluidic chip for rapid testing of DOACs and DOAC reversal agents under whole blood flow.
Aims: Develop a rapid POC detection of DOAC function and DOAC reversal using whole blood.

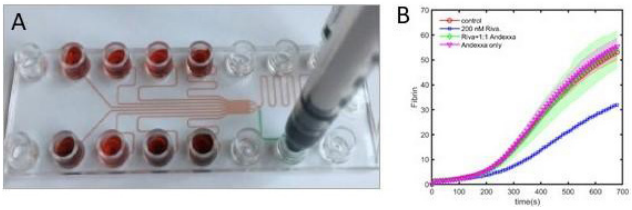

FIGURE 1 Point of Care chip for whole blood testing under flow (A). Detection of DOAC reversal (B)

Methods: We designed an injection-molded 1"x3" device with 8-parallel flow paths crossing over a bioprinted surface of collagen/tissue factor. The device is rapidly primed and compatible with manual or automated pipetting as well as operates under venous or arterial shear rates using constant flowrate or constant pressure-drop modes. Platelet and fibrin deposition were monitored dynamically by small benchtop chip reader.
Results: For whole blood clotting at wall shear rate of 200 s^{-1} for 500 sec, the intrachip CV for platelet and fibrin deposition was 20% (across 8 lanes) and the interchip CV was 25% for platelet and 30% for fibrin deposition (across 10 healthy male and female donors). No significant differences in platelet or fibrin deposition were detected for a blood sample tested on a freshly made chip or a chip stored dry for 6 months. Using the dynamic fibrin signal, dose-response testing of flowing whole blood resulted in an IC50 = 120 nM for the direct oral anticoagulant rivaroxaban (Factor Xa inhibitor). In an ex vivo test for DOAC reversal in whole blood (200 s^{-1}), a single chip test detected within 300 sec the potent reduction in fibrin deposition due to 200 nM rivaroxaban and the complete reversal of rivaroxaban by a 1:1 dose of Andexxa back to the original baseline fibrin deposition (no rivaroxaban).
Conclusions: We demonstrate the ability to manufacture single-use and storage-stable 8-channel chips that operate automatically for rapid detection of DOAC potency and DOAC reversal.

PB0193 | Transplacental Delivery of Fc-fused Factor VIII (rFVIIIFc) in FVIII-Deficient Mice

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Background: The development of neutralizing anti-factor VIII (FVIII) IgG is the major complication of replacement therapy in patients with hemophilia A. We have shown in the past that the administration to pregnant FVIII-deficient mice of Fc-fused A2 and C2 domains of FVIII allows the binding of the fusion proteins to the neoneatal Fc receptor (FcRn) expressed by the syncytiotrophoblast followed by transcytosis from the circulation of the mothers to that of the fetuses. The transcytosed A2Fc and C2Fc were captured by migratory

dendritic cells and reached lymphoid organs where they induced regulatory T cells. The transplacental delivery of A2Fc and C2Fc reduced the immunogenicity of therapeutic FVIII by 10-fold in the offspring. Presumably, the transplacental delivery of the entire FVIII molecule should confer complete immune tolerance.

Aims: To study the transplacental delivery of complete Fc-fused FVIII (rFVIIIFc) in FVIII-deficient mice.

Methods: FVIII-deficient mice at day 17 of pregnancy were treated with rFVIIIFc, rFVIII^{Y1680F}Fc or B domain-deleted FVIII. The amount of FVIII was measured in fetuses' plasma by ELISA.

Results: rFVIIIFc crosses the placenta in a Fc-, dose- and time-dependent manner. The amounts of transcytosed rFVIII are however 100-fold lower than that of a control monoclonal IgG. The use of rFVIII^{Y1680F}Fc, that does not bind endogenous von Willebrand factor, did not increase the amounts of transcytosed FVIII.

Conclusions: rFVIIIFc is transferred from mothers to fetuses albeit at lower amounts than IgG. We now investigate whether the amounts of transcytosed rFVIIIFc are sufficient to induce immune tolerance in the offspring.

PB0194 | Identification of Key Residues in Protein Z Dependent Protease Inhibitor (ZPI) for Efficient Protease Inhibiton

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Background: Protein Z dependent protease inhibitor (ZPI), a natural anticoagulant inhibiting the clotting factors Xa and XIa, is a member of SERPIN superfamily. Inhibitory serpins act through a unique mechanism in which the reactive center loop (RCL) upon cleavage by its target protease inserts into its own central A-β sheet. The covalently attached protease also gets translocated to the opposite end of the A sheet in the process and becomes inactive due to distortion of active site. The highly flexible native conformation of serpins can be readily disrupted by mutations leading to inefficient inhibition of the protease.

Aims: The present study is aimed to analyse the effect of mutation at two structurally important residues, Glu 333 and Ser 321, on the inhibitory activity of ZPI.

Methods: Six systems (WT ZPI, E333K, K280E/E333K, S321F, S321I, S321Y) were subjected to MD simulation using AMBER software.

Results: Our simulation data indicated that the E333K mutation causes the top of the β-sheet to remain in a slightly open conformation in comparison to the WT, due to the loss of the interaction between E333 and K280. However, the mutant also showed an increased stabilization due to interaction between R334 and D332. Thus mutation at 333 may not lead to reduced activity of ZPI, and therefore E333 may not be critical for its activity.

On the other hand, mutation of S321 by hydrophobic residues (S321F and S321I) showed increased flexibility of RCL as was expected due to loss of stabilizing effect of Ser. However, the stabilizing interaction was partly rescued in S321Y as it showed RMSF values comparable to WT. Thus S321 is predicted to be crucial for establishing stable ZPI-protease complex as was reported for antithrombin previously.

Conclusions: In conclusion, our data have identified S321, but not E333, as a critical residue for efficient anticoagulant activity of ZPI.

PB0195 | Protein S Deficiency, Inflammation and Sickle Cell Anemia

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Background: Sickle cell disease (SCD) is a chronic hereditary disease caused by a mutation in the hemoglobin gene. Apart from acute vaso-occlusive and anemic crisis, it is considered as a real prothrombotic state. One of the main risk factors of thrombosis is protein S deficiency, which is frequently observed during SCD.

In this context of chronic inflammation, the interpretation of its decline can be difficult.

Aims: We led a study to know the impact of this proinflammatory state in the PS deficiency observed during sickle cell anemia.

Methods: We included 50 homozygous and 50 heterozygous sickle cell patients. The mean age was 26 years and 20% of the SS had already had a thrombotic accident such as stroke and low limb thrombosis.

Results: The measurement of fibrinogen, as a marker of inflammation, showed hyperfibrinogenemia in 16% of the SS and 10% of the AS. The protein level was significantly lower in the SS than in the AS with 42% and 80% respectively. The prevalence of PS deficiency was thus 52% (SS) and 14% (AS). A weak correlation was found between hyperfibrinemia and protein S deficiency among sickle cell patients: R = 0.068. We can thus say that despite the chronic inflammation state in sickle cell patients linked to illness or other intercurrent disease, protein S deficiency is indeed a risk factor for thrombosis in sickle cell patients, especially homozygous.

Conclusions: In addition to other hematological factors incriminated in the occurrence of thrombotic accidents in sickle cell anemia (APL, PC deficiency) the deficit in PS must be taken into account in the management of sickle cell anemia.

PB0196 | First-in-Human Study to Assess the Safety, Pharmacokinetics and Pharmacodynamics of ONO-7684, an Oral Small Molecule Factor XIa Inhibitor in Healthy Subjects

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Background: Activated blood coagulation factor XI (FXIa) is one of the serine proteases involved in the amplification of thrombin

TABLE 1 Pharmacokinetic parameters following multiple dosing with ONO-7684 (Part B)

Parameter	Treatment					
	80 mg (N = 6)		150 mg (N = 6)		250 mg (N = 6)	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
Cmax (ng/mL)	553 (471, 649)	1041 (764, 1418)	1055 (832, 1338)	2004 (1382, 2906)	1771 (1296, 2422)	3704 (2674, 5131)
Tmax (h)	2.50 (1.00, 4.00)	4.01 (2.00, 12.0)	4.00 (2.00, 6.00)	4.01 (3.00, 8.00)	4.00 (2.00, 4.03)	4.00 (2.00, 12.0)
t1/2 (h)	-	27.5 (22.5, 33.5)	-	21.5 (12.4, 37.4)	-	21.9 (19.4, 24.8)
AUC24 (h*ng/mL)	8107 (7179, 9155)	18853 (13987, 25413)	16345 (12271, 21772)	36290 (23891, 55125)	27863 (21305, 36440)	67048 (45857, 98030)

Cmax, AUC24, t1/2 are presented as geometric mean (95% confidence interval) are presented; Tmax is described as median (range).

TABLE 2 Summary statistics for aPTT and FXI:C following multiple dosing with ONO-7684 (Part B)

Parameter	Treatment							
	Placebo (N = 6)		80 mg (N = 6)		150 mg (N = 6)		250 mg (N = 6)	
	Day 1 24 h	Day 14 24 h	Day 1 24 h	Day 14 24 h	Day 1 24 h	Day 14 24 h	Day 1 24 h	Day 14 24 h
aPTT [ratio to baseline]	1.015 ± 0.0393	1.047 ± 0.0418	1.532 ± 0.1193	1.869 ± 0.1629	1.781 ± 0.1736	2.063 ± 0.2609	2.056 ± 0.0906	2.526 ± 0.1459
Maximum on treatment [ratio to baseline]	1.087 ± 0.0264		2.070 ± 0.1521		2.345 ± 0.2753		2.782 ± 0.1470	
FXI:C [% inhibition]	−4.5 ± 6.80	2.1 ± 5.43	32.7 ± 3.54	61.0 ± 11.14	51.7 ± 11.16	73.0 ± 9.58	66.6 ± 6.49	86.1 ± 5.26
Maximum on treatment [% inhibition]	17.5 ± 3.41		74.7 ± 7.71		84.9 ± 5.16		92.3 ± 2.46	

Data are presented as mean ± SD; aPTT: activated partial thromboplastin time; FXI:C: factor XI clotting activity

production. Inhibition of FXIa reduces thrombin generation and prevents occlusive thrombosis. ONO-7684 is a FXIa inhibitor and novel oral anticoagulant with reduced bleeding risk in preclinical models.

Aims: This study was a first in human investigation into the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of ONO-7684.

Methods: ONO-7684 was assessed in a multi-part, double-blind, placebo-controlled study in healthy adult subjects (ClinicalTrials.gov: NCT03919890) approved by a medical ethics committee. After informed consent was obtained, ONO-7684 or placebo was administered in a 3:1 ratio; in part A subjects were healthy males (single ascending dose: 1-300 mg in the fasted state; food effect assessment at 80 mg), in part B subjects were healthy males (multiple ascending dose, 80, 150 and 250 mg once-daily for 14 days in the fed state). In total, 54 subjects were exposed to ONO-7684 over the 2 parts of the study. The PK, PD, safety and tolerability were assessed throughout the study.

Results: ONO-7684 was well tolerated; there were no signs of bleeding and all adverse events were mild or moderate. ONO-7684 demonstrated dose proportional PK with a mean half-life supportive of once-daily administration (Table 1). Exposure slightly decreased and Tmax was delayed following food. Steady state was reached within 7 days and the accumulation ratio was approximately 2-fold for Cmax and 2.3-fold for AUC. Exposure-dependent changes were observed in the PD parameters, activated partial thromboplastin time (aPTT) and factor XI clotting activity (FXI:C) (Table 2). At 250 mg in part B, the maximum mean aPTT (ratio-to-baseline) and FXI:C (% inhibition) were 2.78-fold and 92%, respectively, with minimal change in PT/INR.

Conclusions: ONO-7684 showed favourable tolerability, PK and PD suitable for further clinical development as a novel antithrombotic agent.

Sponsored by: Ono Pharmaceutical Co. Ltd., Japan

PB0197 | Library Screening and Combinatorial Approaches Identify an Alpha-1 Proteinase Inhibitor Variant with Enhanced Specificity for Factor XIa

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Background: Activated coagulation factor XI (fXIa) promotes clotting by activating factor IX. Targeting FXI(a) may yield improved antithrombotic agents, since FXI-deficient individuals do not bleed spontaneously, and high FXI expressers are prone to thromboembolism. The M358R variant of the serpin alpha-1 proteinase inhibitor (API) potently inhibits not only fXIa, but also other proteases.
Aims: To increase the specificity of API M358R for fXIa.
Methods: Two API M358R T7 phage display libraries separately randomized at reactive centre loop (RCL) positions P13-P8 and P7-P3 were biopanned with fXIa. A third sector (P2'-P3') was randomized and probed in a bacterial expression library. After iterative screening, the most active anti-fXIa candidates were expressed as recombinant proteins in *E. coli* and kinetically characterized.
Results: The most potent fXIa-inhibitory sequences were: P13-P8, HASTGQ (vs native EAAGAM); P7-P3, CLEVE (vs FLEAI); and P2'-P3', TE (vs IP). Second order rate constants (k_2, mean ± SD, n = 5, in $M^{-1}s^{-1}$) for fXIa were: API M358R, 1.69 ± 0.21 X 10^5; CLEVE, 1.53 ± 0.21 X 10^5; HASTGQ 3.38 ± 0.37 X 10^4; and (PRS)TE, 4.08 ± 0.65 X 10^4. Combining 3 (API-RA) or 2 motifs (HASTGQ and CLEVE [API-RB], CLEVE and (PRS)TE [API-RC], or HASTGQ and (PRS)TE [API-RD]) yielded relative fXIa reactivities of API-RC > API-RD > API-RA > API-RB. Rate constants for fXIa inhibition by API-RC or API M358R did not differ. Mean k_2 values for thrombin, fXIIa, fXa, activated protein C (APC), and kallikrein inhibition by API-RC were reduced relative to API M358R by 380-, 180-, 120-, 130-, and 30-fold, respectively.
Conclusions: Construct API-RC (P13-P3' EAAGAMCLEVEPRSTE) rapidly and selectively inhibits fXIa compared to thrombin, fXIIa, fXa, kallikrein, and APC, and is worthy of testing *in vivo*. API specificity can be focused on one target protease by selection in phage and bacterial systems coupled with combinatorial mutagenesis. Funding: Heart and Stroke Foundation of Canada.

PB0199 | The Antiplasmin Activity of the Kunitz Protease Inhibitor Domain of Protease Nexin 2 Affects Thrombolysis and Thrombosis in Mouse Models

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Background: Activated platelets secrete Protease Nexin 2, which contains a 57-amino acid Kunitz Protease Inhibitor (KPI) domain. We previously fused KPI to human serum albumin (KPIHSA). KPIHSA inhibits coagulation factor XIa (fXIa) 6-fold faster than plasmin. KPI residues 11-20 (TGPCRAMISR) contact the fXIa active site in co-crystals.
Aims: To randomize KPI at codon 17, select a variant with no antiplasmin activity, and assess it in murine thrombolysis and thrombosis models.
Methods: Bacterial colony lysates expressing KPI variants randomized at M17 were screened for anti-fXIa or anti-plasmin inhibition. Purified variant and wild-type KPIHSA expressed in *Pichia pastoris* were injected into mice (n = 6 to 8) treated with 10% (w/vol) topical $FeCl_3$ to the carotid artery with (thrombolysis) or without (thrombosis) 0.44 mg long-lasting tissue plasminogen activator (Tenecteplase, TNKase). The time to arterial occlusion (TTO) or reperfusion (TTR) was assessed by Doppler ultrasound.
Results: Lysates containing KPI M17D inhibited fXIa but not plasmin activity. KPIHSA and KPI-M17D-HSA inhibited fXIa indistinguishably (K_i 9 nM). TTR did not differ between mice treated with TNKase or TNKase with 0.94 mg KPI-M17D-HSA (31 ± 16 vs 43 ± 10 minutes, mean ± SD, $p > 0.05$ by non-parametric ANOVA) but was > 60 minutes in all mice treated with TNKase and 0.94 mg KPIHSA ($p < 0.001$ vs TNKase alone). TTO was unaffected by saline control or 0.235 mg KPIHSA (7.3 ± 0.9 vs 10 ± 4 minutes, $p > 0.05$) but was significantly prolonged by 0.235 mg KPI-M17D-HSA (21 ± 5 minutes, $p < 0.01$). Higher doses (0.47 or 0.94 mg) of either protein had indistinguishable effects.
Conclusions: KPIHSA but not KPI-M17D-HSA delayed thrombolysis *in vivo*. At sub-maximal doses, KPI-M17D-HSA delayed thrombotic arterial occlusion more than KPIHSA. The antiplasmin activity of KPI is relevant *in vivo*. Its elimination improves KPI as a potential antithrombotic agent. Funding: Heart and Stroke Foundation of Canada.

PB0200 | Nontoxic Polyphosphate Inhibitor that Reduces Thrombosis without Increased Bleeding

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Background: Polyphosphate (polyP) is a procoagulant polymer secreted by activated platelets. We previously described cationic polyP

inhibitors that were antithrombotic *in vivo*, but with some bleeding risk. We now report a new generation of polyP inhibitors that have both greater efficacy toward polyP and decreased bleeding side-effects. The most promising lead candidate is termed MPI-8.

Aims: Identify a polyP inhibitor that blocks the procoagulant/prothrombotic effects of polyP *in vitro* and *in vivo*, without antihemostatic effects that might cause bleeding.

Methods: Binding of MPI-8 to polyP was evaluated with surface plasmon resonance. *In vitro*, anti-polyP activity was measured using human platelet-poor plasma in clotting assays and calibrated automated thrombography (TG) assays. *In vivo*, bleeding was assessed using a murine tail-transection model, while antithrombotic activity was assessed following laser-induced injury to cremaster arterioles in mice.

Results: MPI-8 bound to long-chain or platelet-size polyP with K_d values of 112 and 37 nM respectively. When added to plasma at concentrations up to 100 μg/mL (10 μM), MPI-8 had no significant effect on tissue factor-induced clotting times or TG. On the other hand, in TG experiments triggered by long-chain polyP, MPI-8 blocked thrombin generation with an IC_{50} of 10 μg/mL. When injected into mice at 100 mg/kg, MPI-8 was without effect on tail bleeding times or amount of hemoglobin lost, while this same MPI-8 dosage significantly reduced accumulation of fibrin and platelets in laser-injured cremaster arterioles.

Conclusions: We have identified a new polyP inhibitor, MPI-8, that binds to polyP with high affinity and abrogates the procoagulant effects of polyP but not of tissue factor. The inhibitor protects mice against arterial thrombosis at a dose that does not increase bleeding. Future studies will focus on determining the minimal anti-thrombotic dose, and evaluating pharmacokinetic/pharmacodynamic properties of MPI-8.

PB0201 | A Comparison of Mass and Potency Equated Bovine and Porcine Heparins

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Background: Heparins are extracted from the mast cell rich mammalian tissues such as lung and intestine. Heparin is primarily derived from porcine mucosa (PMH), bovine mucosal heparins (BMH) are currently being considered by the FDA for re-introduction in medical and surgical procedures as an alternative to porcine mucosal heparins (PMH). We hypothesized that at USP potency equated levels, BMH and PMH should be comparable, however at gravimetric concentrations, mass adjusted PMH should exhibit stronger anticoagulant effects in comparison to BMH.

Aims: The purpose of this study is to compare the clotting profiles and biological properties of mass and USP potency equated BMH and PMH.

Methods: Powdered form of pharmaceutical grade BMH (140 U/mg), PMH (190 U/mg) and protamine sulfate were obtained from commercial vendors. Whole blood thromboelastographic studies were carried out in TEG 5000 Hemostasis system. Clotting (TT and aPTT) and anti-protease (anti-Xa and anti-IIa) assay were measured by using ACL instrument. Relative neutralization by protamine sulfate was carried out at a fixed concentration of 10 ug/ml.

Results: In whole blood TEG, clotting profile and anti-protease, USP potency equated BMH and PMH showed comparable effects. Protamine sulfate at 10 ug/ml completely neutralized BMH and PMH in a comparable fashion except anti-IIa assay. In mass ratio, PMH showed strong effects in all assays compare to BMH. BMH and PMH were completely neutralized by protamine sulfate in clotting assay, however in anti-protease assay BMH was completely neutralized while PMH was only partially neutralized at higher concentrations.

Conclusions: These studies suggest that mass porcine heparin exhibits stronger effects compared to bovine heparin. However, at USP potency adjusted levels, bovine and porcine heparins exhibit comparable effects sand can be considered biosimilar. These studies conclude that bovine heparin can be an alternative source of heparin to the prominent porcine heparin.

PB0202 | Optimisation of Assays for Laboratory Monitoring of Emicizumab in Treated Patients

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Background: Emicizumab comprises a bispecific monoclonal antibody mimicking FVIIIa by bridging FIXa and FX to form the tenase complex with the advantage of a half-life of approx. 30 days versus 12 hr for FVIII. Emicizumab's differing mode of action results in overestimation of activity using existing one stage FVIII assay (OSA) and chromogenic FVIII assay (CSA). CSA using bovine reagents is unaffected by emicizumab and is useful for estimation of baseline FVIII.

Aims: To employ commercial emicizumab calibrator within modified FVIII assays to monitor patients receiving emicizumab.

Methods: OSA was modified by increased pre-dilution of samples and calibration using commercially available emicizumab (r^2 diagnostics). CSA (Hyphen Biomed containing human FX reagent) was also calibrated using emicizumab. The assays were used in conjunction with unmodified CSA employing human and bovine (Siemens) FX reagent to test a range of commercial and patient plasmas. Figure 1 shows results for: Human-derived reference plasma (A); emicizumab reference plasma (B); and 50:50 mixture of the calibrators (C).

Results: CSA (human) showed cross-reactivity of approx. 50% with emicizumab, while no cross-reactivity was observed using CSA (bovine).

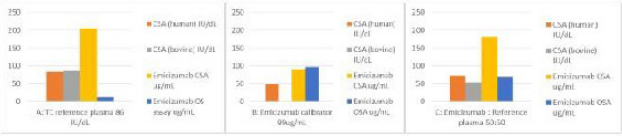

FIGURE 1 Measurement of FVIII / Emicizumab activity in A: Reference plasma (FVIII = 86 II/dL) B: Emicizumab calibrator (99 ug/mL) C: 50:50 R.P.: Emicizumab

CSA containing human FX calibrated with emicizumab showed extensive FVIII cross-reactivity rendering it unsuitable for use in patient samples containing endogenous FVIII.

CSA calibrated using emicizumab was most suitable for assessment of emicizumab in the presence of FVIII, however our results suggest an approx. 10% cross-reactivity.

Conclusions: We demonstrated the most efficient method of monitoring emicizumab therapy combines bovine based CSA to measure endogenous FVIII and modified dilute OSA calibrated using commercial emicizumab reagent. Modified OSA provided good emicizumab accuracy where circulating FVIII was very low or absent. Where FVIII is also present in plasma, cross-reactivity of approx. 10% was observed using the emicuzumab calibrated OSA. With minor modifications this method will be a useful adjunct for haemostasis laboratories managing patients on emicizumab in the emergency setting.

PB0203 | Comparison of Medication Adherence Between Different Oral Anticoagulants: A Propensity Weighted Nationwide Cohort Study

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Background: While warfarin treatment is continuously monitored by regular INR measurements, direct oral anticoagulant (DOAC) treatment is not controlled by any designated monitoring. This may lead to poorer adherence compared to warfarin. Previous studies have yielded conflicting results. Importantly, no study has adequately adjusted for the fact that warfarin dosing is continuously modified according to INR values, while DOACs are prescribed at fixed doses.

Aims: To compare adherence rates between different oral anticoagulants (OACs) in a nationwide cohort.

Methods: Data on all OAC naïve patients who received OAC prescription from 2014-2019 were collected from the Icelandic Medicine Registry and Landspitali University Hospital. We calculated

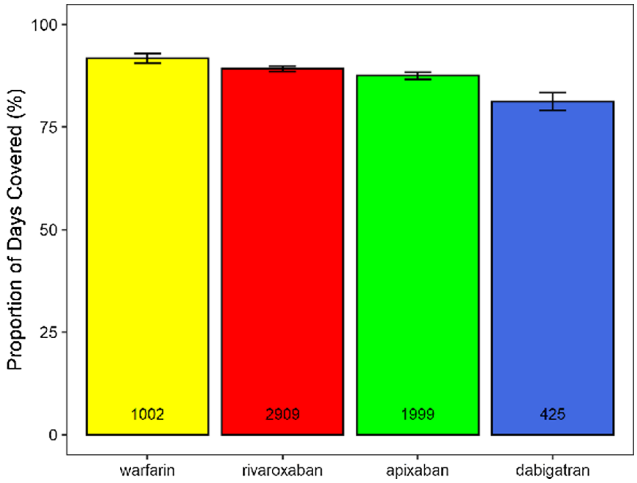

FIGURE 1 Comparison of adherence between different oral anticoagulants using proportion of days covered

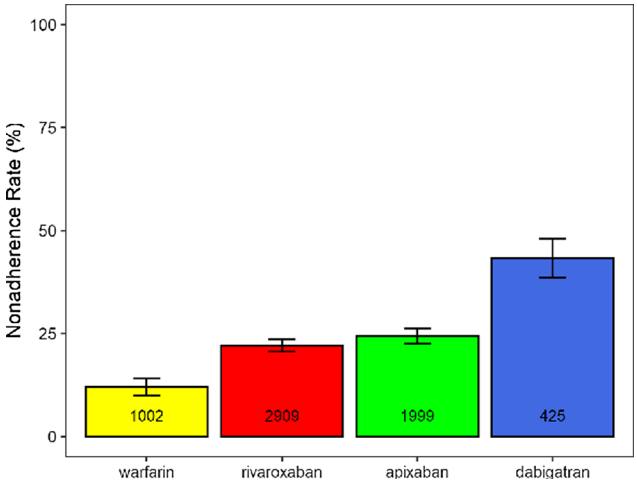

FIGURE 2 Comparison of nonadherence rates between different oral anticoagulants. Nonadherence was defined as proportion of days covered below 80%

the mean daily dose of warfarin for each patient using data from Landspitali Anticoagulation Management Center, which had information on dose adjustments for 72% of patients receiving warfarin in Iceland during the study period. Patients receiving warfarin with missing data on dose adjustments were excluded. Adherence was estimated by mean daily doses using proportion of days covered (PDC). Patients with PDC below 80% were considered nonadherent. Patients receiving warfarin, rivaroxaban, apixaban, and dabigatran were matched for age, gender, comorbidities, and area of residency using inverse-probability weighing.

Results: Our cohort included 1,002 patients receiving warfarin, 2,909 receiving rivaroxaban, 1,999 apixaban, and 425 dabigatran. Warfarin had the highest adherence rate (91.8%, 95% CI 90.6-92.9%), followed by rivaroxaban (89.2%, 88.6-89.9%), apixaban (87.5%, 86.7-88.4%), and dabigatran (81.2%, 79.1-83.4%) (Figure 1). Similarly, rates of nonadherence were lowest for warfarin (12.0%,

95% CI 10.0-14.0%), followed by rivaroxaban (22.1%, 20.6-23.6%), apixaban (24.4%, 22.5-26.3%), and dabigatran (43.3%, 38.6-48.0%) (Figure 2). Using logistic regression, the odds of nonadherence compared to warfarin was 2.1 (95% CI 1.7-2.6), 2.4 (1.9-3.0), and 5.6 (4.2-7.4) for rivaroxaban, apixaban, and dabigatran, respectively.

Conclusions: Warfarin was associated with higher adherence rates compared to DOACs. The highest non-adherence rates were observed with dabigatran, followed by apixaban and rivaroxaban.

PB0204 | The FibWave, a New Sensitive and Relevant Tool for Analysing the fibrin Clot Formation: Comparison with the Calibrated Automated Thrombogram to Assess Direct Oral Anticoagulants

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Background: Direct oral anticoagulants (DOACs) are known to impact coagulation assays. Although many assays are available for the assessment of anticoagulants, limitations have been highlighted like the lack of sensitivity of routine assays or the need of specific calibrators. The thrombin generation assay (TGA) has a great potential for the monitoring of direct oral anticoagulants (DOACs). The FibWave is based on the evaluation of the kinetic of the fibrin clot formation which circumvents the limitations of the TGA without altering its capacity to assess the coagulation potential at the individual level.

Aims:

The study aims:

i) to demonstrate the relevance of the FibWave for assessing the anticoagulant activity and potency of the different DOACs,

ii) to compare the impact of DOACs on the FibWave and the calibrated automated thrombogram (CAT) system and

iii) to investigate the sensitivity of the different FibWave and CAT parameters.

Methods: DOACs were spiked at final concentrations ranging from 0 to 500 ng/mL in normal pooled plasma. Fibrin formation and thrombin generation were measured by FibWave and CAT methodologies, respectively. The coagulation was initiated using either inductor of the intrinsic or extrinsic pathway. The FibWave and CAT parameters were correlated to individual DOAC concentrations.

Results: Parameters of the FibWave and the CAT were more affected when the coagulation process was triggered by the extrinsic pathway (Figure 1). The most influenced FibWave parameters were the FW-Max$_2$, the FW-Min$_2$ and the FW-Ttpeak for factor Xa (FXa) inhibitors and FW-Ttpeak and FW-Max$_2$ for dabigatran while the most influenced CAT parameters were the mVRI and the peak for FXa inhibitors and lag time for dabigatran.

Conclusions: Thanks to its ease of use and its capacity to assess to whole coagulation process, the FibWave could provide the clinicians with a global coagulation test, sensitive at relevant threshold concentrations with a reproducibility similar to the CAT.

FibWave										
Parameters	Apixaban (30ng/mL)		Betrixaban (30ng/mL)		Edoxaban (30ng/mL)		Rivaroxaban (30ng/mL)		Dabigatran (30ng/mL)	
	Intrinsic	Extrinsic	Intrinsic	Extrinsic	Intrinsic	Extrinsic	Intrinsic	Extrinsic	Intrinsic	Extrinsic
FW-Ttpeak	=	=	↑	↑	=	↑	=	↑	↑	↑
FW-Max$_1$	=	=	=	↓	=	↓	=	↓	=	=
FW-Max$_2$	=	↓	=	↓	=	↓	=	↓	=	=
FW-Min$_2$	=	↓	↓	↓	=	↓	=	↓	=	↑
CAT										
Parameters	Apixaban (30ng/mL)		Betrixaban (30ng/mL)		Edoxaban (30ng/mL)		Rivaroxaban (30ng/mL)		Dabigatran (30ng/mL)	
	Intrinsic	Extrinsic	Intrinsic	Extrinsic	Intrinsic	Extrinsic	Intrinsic	Extrinsic	Intrinsic	Extrinsic
Lag time	=	↑	↑	↑	=	↑	=	↑	↑	↑
Ttpeak	=	↑	=	↑	=	↑	=	↑	↑	=
Peak	↓	↓	↓	↓	↓	↓	↓	↓	=	=
ETP	=	=	=	=	=	=	=	=	=	=
mVRI	↓	↓	↓	↓	↓	↓	↓	↓	=	↑

↓: below 0.8 (ratio) in regard to NPP baseline; ↑: over 1.2 (ratio) in regard to NPP baseline; =: between 0.8 et 1.2 included (ratio).
FW-Ttpeak: time to peak (FibWave); FW-Max$_1$: maximum velocity of coagulation; FW-Max$_2$: maximum acceleration of coagulation; FW-Min$_2$: maximum deceleration of coagulation; lag time: time required to reach 10nM of thrombin; Ttpeak: time to peak; Peak: thrombin peak height; ETP: endogenous potential thrombin; mVRI: mean velocity rate index.

FIGURE 1 Summary of DOACs impact on FibWave and CAT parameters with both coagulation pathways

PB0205 | Hypodysfibrinogenemia with a New Heterozygous Mutation in Fibrinogen in a Patient with Thrombotic Complications

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Background: Fibrinogen is a plasma glycoprotein with 340 kDa essential for the fibrin clot formation and platelet aggregation. Hypodysfibrinogenemia is a qualitative and a quantitative deficiency in circulating fibrinogen, with a strong clinical heterogeneity. About half of the cases are clinically asymptomatic and others have a haemorrhagic and sometimes thrombotic tendency. The difference between the level of active fibrinogen and antigen makes it possible to establish the diagnosis of hypodysfibrinogenenia.

Aims: Demonstrate the importance of assaying certain coagulation factors known to be generally responsible for hemorrhagic and rarely thrombotic diseases and to identify the mutations responsible.

Methods: This is a 9-year-old patient, S.K, who was addressed to the haemostasis laboratory for exploration of a disturbed coagulation report. The examination of the patient and his family does not report any hemorrhagic or thrombotic symptomatology in the child, but there is a history of thrombosis in the father.

Results: The haemostasis assessment revealed in the propositus and some members of his family a deficiency in fibrinogen marked by a very large difference between the level of active fibrinogen and antigen which allowed to make the diagnosis of hypodysfibrinogenesis. A thrombosis assessment was performed in the father who thrombosed, and returned without abnormality. The genetic study identified a new mutation in exon 8 of the FGG fibrinogen gene never reported in the literature, found in the propositus and other members of his family with fibrinogen deficiency.

Conclusions: Hypodysfibrionogenemia is a very rare hemostasis abnormality with a clinical haemorrhagic and thrombotic heterogeneity and a significant difference between the level of active fibrinogen and antigen. This study allowed us to identify a new mutation never described in the literature.

PB0206 | Characterisation and Evaluation of a Candidate Bethesda Assay Reference Reagent Developed from Recombinant, Patient-Derived FVIII Inhibitors

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Background: Development of FVIII-neutralising antibodies ('inhibitors') occurs in around one third of severe haemophilia A (HA) patients receiving therapeutic factor VIII (FVIII). Inhibitors limit treatment options and negatively impact patient quality of life. Detecting and measuring inhibitors is notoriously error-prone, with high inter- and intra-laboratory variability. We have developed a candidate reference reagent using patient-derived FVIII-neutralising antibodies and tested this reagent against patient plasmas and a variety of commercially available FVIII activity assays.
Aims: To develop a Bethesda assay reference reagent from patient-derived monoclonal antibodies.
Methods: Recombinant immunoglobulin (IgG)4 was expressed in mammalian cells and purified using protein A. FVIII-neutralising activity was assessed using one-stage clotting assays (OSCAs) and chromogenic assays.
Results: We have generated a panel of neutralising and non-neutralising antibodies that bind the A2, A3, C1 and C2 domains of FVIII. The panel contains both type-1 and type-2 inhibitors, one of which is an immediate acting inhibitor. Due to differences in the biochemical characteristics of the antibodies (Bethesda titres, time-dependency, epitopes) we made type I antibody mixtures to have more confidence of capturing the variety in patient responses. One mixture included the fast-acting antibody ('4-mix') and one did not (3-mix). Both mixtures compared well with patient plasmas (Figure 1A). Antibody titre (potency, BU/ml) for 4-mix was more consistent across the different FVIII assay platforms compared to 3-mix (Figure 1B).
Conclusions: We have a candidate reference reagent that will be further developed as a reference reagent for the Bethesda assay. A mixture of type I antibodies that included an immediate-acting antibody is compatible with, and shows consistency in, a range of commercially available FVIII activity assay kits.

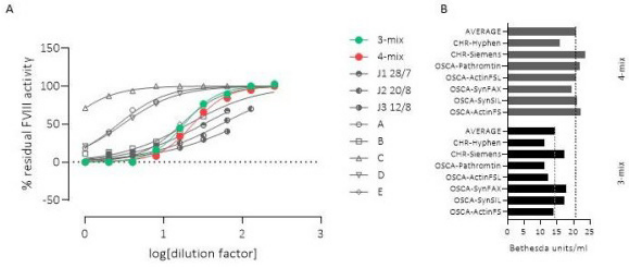

Figure 1. Recombinant anti-FVIII antibody mixtures were compared with patient inhibitor samples (A) and their Bethesda titres were calculated using a range of commercially available FVIII activity assays. (B)The 4-mix (grey) and 3-mix (black) antibody mixes were diluted into FVIII-deficient plasma over an 8-point dilution series (1/2) and incubated with buffered normal pooled plasma for 2 hours at 37°C. Samples were then analysed on a blood analyser according to the manufacturer's instructions.

FIGURE 1

PB0207 | Rare Bleeding Disorders in North India - Study from a Tertiary Care Centre

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Background: Rare bleeding disorders (RBD) comprise of deficiency of FII, FV, FV+VIII, FVII, FX, FXI, FXIII and Fibrinogen. These constitute around 3-5% of all coagulation disorders. The rare factor deficiency (like factor VII) most often do not correlate well with bleeding manifestations. The worldwide prevalence is variable,in India the prevalence is higher in South India due to prevalent consanguinity. In North India only 3-4 centers do the testing for RBD and only a miniscule number were detected amongst a large number of patients screened for bleeding disorders.
Aims: To study the clinical presentation and management of patients provided in cases diagnosed with rare coagulation factor disorders.
Methods: Records of patients diagnosed with RBD in our hospital in past 5 years were retrieved. The bleeding history, family history, bleeding severity, factor levels and follow up treatment history were procured. Complete blood counts, Prothrombin time, Activated partial thromboplastin time, Thrombin time, mixing studies, Factor assays and Clot solubility test using 5 M urea and 1% Monochloroacetic acid were done.
Results: In 47 patients diagnosed with RBD, Male: female ratio was 1.1:1. The Factor VII deficiency was seen in 12 cases,9 cases of FX and FXIII deficiency each. One patient had both FX and FV deficiency. Three cases of FV deficiency, 8 cases of FXI deficiency, 4 cases of FXII and one of FII deficiency were seen. Two patients had combined FV and FVIII deficiency. Mucocutaneous bleeding was the commonest presentation.
Conclusions: The RBD were low in number in our hospital catering to mainly patients from North India where consanguinity is much less common. Factor levels did not correlate well with disease severity.

PB0208 | Discordance Between the Neutralization Profile of Apixaban, Betrixaban, Edoxaban and Rivaroxaban in the Clotting Assays and Anti-Xa Measurements

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Background: Oral factor Xa inhibitors such as Apixaban, betrixaban, edoxaban and rivaroxaban are widely used for the management of thrombotic and cardiovascular indications. Andexanet alfa (AA) is approved for the management of bleeding complications with apixaban and rivaroxaban.

Aims: This study is designed to compare the neutralization efficacy of andexanet alfa for various factor Xa inhibitors in clot-based and anti-Xa assays.

Methods: Citrated pooled plasma was prepared from individual donors. Active pharmaceutical ingredients version of various factor Xa inhibitors were commercially obtained. Each of the factor Xa inhibitor was supplemented in plasma in the concentration range of 0-1 ug/ml. The neutralization profile of AA was studied at 100ug/ml and 200ug/ml and saline was used as a control. Clot-based PT, aPTT and amidolytic anti-Xa assays. The IC_{50} for the Xa inhibitory activity were calculated for the control drugs and after supplementation with AA. The neutralization profile in the clot-based assays was measured in terms of decrease in clotting times.

Results: Apixaban and edoxaban exhibited similar inhibitory profiles in the anti-Xa assays, where as rivaroxaban and betrixaban exhibited much higher IC_{50s}. AA effectively neutralized the anticoagulant effects of all agents at both the 100 and 200 ug/ml, however the neutralization profile in the clot based was not proportional to the anti-Xa effects. At 200ug/ml only betrixaban was completely neutralized whereas all the other agents were partially neutralized. The order of neutralization at 200ug/ml was betrixaban > rivaroxaban > apixaban > edoxaban.

Conclusions: These studies show that the neutralization profiles of various Xa agents by AA exhibit differential characteristic response for each of the individual agent. These results indicate that individual dosing based on clinical endpoints for each agent rather than the neutralization of anti-Xa activity measurement may be more relevant to the clinical outcome.

PB0209 | Circulating T Helper 17 Cells (Th17) Increase at a Higher Rate than Tregs in Previously Untreated Patients with Severe Hemophilia A Who Develop Inhibitors During the First 50 Exposure Days to rFVIII

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Background: The Hemophilia Inhibitor PUPs study (HIPS) is a prospective longitudinal study of previously untreated patients with severe Hemophilia A during their first 50 exposure days (EDs) to a single source of recombinant FVIII, Advate™. Among the 23 HIPS subjects who completed 50 EDs, 4 distinct antibody signatures were identified: Subgroup 1 (7) had no FVIII inhibitors or FVIII-specific antibodies, Subgroup 2 (7) had no inhibitors but had FVIII-Specific IgG1, Subgroup 3 (2) had low titer or transient FVIII inhibitors with FVIII specific IgG1 but without IgG3 or IgG4, and Subgroup 4 (7) had high titer inhibitors with high-affinity IgG1 and class-switched antibodies (IgG3, IgG4; rarely IgG2).

Aims: To compare T-cell subpopulations using highly sensitive epigenetic markers among the 4 subgroups at baseline and throughout the 1st 50 EDs.

Methods: HIPS subjects had blood samples drawn at baseline and after ED1, ED5, ED10, ED20, ED30, ED40, and ED50. Epigenetic studies using DNA markers which are unique and highly specific for $CD3^+$ T cells, $FOXP3^+$ Tregs, $IL\text{-}17A^+$ Th17 cells, follicular helper T cells (Tfh) were used to quantify each cell type (Precision for Medicine; Berlin, GE).

Results: 1. There was a significant difference in baseline % Tfh between subgroups 1, 2 and 4 (Figure 1).

2. There were no differences in baseline % of for total FOXP3$^+$ Tregs, IL-17A$^+$ Th17 cells.

3. There was a significant difference in the rate of increase of % TH17/CD3 cells during 50 EDs between subgroups 1, 2, and 3 and subgroup 4 (Figure 2).

Conclusions: Proportion of Tfh cells is significantly higher at baseline in patients who develop high titer, high-affinity IgG class-switched antibodies. Proportion of Th17 cells increases at a higher rate than FOXP3$^+$ Tregs in patients who develop high titer, high affinity antibodies which likely reflects activation of adaptive immunity predisposing to inhibitor formation.

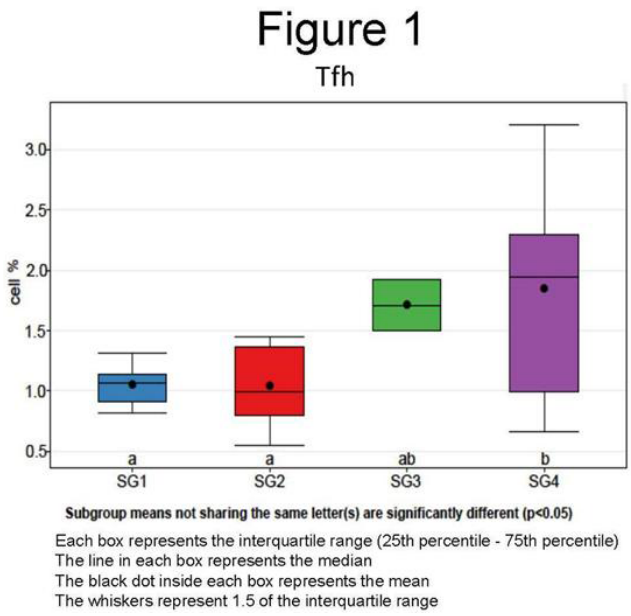

FIGURE 1 Follicular Helper T-cells

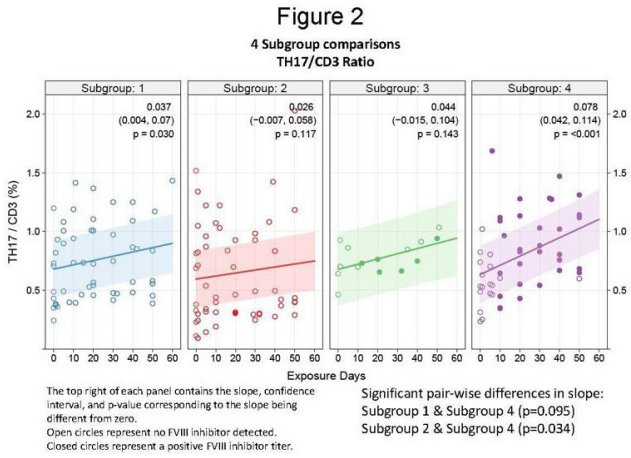

FIGURE 2 TH17/CD3 Ratio

PB0211 | New Peptide Inhibitors of Serine Proteases of the Blood Coagulation System

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Background: Thrombin and FXa are key enzymes of coagulation system. FXa is involved in the generation of thrombin which, in turn, converts plasma fibrinogen into fibrin. Excessive activation of the coagulation system can lead to life-threatening thromboembolic diseases. To reduce the coagulation system activity, heparins, antiplatelet and other agents are used. Recently, the development of new drugs that directly inhibit thrombin and FXa has attracted increasing interest.

Aims: Studying the effect of new peptide inhibitors Trp-Arg-Pip (WR-Pip) and Trp-Arg-OEt (WR-OEt) on intrinsic activity of thrombin and FXa and on selected coagulating parameters.

Methods: Affect the concentration of WR-Pip and WR-OEt on the intrinsic activity of thrombin and FXa and coagulation of fibrinogen by thrombin was studied using kinetic methods, on plasma thrombin generation, thrombin time and prothrombin time was studied using standard methods.

Results: Both peptides reduced amidase activity and were competitive inhibitors of FXa and thrombin. With WR-OEt, the IC_{50} were 1.4 ± 0.1 and 1.1 ± 0.02 mM, and K_i were 0.9 ± 0.1 and 0.67 ± 0.04 mM for FXa and thrombin, respectively. With WR-Pip, the IC_{50} were 10.8 ± 0.4 and 0.30 ± 0.03 mM, and K_i were 6.2 ± 0.4 and 0.15 ± 0.02 mM for FXa and thrombin, respectively. WR-Pip significantly inhibited fibrinogen coagulation by thrombin: 0.25 mM WR-Pip or 2 mM WR-OEt caused two-fold decrease in fibrin formation rate. The double thrombin time elongation was caused by 0.1 mM WR-Pip and 1 mM WR-OEt. At the same 0.1 mM concentration, WR-Pip dramatically reduced plasma thrombin generation, while WR-OEt had virtually no effect. WR-Pip (30 mg/kg) showed 75% antithrombotic effect in experiments on rat's model of thrombosis with both intravenous and oral administration.

Conclusions: WR-Pip inhibits thrombin more strongly, while WR-OEt inhibits FXa. WR-Pip prolongs the thrombin time and prothrombin time, effectively inhibits fibrinogen coagulation, and dramatically reduced plasma thrombin generation, thereby suppressing the external pathway of blood coagulation.

PB0212 | HIPS Qualification Testing (HQT): Qualification of Hemophilia Treatment Centers to Perform PBMC Re-Stimulation for the Hemophilia Inhibitor in Previously Untreated Patients (HIPS) Study

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Background: HIPS is an international multi-center prospective study to assess immune responses during the first 50 exposure days to FVIII replacement therapy in PUPS with severe hemophilia A. The 15 participating Hemophilia Treatment Centers (HTCs) prepared blood samples for plasma, cellular, epigenetic, genomic, and transcriptome analysis at the local site prior to shipment to central laboratories. FVIII-restimulation of PMBCs was performed at each HTC in order to evaluate antigen-specific cellular immune responses.

Aims: HQT procedures were developed to qualify each clinical site to perform the laboratory procedures prior to subject enrollment in HIPS.

Methods: An on-site demonstration and review was conducted at each qualifying HTC. A test qualification kit consisting of whole blood from two CMV sero-positive donors were sent to each operator for PBMC isolation and restimulation with CMV pp65. A sample from each donor was simultaneously processed at the reference lab for comparison. The test samples were assessed for PBMC yield and viability following isolation procedure. Following restimulation with CMV pp65 IFN-γ gene integrity and concentration was measured. The center operator was considered qualified if the ratio of qualification over reference operator was within 0.67-1.5 for pp65 stimulated - and 0.8-1.25 for positive control -stimulated IFN-γ expression.

Results: Forty operators at 15 HTCs were qualified. 11 of the 15 HTCs had acceptable results on first testing. The other 4 HTCs were below acceptable range, and after a review of procedures were permitted to repeat the testing; all 4 HTCs had acceptable results after the 2nd test.

Conclusions: HTCs are capable of performing quality antigen-specific restimulation testing from PBMCs locally. HQT is a useful methodology to assess quality of HTCs to prepare samples suitable for plasma, cellular, epigenetic, genomic, and transcriptome analysis.

TABLE 1 Example of PBMC viability and count

Donor	A			B		
Operator	REF	1	2	REF	1	2
Cell viability [%]	97	95	95	98	93	89
PBMC yield[1E6/ml]	5,1	5,7	4,1	4,5	3,5	5,0

TABLE 2 Sample Table of RNA integrity and Yield

Operator	REF		1		2	
sample	Conc. [ng/ml]	RIN[1]	Conc. [ng/ml]	RIN[1]	Conc. [ng/ml]	RIN[1]
pp65 1	8	n/a	11	9,5	9	n/a
pp65 2	8	n/a	12	9,3	5	n/a
pp65 3	9	n/a	8	n/a	6	n/a
NC 1	7	n/a	13	9,9	9	n/a
NC 2	8	n/a	9	n/a	11	9,6
NC 3	7	n/a	11	9,2	5	n/a
PC 1	4	n/a	12	9,7	9	n/a
PC 2	3	n/a	18	8,4	6	n/a

[1]RIN: RNA Integrity Number
pp65 samples were stimulated with CMV pp65-spanning 15mer peptide pool
PC (positive control) samples were stimulated with Dynabeads Human T-Activator
NC (negative control) samples were not stimulated

PB0213 | Anticoagulant Effect of Novel Nanoparticles Functionalized with Synthetic Piperazinone Derivatives as FXa Inhibitors

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Background: The coagulation cascade is a dynamic physiological process that comprises a sequence of activation reactions for the formation of the blood cloth. This chain of reactions involves the interaction of numerous blood components known as coagulation factors, whose deregulation could generate hemorrhagic or thromboembolic events, such as venous thromboembolism (VTE), deep vein thrombosis (DVT) or pulmonary embolisms (PE).

The coagulation factor Xa (FXa), plays a central role in the convergence of intrinsic and extrinsic coagulation pathways; therefore, it is a critical factor for the research of novel anticoagulation strategies. Moreover, functional nanoparticles as emerged as an innovative therapeutical approach that allows improving the pharmacological properties of diverse types of drugs. In particular, polymeric nanoparticles lead the clinical translation of nanotechnology as drug delivery systems due to its recognized biodegradable and biocompatible properties.

Aims: In this work, we synthesized poly(lactide-co-glycolide) methyl ether-block-poly(ethylene glycol) (PLGA-PEG) nanoparticles functionalized with FXa piperazinone synthetic inhibitors developed in our laboratory.

Methods: Those molecules, as well as the polymeric coupling, were characterized by ^{1}H-NMR. The size (200 nm) and zeta potential (surface charge, −30 mV) of the functional nanoparticles were analyzed by dynamic light scattering, the spherical morphology of nanoparticles using transmission electron microscopy.

Results: The inhibition capacity against FXa enzyme of functionalized nanoparticles was analyzed by SensoLyte ® Rh110 Factor Xa Assay Kit exhibited 70% of inhibition compared with the unbound molecules used as a control.

Conclusions: Our preliminary results strongly support the generation of a novel anticoagulant agent based on functional nanoparticles.

PB0214 | Bisecting the Role of the C-terminal End of Antithrombin: Natural Variants due to a Frameshift at Different Secondary Structures May Exacerbate or Ameliorate a New Dominant-negative Effect

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Background: The C-terminal end (C-term) of antithrombin (p.Val432-Lys464) has no evident functional role, but it has been suggested to be crucial for the folding and secretion of this hemostatic serpin.

Aims: To characterize C-term natural variants of antithrombin.

Methods: Selection of C-term antithrombin variants from 340 unrelated cases (collected from 1998 to 2020). Plasma antithrombin was characterized by functional and antigenic methods. A recombinant model (HEK-EBNA) was used for expressing natural and artificial variants. Intracellular trafficking of antithrombin was analyzed by electron and confocal microscopy.

Results: Nine C-term variants where identified in 14 unrelated cases: 5 missense, 4 small deletions; 6 caused severe type-I deficiency, whilst 3, all missense, caused type-II pleiotropic deficiency (increase of latent antithrombin).

The recurrent p.Arg445Serfs*17 (N = 5) caused an aberrant full-length C-term antithrombin. This variant had a new dominant-negative effect through endoplasmic reticulum aggregation and retention (Figure 1), which explained the severe type-I deficiency and its clinical severity.

Seven additional frameshift variants, with different extension of the aberrant C-term, were analysed, identifying a pleomorphic behaviour:

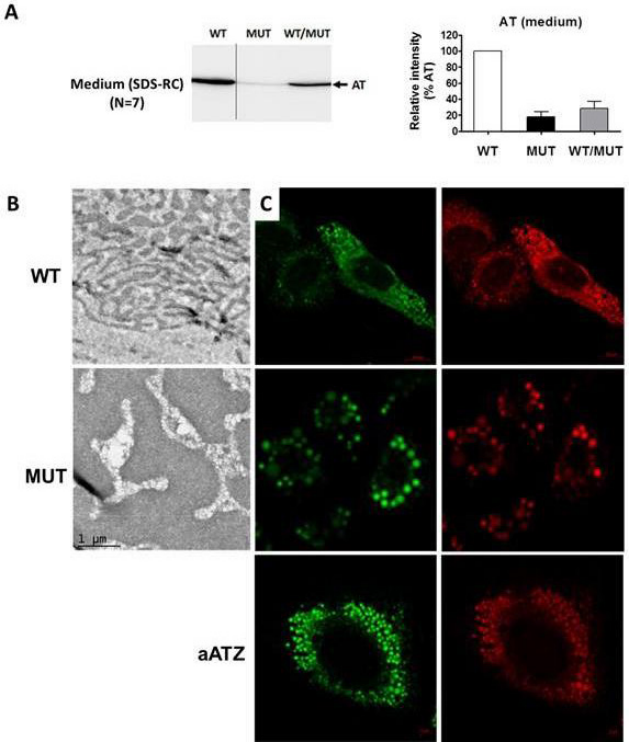

FIGURE 1 p.Arg445Serfs*17 dominant-negative effect (A) and intracellular trafficking by electron microscopy (B) and confocal microscopy (C)

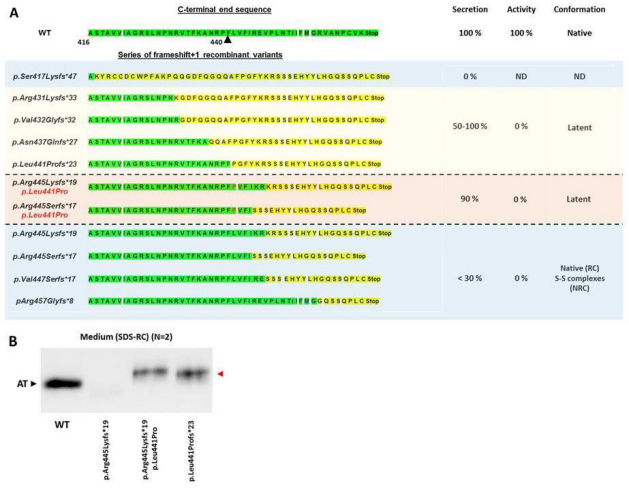

FIGURE 2 Analysis of recombinant C-term, frameshift antithrombin variants (A). The p.Leu441Pro mutation rescued p.Arg445Lysfs*19 secretion (B)

variants affecting Val432-Phe440 were efficiently secreted in an inactive form, whilst frameshift mutants with shorter aberrant C-term had a severely impaired secretion, like p.Arg445Serfs*17. A common aberrant proline shared by the variants efficiently secreted (Pro441) was introduced in two non-secreted variants, resulting in secretory rescue (Figure 2).

Conclusions: The consequences of natural C-term antithrombin variants are highly heterogeneous, strongly related with the secondary structures affected, thus supporting the key structural role of the

C-term of this plasma inhibitor. A completely aberrant C-term caused by frameshift may be secreted in an inactive form, but the resulting protein is folded into polymers if the frameshift takes place after Phe440. The generation of a Pro441 in mutants located between Val432-Phe440 seems to improve their secretion, and may promote novel targets for serpinopathies.

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PB0215 | Deep Vein Thrombosis and Coagulation Factor Deficiency: A Paradoxical Association

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Background: Coagulation factors deficiency is a rare condition. Its clinical presentation is variable. It can be asymptomatic or it can cause severe hemorrhagic complications. Its association with thrombotic events is exceptional.

Aims: To put the accent on a problematic treatement in an unusual presentation of potencially bleeding factor deficiencies with thrombosis.

Methods: We report the observation of a patient with deep vein thrombosis (DVT) and acquired factor V and II deficiency.

Results: A 70-year-old diabetic and hypertensive patient was hospitalized for DVT of the left superficial femoral vein occurring in the context of prolonged bed rest.

The physical examination was normal. Biology showed a low prothrombin time (52.9%) and a prolonged activated partial thromboplastin time (M / T: 1.82) corrected by adding normal plasma.

The rate of coagulation factors showed factor II and V deficiency.

Gynecological examination, oeso-gastro-duodenal endoscopy and thoraco-abdomino-pelvic CT-Scan had not shown signs of an underlying neoplasia.

The patient was initially treated with heparin then relay with Vitamin K antagonist (VKA).

The evolution was complicated by the occurrence of a muscle hematoma related to a VKA overdose, the NIR level was at 7.14, 17 days after the treatment onset.

VKA was stopped, the patient had vitamin K, blood transfusion and prophylactic antibiotic therapy.

In this context, the decision was to definitely stop the VKA especially since the ultrasound Doppler control at 3 weeks showed total venous recanalization of

Thrombosed vein.

Conclusions: Coagulation factor deficiency can be idiopathic or secondary requiring an exhaustive etiological investigation especially solid or hematological neoplasia. It can be a threatening condition due to bleeding complications.

The peculiarity of this observation is the paradoxical and rare association of several deficiencies in coagulation factors.

Although the occurrence of thrombosis was already reported with factors deficiency, treatment however, remains challenging.

PB0216 | Clinical Significance of T-cell Subsets for the Inhibitor Development in Pediatric Hemophilia A Patients

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Background: Although FVIII inhibitors have been extensively studied, much of the basic mechanism of this immune response remains to be uncovered. Th1, Th2, Th17 and Treg cells are expected to influence the process of immunology of both the host immune response and tolerance, but it still need to be verified.

Aims: To investigate the clinical significance of CD4-positive T lymphocyte subsets and cytokines for the development of FVIII inhibitor in pediatric hemophilia A .

Methods: Total122 pediatric HA patients in single center of China were enrolled. The percentages of T-cell subtypes in peripheral blood mononuclear cells were detected by flow cytometry. Cytokines were assayed by Luminex. FVIII inhibitor was detected by modified Nijmegen-Bethesda assay.

Results: There were 36 cases (lower titer 23 cases, higher titer 13 cases) of HA with positive FVIII inhibitor. The percentage of Treg cells were higher in positive inhibitor of HA patients than that in the negative inhibitor of HA group (7.17 ± 1.47% vs. 6.49 ± 1.32%) ($p < 0.05$). Furthermore, compared with the lower titer of inhibitor of HA patients group, the higher percentage of Tc1 was observed in the higher titer of inhibitor group (50.46 ± 24.76% vs. 32.23 ± 16.38%) ($p < 0.05$). Similar tendency of Th1 as well as ratio of Th1/Th2 was found in the higher titer of inhibitor group, however did not achieve the significant difference (For Th1, 25.62 ± 14.23% vs. 16.87 ± 11.50%, p = 0.052; For Th1/Th2, 28.01 ± 31.58% vs. 14.27 ± 11.62%, $p = 0.067$, respectively). The expression level of IL-4, IFN-g, TGF-β in serum were higher in inhibitor-positive HA patients than that of the inhibitor-negative group ($p < 0.05$).In patients with high-titer inhibitor, the TGF-β level were higher than that of patients with low-titer inhibitor ($p = 0.003$).

Conclusions: Our findings suggest that Treg cells might be associated with the development of FVIII inhibitor in pediatric HA. Furthermore, Tc1 might be a risk factor to predict the higher-titer status of inhibitor.

PB0217 | Effect of Mg(II) Ion on the Interaction of Coagulation Factors with Phospholipid Membranes

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Background: Vitamin K-dependent coagulation and anticoagulation factors are indispensible proteins that regualte the coagulation

process. We report $Ca^{2+}$and Mg^{2+}binding sites in the Gla domain of factor IX that stabilize the structure of factor IX upon binding $Ca^{2+}$and Mg^{2+}ions. Physiologic concentrations of Mg^{2+}ions augmented the biological activity of factor IXa, even in the presence of excess concentrations of Ca^{2+}ions. This finding suggests that Mg^{2+}ions also bind to factor IX Gla domain and cause structural changes that contribute to increased activity. In addition to factor IX, several other coagulation and anticoagulation factors (factors VII, X and protein C) are also reported to be affected by the presence of $Mg^{2+}$and Ca^{2+}ions.

Aims: The contribution of $Mg^{2+}$and $Ca^{2+}$to other vitamin K-dependent coagulation factors in membrane-bound processes was evaluated by assessing the affinities of various coagulation factors to phospholipid membranes in the presence of $Mg^{2+}$and Ca^{2+}ions.

Methods: Here, we report on the effect of Mg^{2+}ion on the interaction of coagulation proteins with phospholipid membranes. Phospholipids were immobilized on a sensor chip L1, and the interactions between coagulation proteins and phospholipid membranes were measured by surface plasmon resonance (SPR) using BIACORE.

Results: Factor IX, factor X, and prothrombin bound to phospholipid membrane in the presence of Ca^{2+}ions in a concentration-dependent manner, and the binding affinities were further increased by adding a physiologic concentration of Mg^{2+}ions.

Conclusions: SPR assay results indicate that Mg^{2+}ions have an effect on binding of the main coagulation factors to phospholipid membranes during blood coagulation. Further investigation is now underway to elucidate the effect of Mg^{2+}ions on the coagulation reaction.

PB0218 | Warfarin and Vitamin K Epoxide Reductase: A Mechanism for Inhibition Using Molecular Dynamics Simulation

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Background: Vitamin K epoxide reductase (VKOR), an endoplasmic reticulum membrane protein, is the key enzyme for vitamin K-dependent carboxylation, a posttranslational modification that is essential for the biological functions of coagulation factors. VKOR is the target of the most widely prescribed oral anticoagulant, warfarin. However, the topological structure of VKOR and the mechanism of warfarin's inhibition of VKOR remain elusive. Additionally, it is not clear why warfarin-resistant VKOR mutations identified in patients significantly decrease warfarin's binding affinity, but have only a minor effect on vitamin K binding. Here, Using molecular dynamics (MD) simulations, a T-shaped stacking interaction between warfarin and tyrosine residue 139, within the proposed $TY_{139}A$ warfarin-binding motif, was observed. Our findings provide new insights into the dynamics of the binding of warfarin to VKOR, as well as into warfarin's mechanism of anticoagulation.

Aims: We show that warfarin binds to VKOR based on 3TM model with steered MD simulation.

Methods: MD simulations of warfarin binding to VKOR were performed : step 1, providing an MD-equilibrated 3-TM VKOR in lipid and solvent; step 2, the 40-nanosecond equilibration of warfarin in the ER lumen near a reasonable access point to the interior of VKOR; step 3, steered MD simulation.

Results: As warfarin accesses its binding site, it encounters several hydrophobic residues that are essential for maintaining VKOR's stable structure. We assume that the interactions between these hydrophobic residues must be weakened to allow warfarin to access its binding pocket during the 448-picosecond steered MD simulation. Our results show a dramatic increase in the minimum distance between L15 and L128, suggesting the opening of the warfarin-binding pocket in VKOR. Thereafter, warfarin appears to penetrate into the new cavity of VKOR

Conclusions: The T-shaped stacking configuration between warfarin and Y139 can happen only in the 3-TM model of VKOR, not the 4-TM model.

PB0219 | Are the Plasma Levels Thresholds of DOAC Rationale and Appropriate for Clinical Decision-making? A Reappraisal Using Thrombin Generation Testing

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Background: Monitoring of the anticoagulant activity of direct oral anticoagulants (DOACs) is generally not necessary but a point estimation could be useful in certain circumstances. These specific tests estimate the plasma concentrations of DOAC and report the results in ng/mL. Thresholds were proposed for safe thrombolysis (i.e. 100 ng/mL for rivaroxaban, 50 ng/mL for dabigatran and 10 ng/mL for apixaban). These thresholds are subjective and do not always consider the different pharmacodynamics profile of DOACs. The thrombin generation assay (TGA) has been identified as a useful tool to assess the pharmacodynamics profile of anticoagulants and may better reflect the true anticoagulant activity than measuring the ponderal concentration.

Aims: To assess the impact of DOACs on TGA at relevant threshold used for clinical decision-making.

Methods: DOACs were spiked at 3 relevant thresholds (30, 50 and 100 ng/mL) in normal pooled plasma. Thrombin generation was measured with the calibrated automated thrombogram (CAT).

Results: Thirty, 50 or 100 ng/mL of rivaroxaban did not have the same anticoagulant activity than the same amount of apixaban, betrixaban, edoxaban or dabigatran (Figure 1). Results showed that the effects of FXa inhibitors were more pronounced on the thrombin peak and the

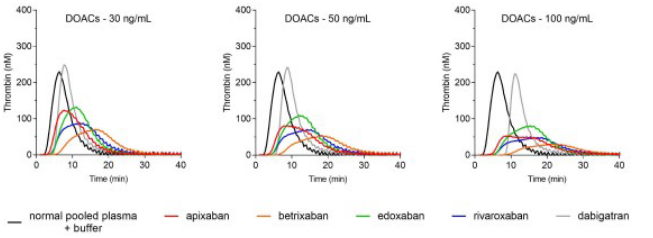

FIGURE 1 Impact of DOACs on thrombin generation (CAT) induced by the extrinsic pathway (5pM of tissue factor)

mean velocity rate index while dabigatran showed its major effects on the lag time and the time to peak. These different TGA profiles, between FXa inhibitors and especially between FXa and thrombin inhibitors, question whether the thresholds recommended by expert guidelines are appropriate and safe whatever the DOAC.

Conclusions: Due to the different targets and inhibition constant (Ki) between DOACs, the use of global parameters in TGA could be better to define safe and appropriate thresholds for thrombolysis. Future research should be performed to address this important clinical question.

PB0220 | Combined Analysis of Dendritic Cell (DC) HLA-class-II (HLAcII) Peptidomic Profiling Data Derived from Different Therapeutic Factor VIII Proteins (tFVIIIs) in Independent Experiments/Laboratories

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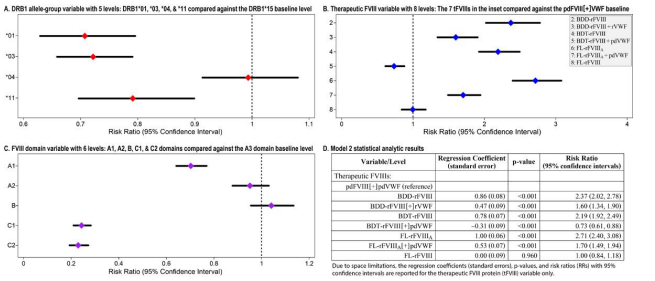
Background: Accurately understanding the immunogenic potential (IP) of different tFVIIIs can inform development of improved biotherapeutics.

Aims: Analyze the HLAcII/tFVIII-derived-peptides presented by DCs from 26 unrelated-donors in three independent protein-processing/presentation assays (PPPAs) performed in two laboratories.



Variable/Level	Regression Coefficient (standard error)	p-value	Risk Ratio (95% confidence intervals)
Therapeutic FVIIIs:			
pdFVIII[+]pdVWF (reference)			
BDD-rFVIII	0.86 (0.08)	<0.001	2.37 (2.02, 2.78)
BDD-rFVIII[+]rVWF	0.47 (0.09)	<0.001	1.60 (1.34, 1.90)
BDT-rFVIII	0.78 (0.07)	<0.001	2.19 (1.92, 2.49)
BDT-rFVIII[+]pdVWF	-0.31 (0.09)	<0.001	0.73 (0.61, 0.88)
FL-rFVIII$_A$	1.00 (0.06)	<0.001	2.71 (2.40, 3.08)
FL-rFVIII$_A$[+]pdVWF	0.53 (0.07)	<0.001	1.70 (1.49, 1.94)
FL-rFVIII	0.00 (0.09)	0.960	1.00 (0.84, 1.18)

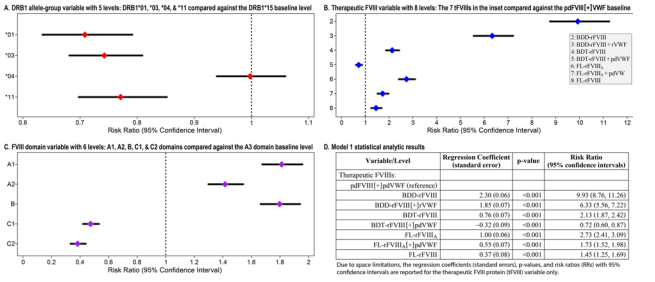
Due to space limitations, the regression coefficients (standard errors), p-values, and risk ratios (RRs) with 95% confidence intervals are reported for the therapeutic FVIII protein (tFVIII) variable only.

FIGURE 1 Risk ratios and 95% confidence intervals for Model 1

Methods: We analyzed "counts" of HLAcII-bound/tFVIII-derived-peptides from distinct tFVIIIs—the dependent variable—using a log-linear mixed model. Donors and DC-PPPAs were modeled as random effects to account for non-independent sampling and heterogeneity. HLAcII allele-groups (AGs), FVIII domains (FDs) and tFVIIIs were modeled as independent predictors. Eight tFVIIIs were examined: plasma-derived (pd) FVIII with von Willebrand Factor (VWF), "pd-FVIII[+]pdVWF"; B-domain-deleted (BDD)-recombinant (r) FVIII, "BDD-rFVIII"; BDD-rFVIII with rVWF, "BDD-rFVIII[+]rVWF"; B-domain-truncated (BDT)-rFVIII, "BDT-rFVIII"; BDT-rFVIII with pdVWF, "BDT-rFVIII[+]pdVWF"; full-length (FL)-rFVIII, "FL-rFVIII$_A$" ('A' based on experiment with the highest tFVIII concentrations); and FL-rFVIII$_A$ with pdVWF, "FL-rFVIII$_A$[+]pdVWF". DR-bound/tFVIII-derived-peptides were analyzed based on which of the: 1) six FDs (A1, A2, B, A3, C1, and C2) they were derived; and 2) five DRB1-AGs identified (*01, *03, *04, *11, and *15) most likely encoded the β-chain of the pertinent HLAcII molecules. We implemented two versions of the model, "Model_1"—which accounted for the different tFVIII concentrations across DC-PPPAs by scaling tFVIII-peptide counts against the experiment with the highest concentration—and "Model_2", which analyzed unscaled tFVIII-peptide counts.

Results: Figures 1 and 2 show the effects of different tFVIIIs, DRB1-AGs, and FDs for Model_1 and Model_2, respectively, using the risk ratios (RRs) and 95%-confidence-interval lower and upper bounds (LB and UB), reported as RR (95%_LB, 95%_UB). In Model_1, RRs are overestimated for BDD-rFVIII and BDD-rFVIII[+]rVWF, likely indicating improper scaling.

Conclusions: Consistent with the literature, we showed that (i) BDT-rFVIII[+]pdVWF has the lowest IP—assuming tFVIII-derived-peptide counts are proportional to IP—and (ii) DR15 presents more tFVIII-derived-peptides than HLAcII-molecules of other DR-serogroups



Variable/Level	Regression Coefficient (standard error)	p-value	Risk Ratio (95% confidence intervals)
Therapeutic FVIIIs:			
pdFVIII[+]pdVWF (reference)			
BDD-rFVIII	2.30 (0.06)	<0.001	9.93 (8.76, 11.26)
BDD-rFVIII[+]rVWF	1.85 (0.07)	<0.001	6.33 (5.56, 7.22)
BDT-rFVIII	0.76 (0.07)	<0.001	2.13 (1.87, 2.42)
BDT-rFVIII[+]pdVWF	-0.32 (0.09)	<0.001	0.72 (0.60, 0.87)
FL-rFVIII$_A$	1.00 (0.06)	<0.001	2.73 (2.41, 3.09)
FL-rFVIII$_A$[+]pdVWF	0.55 (0.07)	<0.001	1.73 (1.52, 1.98)
FL-rFVIII	0.37 (0.08)	<0.001	1.45 (1.25, 1.69)

Due to space limitations, the regression coefficients (standard errors), p-values, and risk ratios (RRs) with 95% confidence intervals are reported for the therapeutic FVIII protein (tFVIII) variable only.

FIGURE 2 Risk ratios and 95% confidence intervals for Model 2

except DR4. Model_2 showed the expected FD-effect with A3 yielding more peptides than the A1-, C1-, and C2-domains.

PB0221 | Perioperative Management of Acquired Factor XI Deficiency: A Case Report and Review of Literature

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Background: Factor XI (FXI) deficiency is a rare bleeding disorder. Only a few cases of acquired inhibitors with a neutralizing activity against FXI have been reported. Bleeding usually occurs in areas of increased fibrinolysis, after trauma, surgery or other challenges to haemostasis but the severity of symptoms is not correlated to FXI levels.
Aims: We report the case of a 87-year-old woman hospitalized for a femoral neck fracture.
Methods: She had no previous bleeding history with normal aPTT in 2017. Since 2018, she was treated by rivaroxaban for atrial fibrillation that was stopped at her arrival due to an indication for partial hip replacement. The only bleeding symptom was an hematoma related to the fracture with a stable hemoglobin concentration (≈110 g/L). A persistent increase of aPTT ratio (2.5) four days after rivaroxaban discontinuation led to the discovery of FXI deficiency (FXI = 7%, HemosIL Synthasil®, ACLTOP700, Werfen) associated to an anti-XI autoantibody titered at 2.5 BU/mL. Factors VIII, IX and XII were in the normal range.
Results: Surgery was performed with a preoperative injection of rFVIIa (30 µg/kg) associated with 500 mg of intraveinous tranexamic acid. Postoperative procedure included tranexamic acid (500 mgx3/day per os, for 10 days), compression stockings and preventive anticoagulation by enoxaparin (20 mg/day) because of her low weight of 39 kgs. No bleeding or thrombotic complications occurred during and after surgery. Thrombography (CAT, Diagnostica Stago) revealed decreased and delayed thrombin generation at baseline that was normalized after rFVIIa injection (Figure 1).

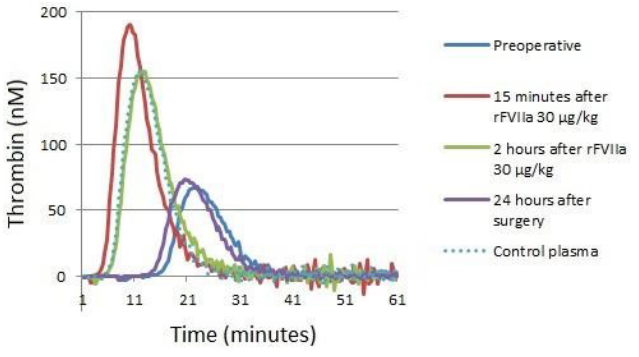

FIGURE 1 Thrombograms before and after rFVIIa injection

Conclusions: This is the third reported case of rFVIIa use in a patient with a FXI inhibitor. Thrombography was correlated to the clinical response to rFVIIa and could be useful to monitor bypass agents in patients with FXI inhibitors.

PB0222 | Probing Interaction of TOS with ATIII Using MD Simulation and Biophysical Studies; Its Effect on Clot Formation and fXa Inhibition

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Background: Global disease burden reported thromboembolic diseases as the third leading cause of human death. Antithrombin (ATIII) is an endogenous anticoagulant protein that prevents hypercoagulability by inhibiting proteases such as factor IIa, factor Xa (fXa). Anticoagulants enhance the activity of ATIII which prevents the activation of procoagulant proteases else leads to abnormal blood clots. To overcome thrombotic complications safer anticoagulants are needed.
Aims: Designing novel Turanose Octasulfate (TOS) and its effect on clot formation and fXa inhibition.
Methods: Synthesis of TOS was achieved using triethylamine-sulfur trioxide and purified by HPLC. Structural modifications were established by FTIR, NMR, and Mass spectrometry. The in vitro efficacy of TOS was tested by clotting assays APTT, PT, and TT. Inhibition of fXa was performed in ATIII and fXa in 30:1 (300 nM:10 nM) molar ratio. Molecular Docking of NOS with ATIII was done using Autodock 4.0 and MD simulation was performed using GROMACS 5.1.5.
Results: Upon incubation of TOS with human plasma, prolonged APTT and a moderate effect on PT and TT were observed, signifies delay in clot formation in plasma treated with TOS. Molecular docking results suggested TOS bind to ATIII with good affinity, in MD simulation the average RMSD value and fluctuation peaks dictate us the complex with TOS provides stability to the ATIII showing stable confirmation. An increase in fluorescence intensity of TOS treated ATIII showed changes in its tertiary structure conformation, while the slight effect on secondary structural change was observed in Far-UV CD spectra. Upon incubation of ATIII treated with TOS demonstrated a moderate effect on fXa inhibition activity.
Conclusions: The results suggest that the effect of TOS on ATIII and clot formation hints its promising anticoagulant potential.

PB0223 | A Novel Coagulation Factor VIII with Reduced Immunogenicity

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Background: Up to 30% of severe Hemophilia A patients develop inhibitory antibodies against therapeutic FVIII. Although several modified recombinant FVIII products are available, a deimmunized FVIII product which reduces the probability of inhibitor development has not yet been reported.
Aims: To develop FVIII with lower immunogenicity by reducing the number of FVIII-peptides presented on immune cells leading to a reduction of FVIII-specific naïve T cell maturation, and hence a reduction in the production of high-affinity inhibitory antibodies.
Methods: Using *in silico* tools, we screened the FVIII sequence and identified FVIII-unique peptides predicted to bind with high affinity to the MHC class II. Amino acid exchanges were incorporated in order to reduce MHC II presentation. Finally, 19 mutations were incorporated into the FVIII coding sequence without altering regions important for activity, folding and binding.
Results: Functional and structural analyses of this deimmunized FVIII variant revealed similarity to a non-modified FVIII reference and other registered rFVIII products. An *in vitro* DC-T cell assay was used to examine the immunogenicity of the deimmunized FVIII variant: DCs derived from healthy donors were co-cultured with autologous $CD4^+$ T cells which were depleted for regulatory T cells in order to allow an anti-FVIII immune response. The DCs were primed with the non-modified and deimmunized FVIII products, and $CD4^+$ T cell proliferation was measured. Using this assay, we demonstrated a significantly reduced immunogenicity of the deimmunized FVIII variant.
Conclusions: Here we present a deimmunized functional recombinant FVIII which was shown to be less immunogenic compared to a non-modified FVIII reference in an *in vitro* assay. When used as factor replacement therapy, this molecule has the potential to reduce the risk of inhibitor development in Hemophilia A patients.

PB0224 | A Sensitive and Specific Assay to Measure TF Activity in Cell-derived Extracellular Vesicles Based on Thrombin Generation

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Background: Most TF activity assays are mainly been based on FX activation by TF in the presence of FVII/FVIIa, a system dependent on 1–2 h incubation followed by measurement of formed FXa in a chromogenic assay. As FVIIa in the presence of phospholipids also has the ability to active FX, the long incubation-times may allow expression of non-TF dependent activities which are not blocked by anti-TF antibodies.
Aims: The aim of this work was to develop a specific and sensitive TF activity assay based on short incubation times of purified clotting factors to avoid non-specific TF activities.
Methods: The TF activity was measured in a two-stage amidolytic assay based on the ability of TF to accelerate the activation of FX by FVIIa, followed by the conversion of prothrombin to thrombin by FXa in the presence of FV/Va and phospholipids. Purified factors, FVIIa, FX, FV/Va, prothrombin, phospholipids and the test sample along with Ca^{2+} were incubated for 4 min at 37 °C. Thrombin generation was quantified by a chromogenic end point assay.
Results: Except for a small background caused by traces of FXa in the FX reagent, the TF activity assay proved high specificity assessed by neutralization of all TF activity by anti-TF antibody. However, the background activity increased with increasing incubaton times. The specificity was further demonstrated by the lack of TF activity in EVs from a knock-out-TF cell line, HAP1/F3, as EVs had no more activity than the control. In contrast, EVs derived from HAP1 cells showed high concentration of TF activity that was totally blocked by anti-TF antibodies. The sensitivity of the assay was in the low fM range.
Conclusions: Our TF activity assay, based on short incubation and thrombin generation, provides a fast, sensitive and specific measurement of TF activity in cell-derived EVs with applicability for laboratory- and clinical research.

PB0225 | Measurement of Polyphosphate in Biologic Samples

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Background: Polyphosphate (polyP), which is secreted by platelets and other cell types, is procoagulant and proinflammatory. Available methods for quantifying polyP are difficult and laborious, severely limiting their use in clinical studies. We developed a microplate-based, ELISA-like assay that allows for rapid and sensitive quantification of polyP in biologic samples.
Aims: To detect polyP in complex biologic samples such as plasma and cell lysates.
Methods: Bacterial lysates were produced from *E. coli* expressing significant levels of polyP. Plasma was collected from 21 healthy volunteers and at various time points from 30 patients with major trauma. All samples were diluted into a high-salt buffer containing EDTA and the detergent, Sarkosyl, then applied to a polyethylenimine-coated microplate well. Wells were then washed to remove interfering proteins, and the captured polyP was detected with a biotinylated polyP-binding protein plus enzyme-linked streptavidin.
Results: PolyP was quantified in bacterial lysates at concentrations between 25 and 232 μM, which agreed with other (more laborious) quantification methods. PolyP was not detected in pooled normal plasma, or any plasma from healthy volunteers. Samples from 6 trauma patients contained detectable polyP at concentrations ranging from 18–43 nM, indicating that 1 of 5 traumatized patients had evidence of circulating polyP.
Conclusions: For the first time, we are now able to detect polyP with sensitivity in the low nM range, using a rapid, multiplexable method that requires only small sample volumes. Under normal physiological circumstances, polyP does not appear to be present in plasma, but in

a pilot study, 1 of 5 patients contained low nM levels of polyP in the plasma at some time following major trauma. This polyP detection method will now allow the investigation of polyP levels in human clinical samples.

PB0226 | Anti-factor V Inhibitor after Liver Transplantation

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Background: Anti-factor V antibodies (anti-FVAb) rarely occur and mostly in association with autoimmune diseases, cancer, infections, and surgery. Half patients develop bleeding complications. Therapeutic approaches to remove the inhibitor include immunosuppressants, intravenous immunoglobulins and plasmapheresis.

Aims::

Methods::

Results: A 63-year-old man underwent a liver transplantation for hepatocellular carcinoma. On day 19 coagulation tests were prolonged, with a PT ratio of 4.70 (mix 1.42) and a aPTT ratio of 2.76 (mix 0.95). Factor V activity was < 1% with an anti-FV antibody (Ab) of 0.75 BU. Patient's therapy included tacrolimus which previously showed to induce an anti-FVAb[1]. Hence, at day 20 tacrolimus was discontinued and prednisone 1 mg/kg/day was started, with a normalization of PT and aPTT ratio and FV activity levels at day 28. During the whole clinical course the patient had no bleeding complications. ELISA assay was used to detect Ig antibodies. Data on FV activity, anti-factor V inhibitor and plasma levels of tacrolimus are reported in figure 1. At variance with previously described[1], no reduction of FV activity was detected when tacrolimus alcoholic solutions were added to patient's plasma (collected when FV levels

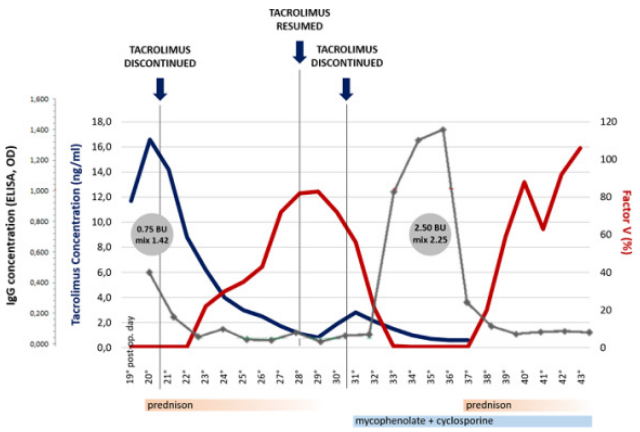

FIGURE 1

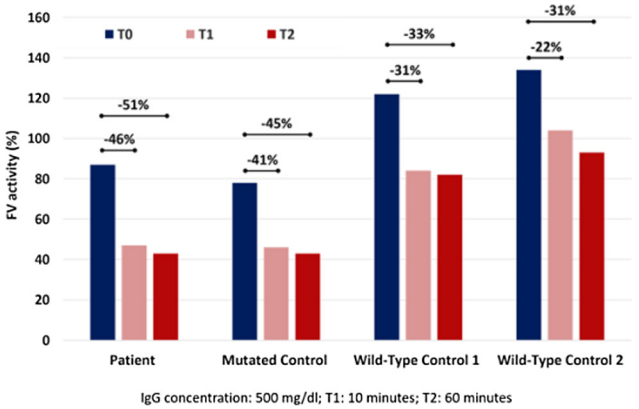

FIGURE 2

were normal). Thus, we hypothesized that a structurally different FV was produced by the transplanted liver and that may have induced the development of the anti-FVAb. Genetic analysis of *FV* gene in the donor and recipient was performed and two discordant single nucleotide variants (SNVs) were observed between donor and recipient DNA. We also observed a slightly reduced inhibitory activity of the anti-FVAb on wild type than on mutated FV (figure 2).

Conclusions: In our patient, the hypothesis of a tacrolimus-dependent acquired inhibitor was ruled out. The genetic variants on *FV* gene might played a role on the anti-FVAb development.

1 Leroy-Matheron C et al. Transplantation. 1999 Oct 15;68(7):1054-6.

PB0227 | Addition of Histone Deacetylase Inhibitors Increases Recombinant Factor V Expression in BHK Cells

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Background: Human factor V (FV) is a 330-KDa glycoprotein that circulates in blood as procofactor and plays a critical role in coagulation. Indeed, defects in FV regulation lead to bleeding or thrombosis. Historically, stable expression of full length (fl)-FV in high yield has been challenging because it is poorly expressed in mammalian cell culture systems.

Histone deacetylase inhibitors (HDACi) increase mRNA and protein expression levels in tissue culture. Of interest, sodium butyrate (NaBu) and N1-hydroxy-N8-phenyl-octanediamide (SAHA) have been demonstrated to increase expression of coagulation factor VIII, which is homologous to FV.

Aims: Here, we tested the possibility that HDACi may increase FV expression.

Methods: Baby Hamster Kidney cells stably transfected with fl-FV (BHK-FV) were used to screen a library of HDACi to identify compounds that increase fl-FV secretion. Compounds of interest were identified in 24-well plates (1.9-cm^2). HDACi expression enhancement

was confirmed in 75-cm^2 and 500-cm^2 flasks. Expression levels were determined using Prothrombin time (PT) and Western blot (WB).

Results: The initial screen identified four HDACi that enhanced fl-FV expression at 24 and 48 hours: NaBu (0.1–2.5 mM), SAHA (0.1–2 μM), valproic acid (0.5–2 mM), and 3-[2-butyl-1-[2-(diethylamino) ethyl]-1H-benzimidazol-5-yl]-N-hydroxy-2E-propenamide (SB939; 0.1–5 μM).

Scale-up to 75-cm^2 confirmed these results and further demonstrated that the addition of 0.75 μM SB939 doubled fl-FV expression (180 mU/mL) over 48 hours compared to untreated BHK-FV. During large-scale collection in 500-cm^2, fl-FV expression in the presence of SB939 increased over 4 days of collection (Day 1: 80 mU/ml - Day 4: 160 mU/ml), whereas fl-FV expression in untreated BHK-FV was stable at 50 mU/ml.

Conclusions: Our results show that SB939 increases the expression of fl-FV in BHK cells. The ability to generate a sufficient amount of purified recombinant fl-FV will advance the characterization of this protein.

PB0228 | The Distribution of Hemophilia A-causing Genetic Variants by Race/Ethnicity and Association with Inhibitors among Enrollees in the Hemophilia Inhibitor Research Study (HIRS)

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Background: Approximately 30% of persons with hemophilia A (HA) develop inhibitors to factor VIII replacement products, rendering those products ineffective. While the underlying cause of the disparity is unclear, inhibitors occur more frequently in Black and Hispanic patients compared to White patients. An important risk factor for inhibitor development is the HA-causing genotype, with null variants conferring higher risk. While previous studies have not identified a significant association between HA-causing genotype, race/ethnicity, and inhibitors, these studies have likely been underpowered.

Aims: The distribution of HA-causing variants by race/ethnicity and inhibitor status among patients with HA enrolled in the Hemophilia Inhibitor Research Study (HIRS) was examined.

Methods: Informed consent was obtained. This investigation includes enrollees in an extension of HIRS, which was designed to over-sample minority populations. The HA-causing variant was determined by inversion screening and *F8* sequencing, and when a variant was not found, duplication screening by multiplex ligation-dependent probe amplification was performed. Association of variant type with inhibitor status was examined using logistic regression.

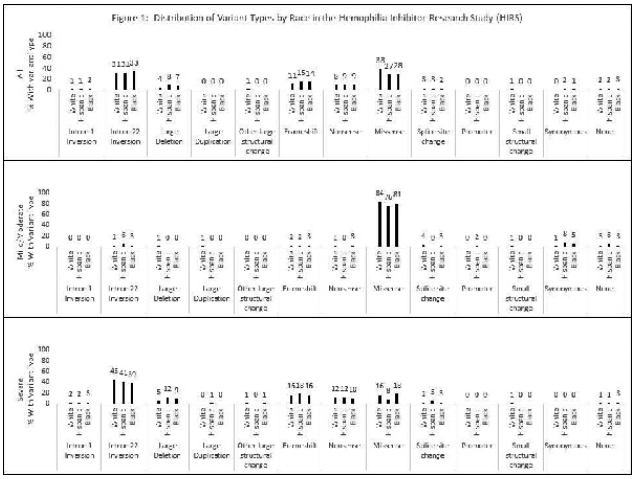

FIGURE 1 Distribution of variant types by race in the Hemophilia Inhibitor Research Study (HIRS)

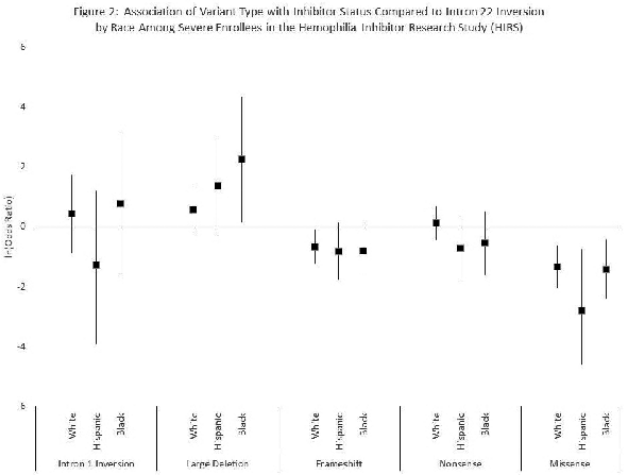

FIGURE 2 Association of variant type with inhibitor status by race in the Hemophilia Inhibitor Research Study (HIRS)

Results: Among 974 White, non-Hispanic (WNH) enrollees, the most common HA-causing variant types were intron 22 inversion (I22INV) (31%) and missense (38%). This distribution was similar among 203 White, Hispanic (WH) and 211 Black, non-Hispanic (BNH) enrollees (Figure 1). Compared to the I22INV, large deletions were associated with increased odds of inhibitors among enrollees with severe HA, although only significantly so among BNH (Figure 2). The confidence limits for the estimated odds ratios comparing odds of inhibitors among variant categories to that of the I22INV overlapped for WNH, WH, and BNH.

Conclusions: The distribution of HA-causing variants among WNH, BNH, and WH was similar, indicating the disparity in inhibitor risk by race/ethnicity observed in this population is unlikely to be due to differences in the distribution of hemophilia genotypes between the groups.

PB0229 | The Anticoagulant Effect of Protamine - A Stabilizer of the Factor V Procofactor State

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Background: Protamine is a mixture of basic, arginine-rich peptides used clinically to neutralize the anticoagulant effect of negatively charged heparin. Paradoxically, protamine can elicit an anticoagulant effect. Previous studies have shown that protamine inhibits factor V (FV) activation; yet mechanistic insights are lacking. FV is activated to FVa through removal of its B-domain and destabilizing interactions between acidic region 2 (AR2; 1493–1538) and the basic region (BR; 964–1008) which are responsible for keeping FV inactive. FV species that lack BR but retain AR2 (FV_{AR}; e.g. FV-short and platelet-FV) are active, but sensitive to inhibition following the addition of BR *in trans*. Here, we hypothesize that protamine mimics FV-BR and inhibits cofactor function of FV_{AR} species.
Aims: To examine how protamine interacts with FV species.
Methods: We used biochemical assays to characterize the interaction between protamine and FV species in a purified system and examined protamine's effects in plasma with or without heparin.
Results: We found that protamine bound tightly to FV-short and inhibited cofactor function (K_i = 2 nM), caused a dose-dependent anticoagulant response on thrombin generation (TG) and impaired the proteolytic processing of FV species by thrombin or FXa. Importantly, no effects were observed in assays employing FVa (lacks AR2). Low concentrations of protamine restored TG in heparinized normal human plasma (NHP) and FV deficient plasma reconstituted with FV-short or FVa. However, high concentrations resulted in the progressive reduction of TG in NHP and FV-deficient plasma reconstituted with FV-short. Similar results were obtained using Poly-Arginine, however other basic molecules (e.g. histone H3, Poly-Lysine or platelet Factor 4) failed to replicate protamine's effects.
Conclusions: These results suggest protamine exerts its anticoagulant effect by interacting with physiological relevant FV_{AR} species. The interaction of protamine is specific and similar to how the endogenous FV-BR keeps FV inactive or how TFPIα blocks the procoagulant effects of FV_{AR}.

PB0231 | To Determine the Effect on Gender and Age of Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT) Levels and International Normalized Ratio (INR)

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Background: (PT)and aPTT assay is widely used test for diagnosing coagulation disorders.(INR) has been used to standardize PT value. Laboratory-specific reference intervals are essential for appropriate interpretation of test results.
Aims: The objective of our study is to determine whether PT, INR and aPTT levels differ between the genders and ages in patients without any known liver disease or anticoagulant use.
Methods: This retrospective study consist of PT, INR and aPTT levels of patients without any history of bleeding admitted in the civil hospital Hyderabad for minor surgical interventions (between April 2019 to July 2019 were included. There were 2274 patients comprises 912 males and 1362 females. Patients were classified into three groups according to age.
0–14 age group was consisted of 268 males and 22 females;15–50 age group was consisted of 222 males and 1335 females and there were 151 females and 276 males in the third group (over the age of 50 year). Plasma PT and aPTT levels were measured by SYSMEX CA-500 auto analyzer.
Results: The PT and INR levels of Females were significantly higher compared to males. The PT, INR and aPTT levels of males and females aged between 15–50 years were found to be significantly higher compared to other groups. The aPTT levels of male and female of the age of 0–14 were lowest. PT, INR and aPTT levels of women aged between 15 and 50 were significantly higher than females over the age of 50.PT, INR and aPTT levels of men above 50 were significantly higher than other group.PT, INR and aPTT reference ranges of male and female subjects, based on 95% confidence interval, and according to gender and age groups.
Conclusions: It would be more exact to take into account the gender and age differences for formative reference ranges of PT, INR and aPTT in clinical use.

PB0232 | Lysine Residues Impair the Efficacy of N-glycosylation: Pathological and Physiological Relevance in Antithrombin

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[1]*Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional, Murcia, Spain;* [2]*Aix-Marseille Univ, INSERM, INRA, C2VN, Laboratory of Haematology, CRB Assistance Publique - Hôpitaux de Marseille, HemoVasc (CRB AP-HM HemoVasc), Marseille, France;* [3]*INSERM UMR_S 1219, Bordeaux Population Health Research Center, University of Bordeaux, Bordeaux, France;* [4]*Institute of Clinical Medicine, University of Oslo; Research Institute of Internal Medicine, Oslo University Hospital, Norway; Department of Haematology, Oslo University Hospital, Oslo, Norway*

Background: N-glycosylation is a postranslational modification crucial on the correct folding, secretion, function and half-life of N-glycoproteins. Oligosaccharyltransferase decorates the Asn located in Asn-X-Ser/Thr sequons with oligosaccharides. Antithrombin,

the main endogenous anticoagulant, has 4 sequons. Asn167 is inefficiently glycosylated rendering two glycoforms: alpha and beta. Previously, we found two thrombophilic families carrying new *SERPINC1* variants in the Asn224 sequon (p.Asn224Thr and p.Glu227Lys) with no apparent antithrombin deficiency.

Aims: To study the effect of Lys residues on the efficacy of N-glycosylation and its pathological and physiological relevance.

Methods: Human recombinant antithrombins (rAT) were expressed in HEK293T-EBNA cells and purified by chromatography (FPLC). Glycosylation was studied by western-blot/silver staining and PNGase-F treatment. Functional analysis included chromogenic assays and identification of thrombin-antithrombin complexes by western blot. Heparin affinity was evaluated by endogenous fluorescence titration.

Results: $rAT^{p.Glu227Lys}$ in beta context (avoiding heterogeneity) generated two glycoforms due to inefficient N-glycosylation at Asn224. This result and the presence of 3 electropositive Lys flanking Asn167, suggested a negative role for these residues on the N-glycosylation. The mutation of any Lys flanking Asn167 ($rAT^{p.Lys165Ala}$; $rAT^{p.Lys168Ala}$; $rAT^{p.Lys171Ala}$) improved the glycosylation efficacy. In contrast, generation of Lys residues in different positions at Asn187 sequon, which lacks Lys, impaired its N-glycosylation.

Functional studies of the glycoform lacking glycosylation at Asn224 ($rAT^{p.Asn224Thr}$ in beta context) demonstrated higher heparin affinity compared to rAT^{bWT} (Kd:22.1±2.9nM *vs* 36.8±1.2nM, respectively) and null anti-FXa and anti-FIIa activity. The mixture of glycoforms expressed by $rAT^{p.Glu227Lys}$ had almost null anticoagulant activity.

Conclusions: The electropositive residue Lys modulates the N-glycosylation efficacy. Lys at +1, +3, +4, −2 and −4 of three different sequons of antithrombin significantly impaired the efficacy of this process. Moreover, antithrombins lacking two N-glycans (physiological at 167 and by natural variants at Asn224) have increased heparin affinity but null anticoagulant activity, which might contribute to the thrombotic risk.PI18/00598 (ISCIII&FEDER); 19873/GERM/15 (Fundación Séneca)

FIGURE 1 Efficacy of N-glycosylation in recombinant antithrombin by mutating Lys residues

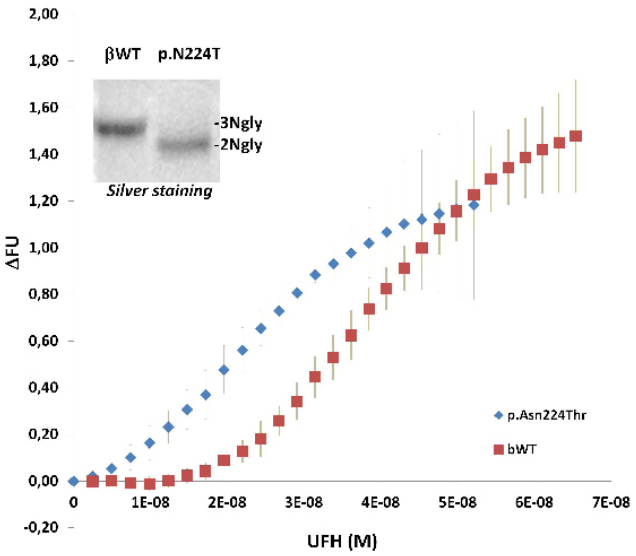

FIGURE 2 Heparin affinity measured by intrinsic fluorescence by heparin titration of p.Asn224Thr in beta context vs betaWT antithrombin

PB0233 | Whole Genome Sequencing Study of Coagulation Factor VIII and von Willebrand Factor Reveals New Genetic Associations

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The University of Texas Health Science Center at Houston, Houston, United States

Background: Coagulation factor VIII (FVIII) and its carrier protein von Willebrand factor (vWF) are implicated in increasing the risk of thrombotic events.

Aims: We aimed to combine whole genome sequencing data from NHLBI's TOPMed program with TOPMed-based imputation of genotypes in the CHARGE consortium to identify genetic associations with plasma levels of FVIII and vWF.

Methods: We included 9 TOPMed studies with European, African, Asian, and Hispanic ancestry participants. Association analyses in TOPMed were conducted across all individuals adjusting for age, sex, ancestry, principal components, and a kinship matrix. Using TOPMed as a reference panel, we imputed genotypes in 12 CHARGE studies. Association analyses in CHARGE were conducted separately within each study, stratified by ancestry group. Our primary analysis consisted of a meta-analysis of TOPMed and CHARGE (N=35,006 for FVIII and N=35,362 for vWF).

Results: We identified associations (P< 5E-9) with variants at 11 known loci for FVIII and 12 for vWF, of which 10 overlapped. Additionally, 4 new loci were associated with FVIII (*F12*, *KNG1*, *ASGR1*, and *CD36*). *F12* and *KNG1* encode kininogen 1 and factor XII, which are both involved in the contact activation pathway that FVIII also participates in. Variants at *F12* and *KNG1* were not associated with vWF (P>0.05). At the *ASGR1* locus, top variant rs62061426 is associated with *ASGR1* expression in liver. *ASGR1* encodes a protein

that degrades glycoproteins like FVIII and vWF. Consistent with this, the ASGR1-decreasing allele is associated with increased levels of FVIII. The driving variant in *CD36*, rs3211938, is a loss-of-function variant that causes CD36 deficiency and is associated with other hematological phenotypes. The loss-of-function allele was only found among the African ancestry participants (frequency = 11.6%). Top variants at *ASGR1* and *CD36* were suggestively associated with vWF (P< 0.05).

Conclusions: Four new loci were identified for FVIII, including 2 loci that were independent of vWF.

PB0234 | Baseline VWF Antigen Level Is Associated with the Outcome of Immune Tolerance Induction Using pdFVIII/VWF Concentrate in Hemophilia A with Inhibitor

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Background: The effect of Von Willebrand factor (VWF) on immune tolerance induction (ITI) in severe hemophilia A patients with inhibitor had not been clearly unveiled. Clinical observations also could not demonstrate ITI outcome is better when using concentrates containing VWF than avoiding VWF.

Aims: To describe the relationship between baseline VWF: Ag and ITI outcome under low-dose ITI/immunosuppression (IS) strategy.

Methods: A total of 38 pediatric study subjects (age, 0.8 to 13.2) with severe hemophilia A was recruited from Beijing Children's hospital in China between September 2016 to July 2019. Subjects had a high titer inhibitor and carrying high inhibitor risk of F8 mutation. VWF: Ag was tested before ITI initiated. Subjects received ITI at ~50 FVIII IU/kg (domestic pdFVIII/VWF) every-other-day alone, or in combination with IS (rituximab and prednisone).

Results: In these subjects, baseline VWF: Ag was median 105.7% (range 63.8–229.1%) and peak historic inhibitor titer of was median 28.4 (range 6.8–416) BU. Seventeen subjects with O type blood group, 21 subjects with non-O type blood group. ITI-alone treatment was given to 19 subjects, ITI-IS to another 19 subjects. The overall success (negative inhibitor) rate was 89.5% in median 8.7 months, no significant difference between two treatment groups. We observed that only baseline VWF: Ag (≥or < 110.5%, HR=0.451, P=0.035) was the predictor of ITI outcome in multivariate analysis rather than inhibitor titer and age. The baseline VWF:Ag also correlated with time to ITI success in patients with non-O blood type (r= 0.723, P<0.001), but no correlation was observed for type O patients.

Conclusions: Baseline VWF:Ag is a regulator of ITI outcome in low-dose ITI/IS strategy using pdFVIII/VWF concentrate especially when modified by the ABO blood group.

PB0236 | Assessment of Four Factor Prothrombin Complex Concentrate (4F-PCC) Usage in Emergency Department Patients: A Single Center Experience

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Background: 4F-PCC is commonly used in non-VKA anticoagulated patients. The clinical efficacy of this practice is still in question.

Aims: Retrospective assessment of the efficacy of 4F-PCC in non-VKA-anticoagulated patients within our emergency department (ED).

Methods: Data of patients who were given 4F-PCC upon admission to the ED over a 3-year period were collected and evaluated for VKA-treated (group A) and non-VKA-treated patients (group B). Changes in INR, bleeding events, and clinical outcome were assessed in both groups.

Results: Over 3 years, 147 patients received 4F-PCC at time of ED admission, of whom 71% (n=105) were from group A and 29% (n=42) were from group B. Vitamin-K was administered in 17 (16%) of group A patients and 8 (19%) of group B patients. The most common reason for 4F-PCC administration in both groups was correction of INR (67% and 71%, respectively). Bleeding at time of admission was less common in both groups (33% and 29%, respectively). A significant correction of INR was observed in group A (3.5±1.9 to 1.4±0.3, p< 0.05) but no significant changes were detected in group B (1.9±1.1 to 1.6±1.2, p=0.78). Bleeding was significantly controlled in group A patients over the first 24 hours (94% vs. 17%). Four patients (33%) died within 24 hours of admission in group B vs 2 patients (6%) in group A. No thrombotic events were reported in either group.

Conclusions: Our data showed that almost one third of patients treated with 4F-PCC are non-VKA treated patients. As expected, significant changes in INR were detected in the VKA-treated patients but not in the non-VKA-treated patients. A 24-hour assessment of bleeding and clinical outcomes revealed better responses in the VKA-treated patients. Although we report no direct adverse effects as a result of administration of 4F-PCC in our non-VKA-treated patients, no significant clinical benefits were detected either.

PB0237 | Sequence Mismatch Between Therapeutic (t) and Endogenous (e) Factor VIII Proteins (FVIIIs), and HLA-class-II (HLAcII)-binding in the Development of Neutralizing Anti-tFVIII-Antibodies in Hemophilia A (HA): Evidence from the PATH Study

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Background: Our group presented evidence supporting the "Sequence Mismatch" hypothesis that sequence-mismatches in tFVIIIs and corresponding sites in the dysfunctional/deficient eFVIIIs expressed by patients with pharmacogenetically-relevant HA-causing *F8* mutations may elicit inhibitor development.

Aims: Analysis of PATH data showed—using an area-under-the-curve (AUC) estimated from receiver operator characteristic (ROC) curve analysis—that HLAcII binding-affinities estimated with the NetMHCIIpan algorithm applied to overlapping-sets of 15-mer peptides spanning sequence-mismatches predicted inhibitor-status in 25 patients with the intron (I)22-inversion. Our aim here was to investigate—in a large cohort of mainly severe HA patients—how HLAcII-binding of peptides potentially derivable from sequence-mismatches contributes to inhibitor development while also accounting for genetic-relatedness and *F8* mutational-heterogeneity.

Methods: 442 North American HA patients (237 White and 205 Black) were: 1) Immuno-Chip genotyped at ~167,000 SNPs in autoimmune disease genes; 2) Evaluated by Sanger DNA-sequencing and PCR-/RT-PCR-based assays for the recurrent I1- and I22-inversions to identify causal-*F8*-mutations; and 3) Tested with the Nijmegen-modified Bethesda assay to determine inhibitor status. Immuno-Chip genotypes were used to construct a genetic-relationship matrix; the *F8* sequence and I1-/I22-inversion assay data were used to construct a shared *F8*-mutation matrix. These matrices allowed estimation of heritability and *F8*-mutation effects. We performed Cholesky decomposition of the phenotypic covariance matrix expressed as a function of the matrices, heritability, and *F8*-mutation effect estimate. The derived Cholesky factor was used to decorrelate the HLAcII-binding data, enabling unbiased ROC curve analysis and AUC estimation.

Results: We found that *F8*-mutation effect accounted for ~23% of the variability in inhibitor development ($p < 0.0001$) and that non-HA heritability accounted for ~50% ($p < 0.0001$). Here we report results demonstrating the validity of the Sequence Mismatch hypothesis while modeling the effects of genetic-relatedness, mutational-heterogeneity, and HLAcII-binding affinity.

Conclusions: This method accurately accounts for genetic-relatedness, mutational-heterogeneity, and HLAcII-binding in the development of inhibitors in severe HA patients with identifiable FVIII-sequence-mismatches.

PB0238 | Inactivation of Tissue Factor Pathway Inhibitor through Specific Citrullination by Peptide Arginine Deiminase 4

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Background: Tissue factor pathway inhibitor (TFPI) is an important regulator in hemostasis and a link between inflammation and thrombosis. Neutrophil extracellular traps (NETs) formed by neutrophils in a process termed "NETosis" can bind TFPI which is cleaved and inactivated by elastase during thrombotic events. In neutrophils, the enzyme peptide arginine deiminase 4 (PAD4) is crucial for NETosis.

Aims: To demonstrate that PAD4 regulates the activity of TFPI by posttranslational modification of its functional arginine residues into citrulline.

Methods: Citrullination of TFPI by PAD4 was monitored by FXa inhibition and plasma thrombin generation. Full-length (fl)TFPI, Kunitz domains K1K2, K2, and TFPI1-160 were used to study the effects of citrullination on FXa inhibition. Mass spectrometry was used to locate sites of citrullination.

Results: Experiments indicated that flTFPI was rapidly citrullinated by low concentrations of PAD4 (< 0.1nM). Citrullination of TFPI by PAD4 was calcium-ion, time- and concentration-dependent and was confirmed by mass spectrometry, revealing a preference for particular functional residues in TFPI. Citrullination of different TFPI fragments showed that the K2 domain was less efficiently inactivated by PAD4 than K1K2, TFPI 1-160, and flTFPI, which suggested an interaction between PAD4 and the C-terminus of TFPI. The presence of phospholipids inhibited the citrullination reaction only in flTFPI.

Thrombin generation in TFPI-deficient plasma triggered with TF or snake venom showed almost complete inactivity of added citrullinated flTFPI. The presence of nucleic acids, but not histones, appeared to accelerate the citrullination of TFPI by PAD4.

Conclusions: Taken together, flTFPI was shown to be very sensitive to citrullination by PAD4. Citrullinated TFPI lost its ability to inhibit FXa. This process might play a role in the observed increased thrombosis risk accompanied with inflammation or sepsis. Additional experiments are needed to determine the physiologic or pathologic relevance of this observation.

PB0239 | Always Ask Relevant Questions to Patients Might Be They Tell You the Diagnosis

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Background: Listen to patients; it explains that medicine should be a partnership between doctor and patient. The doctor will get a good picture, not only of your symptoms but also of their importance to you.

Aims: Aim of this survey was how many patients were neglected in Thrombosis and Hemostais unit at the time of requisition for testing of suspected hemostatic disorders by clinician.

Methods: The survey was approved by the ethics committee of the NIBD, Karachi, Pakistan, in accordance with the declaration of Helsinki. The contents of structured questionnaire were followed. It was an observational and time prospect, conducted from Feb 2019 till December 2019. Data was collected from Thrombosis and Haemostasis units of public and private sectors in all 4 provinces from in and out patients department. Simple descriptive was applied for data analysis.

Results: Total 200 cases were observed with different challenges like lacking of: **Bleeding Assessment:** 20% especially in females. **Drug History:** 20% like long term use of Hydrea, Warfarin, Heparin, clopidogrel, aspirin, site of blood collection, inappropriate reagents, vitamin K and inappropriate doses of anticoagulants. **Family History:** 20% inherited disorders like Von Willebrand Disease labelled as Hemophilia A, F.IX deficient labelled as F.VIII, Inherited Macro thrombocytopenia as ITP, Afibrinogenemia cases, Combined Clotting Factor Defects, Type 2 N VWD, **Infusion History:** 10%. Blood products, vitamin K especially inappropriate doses.

Concomitant Factors: 30% like Acidosis, haematological malignancies/benign disorders, Beta Thalassemia, Pregnancies, cardiac Problems, hypersplenism.

Example: 15 years old boy, known case of beta thalassaemia intermedia presented with nasal bleed and on hydrea since 13 years but nothing was mentioned on his request form. After lab investigation he labelled as acquired F.IX deficiency.

Conclusions: Trained clinicians in the field of thrombosis and haemostasis are scanty. Need training to follow structured questionnaire and understand importance of listening. Great clinician treats the patient with disease.

PB0240 | X-ray Crystallography and Hemostatic Activities of a Neutralizing Anti-TFPI Antibody, Marstacimab

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Background: An approach for achieving hemostatic pharmacology in hemophilia is neutralization of Tissue Factor Pathway Inhibitor (TFPI) to augment extrinsic coagulation pathway activity. TFPI, a kunitz-type domain (K1, K2 and K3) protease inhibitor of the extrinsic coagulation pathway, modulates coagulation by directly binding and inhibiting the Tissue factor / Factor VIIa / Factor Xa (FXa) complex. Understanding the interaction of TFPI with FXa at the molecular level would aid in the development of effective TFPI inhibitors and provide a molecular basis for the identification of novel neutralizing TFPI monoclonal antibodies.

Aims: The aim of these studies was to determine the structures of recombinant TFPI-K2 in complex with human Gla-domainless FXa and anti-TFPI (marstacimab)-TFPI-K2 complexes by X-ray crystallography

Methods: Binding affinities were determined using Surface Plasmon Resonance (Biacore). The dilute prothrombin time (dPT) and the thrombin generation (TGA) assays measured hemostatic activity in hemophilia plasma.

Results: The co-structure of TFPI-K2 with FXa showed interactions between TFPI-K2 and four surface loops in FXa. This included large conformational changes in two loops of FXa upon K2 binding, and the key interaction sites between R107 (TFPI-K2) and D189 (FXa), critical for the FXa inhibition by TFPI-K2. Marstacimab is a fully human neutralizing monoclonal antibody with an affinity (Kd) of 3.7nM to human TFPI-K1K2 domain. The co-structure of marstacimab Fab and TFPI K2 complex showed interaction of VH (heavy chain variable region) of the marstacimab Fab with the first R107-centered loop of the K2 domain in TFPI. The overlap of these two crystal structures indicated that marstacimab inhibits TFPI-FXa binding preventing the interaction between R107 and D189. This was further supported by additional in vitro studies. Marstacimab restored hemostatic activities in hemophilia plasma, including the shortening of dPT (IC_{50} of 1.3 nM) and increased thrombin generation (IC_{50} of 2.8 nM).

Conclusions: Marstacimab is currently in development for treatment of hemophilia.

CONTACT PATHWAY

PB0241 | Allosteric Modulation of Exosite 1 Attenuates the Enhancing Effect of Polyphosphate on FXI Activation by Thrombin

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Background: There is mounting evidence that feedback activation of factor (F) XI by thrombin is important for thrombus stabilization and growth. Polyphosphate (polyP) released from activated platelets enhances this reaction by binding FXI and thrombin and bridging them together.
Aims: To determine the contribution of thrombin exosites 1 and 2 to the potentiation of thrombin activation of FXI by polyP.
Methods: FXI was incubated with α-thrombin or a recombinant thrombin exosite 1 variant (K109E/K110E) in the absence or presence of polyP (6-mer, 20-mer, or 70-mer) and FXIa generation was quantified by measuring S2366 hydrolysis. The effect of HD1 and HD22, DNA aptamers that bind specifically to exosite 1 and 2 on thrombin, respectively, on these reactions was then determined.
Results: Whereas 70-mer polyP accelerated the rate of FXI activation by thrombin up to 1500-fold in a bell-shaped and concentration-dependent manner, shorter polyP polymers did not, suggesting that the template effect of polyP requires more than 20 phosphate units. In the presence of 70-mer polyP, HD1 and HD22 saturably attenuated polyP catalyzed FXI activation by thrombin by 50% and 70%, respectively. In contrast, in the absence of polyP, HD1 had no effect on FXIa activation, suggesting that HD1 is unlikely to sterically inhibit FXI binding to thrombin. With 70-mer polyP, the maximal activation of FXI by K109E/K110E thrombin was 30% less than that with α-thrombin.
Conclusions: When composed of at least 70 phosphate units, polyP serves as a template and binds FXI and thrombin and promotes their interaction. HD22 attenuates the reaction because exosite 2 mediates the interaction of thrombin with polyP. The attenuating effect of HD1 likely reflects allosteric modulation of exosite 2 and/or the active site. These findings suggest that allosteric modulation of thrombin's exosites may represent a novel strategy for downregulating FXI activation.

PB0242 | Histidine-Rich Glycoprotein Attenuates the Prothrombotic Activity of Polyphosphate in a FXII-Dependent Manner

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Background: Histidine-rich glycoprotein (HRG) is an abundant plasma protein that binds factor (F) XIIa and inhibits FXII autoactivation and FXIIa-mediated activation of FXI. Polyphosphate (PolyP) is a potent procoagulant released by activated platelets that serve as a physiological activator of the contact pathway. Previously, we showed that HRG binds DNA and neutralizes its procoagulant activity, so the capacity of HRG to bind polyanions may endow it with a regulatory role in thrombosis. Therefore, we hypothesized that HRG would also bind PolyP and attenuate its procoagulant activity.
Aims: To determine whether HRG binds PolyP and attenuates its prothrombotic activity in a FXII dependent manner.
Methods: Binding of immobilized short-chain PolyP (70-mer) to HRG was measured using an enzyme-linked immunosorbent assay (ELISA). PolyP-induced thrombin generation was performed in HRG-depleted and control plasma. HRG-knockout ($HRG^{-/-}$) and wild-type (WT) mice were given intraperitoneal PolyP (100 mg/kg) or an equivalent volume of saline. Blood collected at intervals was used for the determination of thrombin generation and thrombin-antithrombin complexes (TAT) levels. After 60 min, lung perfusion and fibrin deposition were assessed with Evans blue dye and immunohistochemistry, respectively. To examine the role of FXII, $HRG^{-/-}$ and WT mice were given a FXII-specific antisense oligonucleotide (ASO) (40 mg/kg/wk) for four weeks before PolyP administration.
Results: HRG binds PolyP with a K_d value of 152 ± 6 nM. PolyP-induced thrombin generation is 2-fold greater in HRG-depleted plasma than in control plasma. Intraperitoneal PolyP enhances peak thrombin of thrombin generation, increases TAT levels, reduces lung perfusion, and promotes pulmonary fibrin deposition to a greater extent in $HRG^{-/-}$ mice than in WT mice and these effects are attenuated with FXII knockdown.
Conclusions: HRG binds PolyP and attenuates its prothrombotic activity in a FXII dependent manner. These findings provide evidence of another mechanism via which HRG modulates the intrinsic pathway of coagulation.

PB0243 | First Evaluation of the Safety, Pharmacokinetics and Pharmacodynamics of BAY 2433334 a Small Molecule Targeting Coagulation Factor XIa in Healthy Young Male Participants

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Background: Anticoagulants are the cornerstone of prevention and treatment of thrombotic disorders, even though those currently in clinical use are associated with risk of bleeding, limiting their antithrombotic potential. Coagulation factor XI (FXI) contributes to the development of thrombosis but plays only a minor role in hemostasis and is therefore an attractive target for novel antithrombotic therapy without the risk of relevant bleeding.

Aims: To evaluate the safety, pharmacokinetic (PK) and pharmacodynamic (PD) properties of BAY 2433334, a small molecule targeting FXIa, in healthy male participants.

Methods: This Phase I study was divided into two parts. A single dose escalation with a focus on safety, tolerability, PK, and PD properties of BAY 2433334. Oral doses of 5 mg up to 150 mg were administered orally. Second part investigating the effect of food on the PK of BAY 2433334. Adverse events were closely monitored. Key PK parameters included exposure, maximum plasma concentration and terminal half-life. The coagulation properties were assessed using activated partial thromboplastin time (aPTT), rotational thromboelastometry, bleeding time and FXIa activity. The study was approved by the local ethics committee and all volunteers provided written informed consent.

Results: BAY 2433334 demonstrated a favorable safety profile. BAY 2433334 showed a terminal half-life of approximately 15 hours allowing for a once daily administration. The intake of food decreased the peak concentrations by 31% compared to fasted state with a minor effect on exposure (decrease by 12.4%). Dose- and time dependent inhibition of FXIa activity and increase in aPTT were observed. There was no increase in mean bleeding time assessed by Surgicutt®.

Conclusions: BAY 2433334 was safe and well tolerated and demonstrated a favorable PK/PD profile in healthy volunteers with once-daily dosing potential. It is currently being evaluated in several Phase II dose finding programs.

PB0244 | Are Therapeutic Immunoglobulins Free from Factor XIa?

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Background: In 2010, an intravenous immunoglobulin (IVIG) product was temporarily removed from the market due to an association with serious thromboembolic events. Investigations by manufacturers and regulators revealed that factor XIa (FXIa) was present as a process-related impurity. Subsequently, efforts have been made by manufacturers to reduce or remove procoagulant activity from these therapeutics.

Aims: This study investigates levels of FXIa present in current marketed immunoglobulin products.

Methods: Nine immunoglobulin products (comprising IVIG and subcutaneous immunoglobulins) from 5 manufacturers were tested for FXIa content using the Rossix chromogenic assay. For 3 products, 2 different lots were tested. Samples were spiked with 1 mIU/ml FXIa, or left unspiked, and subsequently tested in duplicate across a minimum of 3 dilutions. Assays (n≥2 for each product) used the 1st International Standard (IS) for FXIa (13/100, NIBSC) as the standard.

Results: Results were analysed by parallel line assay and are shown in Tables 1 (IVIG) and 2 (subcutaneous immunoglobulins). Six of the products tested unspiked showed FXIa levels below the limit of quantification (LOQ) of 0.03 mIU/ml, with the corresponding spiked samples showing near 100% recovery of FXIa. One product had detectable and quantifiable levels of FXIa without spiking. Two products, unspiked, showed a dose response that was not parallel. Here, results ranges are reported, representing the 95% confidence limits. These products showed a FXIa recovery above 1 mIU/ml when spiked, the difference reflecting the level measured in the unspiked sample.

Conclusions: This study shows that current, marketed, immunoglobulin products can contain detectable levels of FXIa. Where valid results cannot be obtained from unspiked samples, this study has shown that spiking with FXIa brings the levels into the quantifiable range of the assay. Accurate measurement is important to inform on "safe" levels of FXIa in these products and allow future safety guidelines to be set.

TABLE 1 FXIa in intravenous immunoglobulins

Manufacturer	IVIG Product	FXIa level in mIU/ml (95% confidence limits) Unspiked sample	Sample spiked with 1 mIU/ml FXIa
A	1	<LOQ	1.06 (0.97–1.15)
	2A	- (0.26–0.31)	1.30 (1.29–1.31)
	2B	- (0.16–0.52)	1.26 (1.07–1.49)
B	1	<LOQ	0.95 (0.94–0.95)
C	1A	<LOQ	1.00 (0.76–1.32)
	1B	<LOQ	0.98 (0.75–1.30)
	2	<LOQ	0.96 (0.79–1.17)
D	1	<LOQ	0.97 (0.70–1.35)

TABLE 2 FXIa in subcutaneous immunoglobulins

Manufacturer	Subcutaneous Immunoglobulin Product	FXIa level in mIU/ml (95% confidence limits) Unspiked sample	Sample spiked with 1 mIU/ml FXIa
A	3A	4.39 (4.29–4.49)	Not required
	3B	1.52 (1.41–1.64)	Not required
B	2	<LOQ	0.94 (0.84–1.06)
E	1	- (0.06–0.28)	1.11 (0.93–1.32)

PB0245 | ONO-1600586, an Oral Direct Factor XIa Inhibitor, Suppresses Thrombus Formation in Rabbit Jugular Vein without Bleeding Tendency

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Background: Current anticoagulants can reduce risks of venous thromboembolism and ischemic stroke, however, they have a certain risk of critical bleeding. Activated blood coagulation factor XI (FXIa) is a serine protease involved in the amplification of thrombin production. Elevated levels of plasma factor XI are associated with venous thromboembolism and ischemic stroke, while subjects with congenital FXI deficiency have less bleeding tendency.
Aims: To evaluate the effect of ONO-1600586, an oral direct FXIa inhibitor, in a rabbit model of venous thrombosis and skin bleeding.
Methods: Rabbits with fasting condition were orally administered a solvent as a control, a FXIa inhibitor (ONO-1600586, 50mg/kg), or a factor Xa inhibitor (rivaroxaban, 15 mg/kg) 90 minute before thrombus formation. The venous thrombus was induced by a balloon injury and stenosis in rabbit jugular vein, and was weighted 3 hours after the thrombus formation. The bleeding time and volume were evaluated with 3 cm length of skin incision on thigh. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured before, 75 minutes and 4.5 hours after the drug administration.
Results: ONO-1600586 prolonged aPTT 2.7 times but not PT at 4.5 hours. Rivaroxaban prolonged PT 1.5 times and aPTT 1.5 times at 4.5 hours. ONO-1600586 (81 +/- 65 mg, n=11, p< 0.01) and rivaroxaban (64 +/- 51 mg, n=9, p< 0.01) similarly reduced venous thrombus weight, compared with that of control (207 +/- 91 mg, n=9). Rivaroxaban prolonged the bleeding time (20 +/- 8 minutes, n=9, p< 0.05) and increased the bleeding volume (185 +/- 86 µL, n=9, p< 0.05), compared with that of control (7.8 +/- 4.7 minutes, 83 +/- 30 µL, n=9) and ONO-1600586 (8.3 +/- 4.2 minutes, 94 +/- 46 µL, n=11).
Conclusions: ONO-1600586 may be a safer anticoagulant targeting FXIa for venous thromboembolism.

PB0246 | Detecting Oral Kallikrein-Targeting Therapy through Triggered Contact Activation: A Phase I Study

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Background: Plasma kallikrein (PKa) is a critical disease mediator in hereditary angioedema caused by C1 esterase inhibitor deficiency (C1INH-HAE). This disorder is characterized by dangerous attacks of bradykinin-mediated tissue swelling. PKa cleaves high-molecular-weight kininogen (HK) into a cleaved form (cHK), which liberates bradykinin. The oral PKa inhibitor ATN-249 is under clinical development for treatment of C1INH-HAE, and was investigated in a phase I randomized, double-blind, placebo-controlled single ascending dose study (ACTRN12618000430235).
Aims: To monitor the biological activity of ATN-249 in plasma.
Methods: Blood samples were collected from 48 healthy male participants at predose, 2, 4, 9, 12 and 24 hours after intake of a single dose ATN-249 (50–800 mg). Each dose cohort consisted of 6 subjects receiving active compound and two subjects receiving placebo. Contact activation was triggered with ellagic acid in subject plasma samples. We monitored PKa activity with a fluorogenic substrate, and cHK generation by ELISA.
Results: Consecutive samples (collected over 24 hours) of placebo-treated subjects developed comparable levels of PKa activity and cHK after *ex vivo* stimulation. However, we found a surprisingly large inter-individual variation (coefficient of variation of 41.7% for PKa activity, and 27.4% for cHK generation) between study subjects. We subsequently normalized data to predose levels to facilitate grouped comparisons. ATN-249 dose-dependently attenuated triggered PKa activity and -cHK generation. Two hours after intake, 800 mg ATN-249 inhibited triggered PKa activity by 92.5% and -cHK generation by 41% (Fig. 1). Statistically significant effects were detectable until 24 hours and 9 hours after intake, respectively.
Conclusions: The considerable inter-individual variability in triggered PKa activity and cHK generation argues against a generalized cutoff value for these parameters in C1INH-HAE management and motivates development of personalized approaches. These assays are useful to monitor the biological activity of the oral PKa inhibitor ATN-249 in plasma.

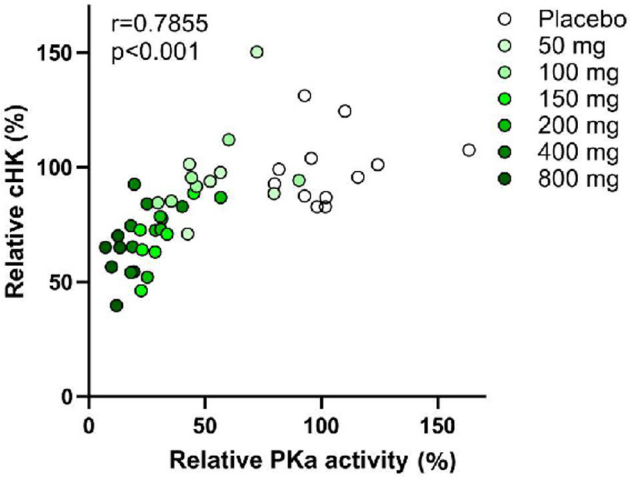

FIGURE 1 ATN-249 attenuates PK activity and cHK generation (normalized) in plasma samples of healthy study subjects

PB0248 | A Critical Role of Carboxyl-Terminal Alpha-Helix in the Secretion of Coagulation Factor XI

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Background: Coagulation factor XI (FXI) is a plasma serine protease zymogen that belongs to the intrinsic pathway of blood coagulation. However, a molecular machinery of FXI secretion has remained unclear.

Aims: To disclose a molecular basis of FXI secretion by characterizing a novel FXI mutant identified in a Japanese FXI deficiency patient.

Methods: The patient is a 75-year-old Japanese male with FXI:C < 3%. Genetic mutation of FXI gene (*F11*) was analyzed with direct sequencing. Mutant FXI corresponding to the identified *F11* mutation was overexpressed in HEK293 cells and examined the extracellular secretion with western blotting. Subcellular localization of the mutant FXI was investigated with immunocytochemistry.

Results: We identified a novel homozygous frameshift mutation in *F11* (c.1788dupC: p.Glu597Argfs*65), resulting in a C-terminal extension. The C-terminal extended FXI was intracellularly synthesized, but represented an extracellular secretion defect. Subcellular localization analysis showed that the extended mutant FXI was not detected at the Golgi apparatus, indicating an abnormal retention within the endoplasmic reticulum. To further investigate the roles of C-terminal region in the FXI secretion, we generated a series of recombinant FXIs gradually truncated at the C-terminus. The truncated FXIs revealed that a preservation of threonine at position 622, a fourth residue from the C-terminus, was essential for the FXI secretion. *In silico* structure simulation predicted that the residue at position 622 engaged to constitute an alpha-helix, suggesting an importance of the C-terminal alpha-helix motif for the FXI intracellular behavior and secretion.

Conclusions: Given the observation of the secretory defect in the C-terminal extended FXI mutant (p.Glu597Argfs*65), we demonstrated the critical role of C-terminal region, especially alpha-helix motif, in the FXI extracellular secretion.

PB0249 | Activation of Coagulation by 5.8S RNA

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Background: Hemolytic disorders are prone to thrombosis. Previous work showed the RNA released from damaged cells activates clotting. The type of RNA released from hemolysis and its coagulation function is not known.

Aims: To test whether the hemolysis releases coagulation-inducing RNA and identify that RNA.

Methods: Zebrafish red cell lysate was gel-electrophoresed, and RNA-Seq was performed. RT-PCR, *in vitro* transcription, and restriction digestions were performed according to the manufacturer recommendations. Deoxy-oligonucleotides were synthesized. Coagulation assays and laser-induced thrombosis assays were according to published methods. Student's t-test was used in statistical analysis.

Results: The lysate from zebrafish red cells showed a single band of RNA on gels and was identified as 5.8S RNA (5.8SR) by RNA-Seq analysis. 5.8SR activated coagulation in zebrafish plasma. *In vitro* synthesized 5.8SR, its shorter fragments and mutated versions were made using DNA templates produced by RT-PCR or synthetic deoxyoligonucleotides. We found 3′-end 26 nucleotides (3′-26) with the stem and loop were sufficient for inducing clotting activity. Similar results were obtained with human RBC lysates and plasma. We found prolonged clotting time and time to occlusion in laser-induced thrombosis assays in the presence of corn trypsin inhibitor and 5.8SR monoclonal antibody (5.8SRmAb). Similar inhibitions were found with human plasma. 5.8SRmAb injections into adult fish resulted in higher bleeding in mechanical injury assay. Since zebrafish has no factor XII and hepatocyte growth factor activator (HGFA) has similarities to factor XII, we knocked down the HGFA in adult zebrafish and found that the plasma from this fish does not respond to 3′-26.

Conclusions: We identified 5.8SR released in hemolysis to activate clotting in human and zebrafish plasma. Only 3′-26 is needed for the activity. 5.8SRmAb injections cause bleeding. Thus, it may have clinical utility in treating thrombosis. Furthermore, we showed that fish HGFA activates coagulation. Thus, factor XII may have evolved from HGFA.

PB0250 | Coagulation Assays Based on Surface-Mimicking Antibodies against Factor XII

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Background: Blood coagulation testing is useful clinically as a screening test for bleeding and hypercoagulable disease states. The activated partial thromboplastin time (aPTT) is one of the most commonly used coagulation tests, performed over 5 billion times annually. The aPTT assay measures the time it takes for recalcified plasma to clot when exposed to substances that activate coagulation factor XII (FXII), a process known as "contact activation".

Aims: To identify the mechanism of FXII contact activation and establish coagulation diagnostics based on surface-mimicking antibodies that activate FXII.

Methods: Recombinant FXII deletion mutants were used to systematically scan for sequences that are involved in contact activation *in vitro* and *in vivo*. Antibodies against the contact activation site on FXII were generated and used for regulatable plasma contact activation.

Results: Reconstitution of FXII-deficient plasma with FXII mutants identified that residues Gln317-Ser339 in the proline-rich domain were essential for foreign- and physiologic surface-driven zymogen FXII activation. FXII deficiency in this 23-residue segment erased surface-triggered thrombin formation and coagulation, however FXII mutants lacking the contact activation site were activatable by proteolysis. A peptide containing Gln317-Ser339 residues interfered with surface-induced coagulation. Thrombus formation was defective in FXII gene-deleted (*F12*$^{-/-}$) mice. Reconstitution of *F12*$^{-/-}$ mice with full length FXII but not with FXII variants lacking the contact activation site restored defective thrombus formation. Based on these studies, we developed antibodies against the contact activation site on FXII. These surface-mimicking antibodies initiated FXII activation, induced thrombin generation and triggered the intrinsic coagulation pathway in a regulatable manner. FXII-activating antibodies enabled us to standardize aPTT reagents and allowed for improved coagulation assays in the absence of particulate surfaces.

Conclusions: The present study adds mechanistic insight into FXII activation *in vitro* and *in vivo* and translates the findings to establish antibody-driven controllable aPTT coagulation assays.

PB0251 | Factor XII and Kininogen Complex with gC1qR/C1QBP/P32 Is Governed by Zinc Ions

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Background: A key receptor for appropriate assembly and activation of the contact system (consisting of factor XII (FXII), prekallikrein (PK) and kininogen (HK)), is trimeric gC1qR/C1QBP/P32.

Aims:

To understand::

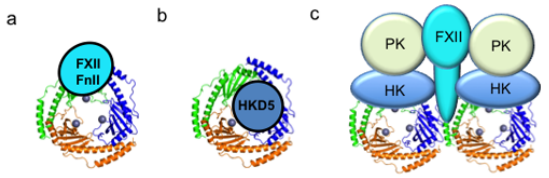

Figure 1: Schematic diagram of a (a) FXIIFnII domain-gC1qR complex (b) HKD5-gC1qR complex and (c) FXII-HK-gC1qR-PK complex with a 1:2:6:2 stoichiometry. In this model gC1qR is capable of stimulating reciprocal FXII-PK activation by aligning the activation loops and active sites of FXII and PK

FIGURE 1

(1) the structure of the complex formed between FXII, HK and gC1qR,: (2) how Zn^{2+} ions regulate complex formation.

Methods: The key residues involved in the FXII interaction with gC1qR were analysed by protein crystallography coupled with ITC and SPR. The functional consequences of the gC1qR-FXII interaction were studied using thrombin generation and clotting assays.

Results: The FXIIFnII-gC1qR-Zn^{2+} complex crystal structure provides the first insight into a gC1qR ligand interaction and shows a novel asymmetric stoichiometry of one FXIIFnII bound to the gC1qR trimer. The structure reveals key gC1qR residues coordinate a Zn^{2+} ion adjacent to the FXII binding site and a comparison with the Zn^{2+} free gC1qR crystal structure reveals the crucial conformational change underlying the Zn^{2+} dependent ligand binding. We also studied HKD5 binding and discovered that this is also asymmetric driven by the central location of a critical negatively charged loop in the gC1qR trimer and thus steric occlusion. We tested the effects of gC1qR on plasma coagulation to establish whether contact activation can occur under conditions where both gC1qR and Zn^{2+} are added. In the absence of additional stimuli, a shortened clotting time with pooled normal plasma was observed in a dose and FXII-dependent manner. Gel filtration experiments reveal that gC1qR clusters FXII and HK into a higher order 500kDa ternary complex a schemtic of which is should below in Figure 1.

Conclusions: The crystal structure of FXII in complex with gC1qR reveals an asymmetric interaction and bound Zn^{2+} ions. gC1qR undergoes a major conformational change upon FXII binding and the gC1qR-FXII-HK complex forms a higher order 500kDa complex.

PB0252 | Importance of the Non-Catalytic Heavy Chains of the Homologs Factor XII and HGFA in Protease Function

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Background: The proteases factor XII (FXII) and its homolog hepatocyte growth factor activator (HGFA) contain non-catalytic heavy chains (HC) and catalytic light chains (LC). FXII-HC binds to negatively charged surfaces such as heparin or polyphosphate, enhancing FXII activation by kallikrein and autoactivation. HGFA activation by thrombin is also surface-dependent. In the absence of surface FXII-HC down-regulates FXII activation by kallikrein.

Aims: To compare FXII-HC and HGFA-HC in interactions with charged surfaces and protease activation.

Methods: Recombinant FXII, HGFA, a chimera containing HGFA-HC and FXII-LC ($HGFA^{HC}FXII^{LC}$), and a chimera containing FXII-HC and HGFA-LC ($FXII^{HC}HGFA^{LC}$) were prepared. Protein binding to heparin-Sepharose and protein activation were compared.

Results: The human FXII-HC carries a positive charge while FXII-LC is negatively charged. The situation is reversed in HGFA (HGFA-HC negatively charged; HGFA-LC positively charged). The order of protein affinity for heparin was $FXII^{HC}HGFA^{LC}$ > FXII > HGFA. $HGFA^{HC}FXII^{LC}$ did not bind heparin. These results are consistent with the charges on the different chains. Interestingly, while neither chimera autoactivated in the presence of polyphosphate, $HGFA^{HC}FXII^{LC}$ autoactivated on a positively charged surface (polyethyleneimine). In the absence of surface, $HGFA^{HC}FXII^{LC}$ activation by kallikrein and $FXII^{HC}HGFA^{LC}$ by thrombin were substantially faster than FXII and HGFA activation by the respective proteases. Unlike FXII and HGFA activation, $HGFA^{HC}FXII^{LC}$ and $FXII^{HC}HGFA^{LC}$ activation were not enhanced by surface.

Conclusions: While FXII binds surfaces via positive charges on its HC, HGFA probably binds through positive charges on its LC. The capacity of $HGFA^{HC}FXII^{LC}$ to autoactivate on polyethyleneimine emphasizes the importance of charge interactions in surface-dependent FXII activation. Enhanced activation of $HGFA^{HC}FXII^{LC}$ and $FXII^{HC}HGFA^{LC}$ relative to FXII and HGFA in the absence of surface indicates the HCs of both FXII and HGFA normally inhibit surface-independent activation, probably by masking the activation cleavage sites, and that this process is disrupted in the chimeras.

PB0253 | Structure-Function Relationships in Factor XII Studied with Factor XII-HGFA Chimeras

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Background: Factor XII (FXII) is proteolytically converted to the protease (FXIIa) by kallikrein or autoactivation on negatively charged surfaces. The FXII non-catalytic heavy chain (HC) contains six domains: (from the N-terminus) the fibronectin type II (FnII), epidermal growth factor-1 (EGF-1), fibronectin type I (FnI), EGF-2, and kringle domains, and the proline-rich region (PRR). FXII shares this arrangement with its homolog hepatocyte growth factor activator (HGFA). The FXII-HC mediates binding to surfaces during contact activation, and inhibits FXII activation by kallikrein in the absence of surface by blocking access to the activation cleavage site. The roles of the individual HC domains in these processes is incompletely understood, but replacing the FXII-HC with the HGFA-HC disrupts both functions.

Aims: Determine the importance of FXII-HC domains to FXII autoactivation and activation by kallikrein.

Methods: A panel of FXII variants with one HC domain replaced with the corresponding HGFA domain was prepared. FXII with the FnII replacement did not express, so FXII lacking FnII (DFnII) was used. Protein activation was assessed with chromogenic assays.

Results: The FXII/HGFA EGF-1 and kringle chimeras demonstrated defects in autoactivation in the presence of polyanionic polyphosphate. Interestingly EGF-1 autoactivated on a positively charged surface (polyethyleneimine). Other chimeras activated similarly to FXII. DFnII and the EGF-1, FnI, EGF-2 and kringle chimeras, but not the PRR chimera, showed significantly enhanced activation by kallikrein in the absence of polyphosphate compared to FXII.

Conclusions: The FXII EGF-1 and kringle domains appear to play important roles during autoactivation on polyphosphate. In the case of EGF1, this may include a charge interaction with the surface. The ability of FXII-HC to restrict activation by kallikrein in the absence of surface is sensitive to perturbations throughout the HC, suggesting an alteration in the precise positioning of the HC relative to the activation cleavage site.

PB0254 | Effects of Plasmin on Factor XII Structure and Activation

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Background: As a component of the kallikrein-kinin system, factor XIIa (FXIIa) contributes to bradykinin generation by converting prekallikrein to kallikrein. Kallikrein, in turn, cleaves the precursor FXII after Arg353, forming FXIIa. FXII variants with Lys/Arg substitutions for Thr309 are cleaved by several proteases after Lys/Arg309, forming truncated FXII (Thr310-Ser596) that is activated by kallikrein more rapidly than full-length FXII. This explains why patients with Thr309Arg/Lys are prone to bradykinin-mediated soft tissue swelling (angioedema). Plasmin also activates FXII by cleavage after Arg353, although the reaction is relatively slow. Angioedema occasionally occurs in patients receiving fibrinolytic therapy. While this may reflect plasmin cleavage of FXII after Arg353, proteolytic FXII truncation, even in the absence of Thr309Arg/Lys, is also possible.

Aims: To study the effects of plasmin on FXII structure and activation.

Methods: FXII cleavage and activity were assessed with western blots and chromogenic substrate assays.

Results: Plasmin cleaves FXII at several locations. Cleavage after Arg353 appears to be a relatively minor event. Major cleavage events occurred in the FXII proline-rich region after Arg334, Arg326 and Arg291. Truncated recombinant FXII lacking sequence N-terminal to Arg334, Arg326 and Arg291 are activated by kallikrein ~15-times faster than full-length FXII. When added to plasma, truncated species, but not full-length FXII, induced rapid cleavage of high-molecular-weight kininogen in a manner not dependent on a surface/polyanion to induce contact activation.

Conclusions: In addition to converting FXII to FXIIa by cleavage after Arg353, plasmin may cleave FXII at other sites, creating truncated

FXII forms that are activated by kallikrein more rapidly than full-length FXII. This observation may be relevant to the angioedema that occurs in occasional patients treated with fibrinolytic therapy to alleviate blood vessel obstruction. If this mechanism is relevant to angioedema, it remains to be determined why it occurs in some patients, but not others.

PB0255 | Evolutionary History of the Kallikrein-Kinin System and Factor XI

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Background: Whole genome sequences have facilitated reconstruction of the natural histories of complex processes, such as vertebrate blood coagulation (Figure).

Aims: To update and correct our prior work on the evolution of the kallikrein-kinin system (KKS) proteases prekallikrein and factor (FXII), and their connection to coagulation through factor XI (FXI).

Methods: Genomes of multiple species of fish, amphibians, reptiles, birds and mammals were surveyed with BLAST searches using human protein sequences for prekallikrein, FXII, and FXI.

Results: Prekallikrein is not found in common ray-finned or cartilaginous fish, but is present in the lobe-finned coelacanth (*Latimeria*) and lungfish (*Protopterus*). Lobe-finned fish are ancestral to terrestrial vertebrates (tetrapods: amphibians, reptiles, birds, and mammals). Prekallikrein is found in all tetrapods, except for diving mammals (cetaceans), where the gene has been lost. FXII was not identified in common fish or *Latimeria*, but was present in *Protopterus*, amphibians, reptiles and most mammals. It has been lost in birds and cetaceans. FXI arose either in a reptile-like proto-mammal or primitive egg-laying mammal (monotreme) by duplication of the prekallikrein gene.

Conclusions: Our analysis indicates prekallikrein and FXII appeared in lobed-finned aquatic ancestors of tetrapods. This suggests a functional KKS may have been present earlier than previously suspected. Interestingly, parts of the KKS are missing in some groups, suggesting the proteins can function independently of each other. For example, prekallikrein is present without FXII in birds and *Latimeria*, consistent with the observation that prekallikrein is activated by FXIIa-independent processes in humans. FXI is found only in mammals, explaining why KKS activation by contact activation induces clotting in plasma from mammals, but not other tetrapods. It also

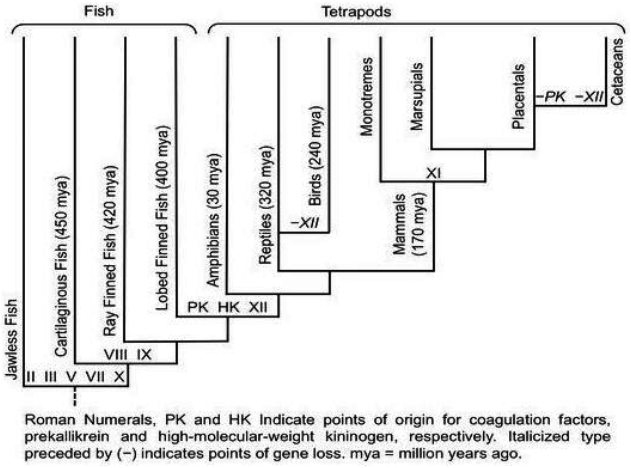

FIGURE 1 Evolution of the vertebrate plasma coagulation and kallikrein-kinin systems

appeared earlier than previously suspected. The presence of FXI in cetaceans in the absence of prekallikrein and FXII supports the hypothesis that FXI can be activated independently of FXIIa and contact activation.

PB0256 | Neutralization of Osocimab-Induced Anticoagulation with Prothrombin Complex Concentrate, Activated Prothrombin Complex Concentrate and Recombinant Activated FVII in vitro

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Background: Coagulation factor XI (FXI) contributes to the development of thrombosis but plays only a minor role in hemostasis and is therefore an attractive target for antithrombotic therapy. Inhibition of FXIa offers the potential to reduce the risk of thrombosis without increasing the risk of bleeding. Osocimab is a fully human antibody which binds specifically to activated FXI and shows anticoagulant efficacy *in vitro* and *in vivo*. A neutralization of the anticoagulant effect might be desirable in emergency situations.

Aims: To evaluate the *in vitro* reversal effect of prothrombin complex concentrate, activated prothrombin complex concentrate and recombinant activated FVII on osocimab-induced anticoagulation.

Methods: Neutralization of osocimab (0.1–1.5µM)-induced anticoagulation with prothrombin complex concentrate (Beriplex®, 0.04–5.0 U/ml), activated prothrombin complex concentrate (FEIBA®, 0.004–2.0 U/ml) and recombinant activated FVII (NovoSeven®, 0.1–1500nM) was assessed in activated partial thromboplastin time (aPTT), thrombin generation assay (TGA) in human plasma and in thromboelastometry clotting time (CT) in human whole blood *in vitro*.

Results: The three agents, prothrombin complex concentrate, activated prothrombin complex concentrate and recombinant activated FVII, showed different profiles to reverse the osocimab-induced

anticoagulant effects *in vitro*. FEIBA and rFVIIa fully reversed prolongations of aPTT and thrombeoelastometry CT, whereas PCC did not show any effect. FEIBA had a superior effect to NovoSeven and PCC in reversing the osocimab-induced changes of the thrombin generation parameters.

Conclusions: The extent of neutralization by PCC, aPCC and rVIIa was dependent on the parameter measured in osocimab-anticoagulated human plasma or blood. aPCC and rVIIa effectively neutralized most of the evaluated parameters.

PB0258 | Identification of the Histidine-rich Glycoprotein Domain Responsible for Contact Pathway Inhibition

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Background: Previously, we showed that histidine-rich glycoprotein (HRG) binds factor (F) XIIa with high affinity, inhibits FXII autoactivation and FXIIa-mediated activation of FXI, and attenuates ferric chloride-induced arterial thrombosis in mice. Therefore, HRG downregulates the contact pathway in vitro and in vivo.

Aims: To identify the domain on HRG responsible for contact pathway inhibition.

Methods: PCR-amplified HRG domain constructs (N-terminal [N1, N2, and N1N2], proline-rich regions [PRR1, PRR2], histidine-rich region [HRR], and C-terminal [COOH]) were subcloned into maltose-binding protein (MBP)-containing pMALc5e vectors and expressed in *E. coli*. Plasma-derived HRG and recombinant domain constructs were examined for their (a) affinities for FXII, FXIIa, and polyphosphate by surface plasmon resonance (SPR) and immunoassay, and their effects on (b) polyphosphate (70mer) mediated FXII autoactivation and (c) the activated partial thromboplastin time (APTT) in HRG-depleted human plasma.

Results: Using SPR, plasma-derived HRG and recombinant domain constructs bind FXIIa, but not FXII or β-FXII. HRR binds FXIIa with a Kd of 129 nM, similar to that of HRG (Kd 51 nM). By immunoassay, HRR and PRR2 bind immobilized biotin-polyphosphate with high affinity (Kd 12 nM and 26 nM, respectively), whereas HRG binds with a Kd of 152 nM. HRG and HRR inhibit polyphosphate-mediated FXII autoactivation by over 85% with IC50 values of 200 and 500 nM, respectively. PRR2 has an IC50 of 652 nM, whereas the remaining domains have significantly lower potencies (IC50 >800 nM). In the APTT, each of the constructs has little effect (prolongation < 2-fold), except HRR which, like HRG, significantly prolongs clotting in HRG-depleted plasma by 7-fold.

Conclusions: The interaction of HRG with FXIIa and polyphosphate is predominantly mediated by the HRR domain. Therefore, by downregulating the contact system, HRR is the domain responsible for the anticoagulant properties of HRG.

PB0259 | Design of Polyphosphate Inhibitors that Reduce Thrombosis without Increased Bleeding

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[1]*University of British Columbia, Centre for Blood Research, Vancouver, Canada;* [2]*University of British Columbia, Department of Chemistry, Vancouver, Canada;* [3]*University of Michigan, Department of Biological Chemistry, Ann Arbor, United States;* [4]*University of British Columbia, Department of Pathology and Laboratory Medicine, Vancouver, Canada;* [5]*University of British Columbia, Department of Chemical and Biological Engineering, Vancouver, Canada*

Background: Polyphosphate (polyP) has been shown to be a potent procoagulant polyanion. We present a strategic design of inhibitors with high specificity to polyP and minimized non-specific interactions. A switchable protonation state has been utilized, whereby the inhibitors present a low charge state in solution prior to polyP binding but increase its cationic charge upon binding to polyP thereby increasing its potency.

Aims: Design a polyP inhibitor that exhibits a tailored affinity for polyP and minimized non-specific interactions with blood components to prevent adverse effects on bleeding.

Methods: Inhibitors were synthesized using a systematic increase of varied cationic moieties (ligands) to generate a library of compounds on a polymer scaffold. The library was characterized by their charge content in solution by conductometry and potentiometry. The binding events between polyP and inhibitors was studied by isothermal calorimetry (ITC). The activity of inhibitors were assessed using plasma clotting activity.

Results: A library of synthesized compounds ranging from 10 kDa to 23 kDa were functionalized with a range of 10–30 ligands. Protonatibility of inhibitors confirmed both strongly cationic (pKa > 8) and switchable protonation states (pKa 6.5–7.5) were successfully incorporated. Upon binding to polyP, inhibitors were able to recruit protons from its surroundings to satisfy the charge requirement to inhibit polyP as determined by ITC. Plasma clotting assays confirmed the activities of these new generation polyP inhibitors.

Conclusions: We present the strategic design of potent polyP inhibitors that were antithrombotic without the potential of increased bleeding. We confirm the successful utilization of a switchable protonation state to increase specificity and reduce non-specific interactions of our new generation polyP inhibitors.

PB0260 | Plasma Contact Activation through T Cell-Derived Polyphosphate

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Background: Inorganic polyphosphate (polyP) is abundantly found in every cell in nature and acts as phosphate and energy storage molecule. PolyP released from platelet granules initiates FXII-driven contact activation. The contribution of polyP from other immune cells is unknown.

Aims: To analyze plasma contact activation mediated by polyP derived from CD4+ T cells.

Methods: We quantified and compared polyP levels from different CD4+ T cell subsets using negative DAPI staining and malachite green assays. Stimulation and activation of T cells in vitro was performed to study mechanisms that increase polyP levels and mediate its release. We utilized chromogenic substrate cleavage assays to analyze contact activation by T-cell derived polyP.

Results: We found that pro-coagulant polyP increases in T cell antigen receptor stimulated CD4+ T cells. Inhibition of mitochondrial oxidative phosphorylation decreased intracellular polyP levels in CD4+ T cells. Comparison of T cell subsets ex vivo revealed that polyP levels are higher in anti-inflammatory CD4+CD25+ T cells than in naive CD4+CD25- T cells. Moreover, preliminary data showed that T cell differentiation and T cell polyP levels mutually control each other. To assess the contribution of T cell polyP for contact activation, we tested the capacity of T cell polyP to initiate FXII activation. We found that isolated T cell polyP in cell-free systems as well as polyP released from activated T cells promote FXII activation in vitro.

Conclusions: The accumulation of polyP in T cell antigen receptor stimulated CD4+ T cells and the activation-dependent release of T cell polyP indicates that CD4+ T cells may play a role in polyP-mediated contact activation. Further studies will reveal the potential implication of T cell polyP for thrombotic diseases.

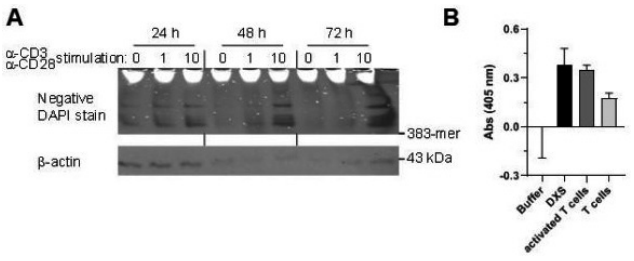

FIGURE 1 PolyP accumulates A) in stimulated CD4+ T cells and promotes B) FXII-driven contact activation

PB0261 | Quantification of Free FXIa by an Innovative "Catch and Release" Assay

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Background: Reduced in vivo levels of FXI have been associated with a lower thrombosis risk in absence of an increased bleeding risk. However, it is not known if increased FXIa levels lead to a higher risk on thrombosis. Previous studies have described methods for indirect measurement of FXIa either by quantification of FXIa-inhibitor complexes or through thrombin generation based assays. However, complex ELISA's can be influenced by contact activation during blood drawing, while thrombin generation assays are hindered by natural inhibitors of FXIa, resulting in a need for an improved assay to quantify FXIa in the circulation.

Aims: To developed an assay to measure the amount of FXIa present in blood, aiming at isolation of circulating FXIa homodimers through complexation with a multimeric form of the natural FXIa inhibitor Fasxiator.

Methods: Fasxiator (*Bugarus Fasciatus*: 62 amino acids; 3 disulfide bonds) was synthesized using tBoc-solid phase peptide synthesis protocols in two parts, subsequently ligated using native chemical ligation (NCL) and folded to obtain the native protein containing 3 disulfide bonds. The inhibitor was modified with a desthiobiotin tag to enable tetramer formation of Fasxiator with Avidin. In a proof of concept study desthiobiotin-Fasxiator was coupled to avidin and subsequently used to inhibit and isolate FXIa from plasma. To release FXIa, the multivalent enzyme-inhibitor complex was disrupted by incubation with biotin. Lineweaver Burk plots were made and Biacore assays were performed to study the kinetic parameters of FXIa inhibition.

Results: Multimerization of Fasxiator increased the affinity for FXIa 10-fold, which makes a tetrameric Fasxiator a suitable candidate for FXIa isolation. Using this assay set up We were able to measure FXIa down to 50 pM in blood plasma.

Conclusions: The so designed "catch and release" FXIa assay is a promising approach to isolate and measure FXIa in circulation.

PB0263 | The Misfolded Protein Glycated Albumin Activates the Contact System Leading to Plasma Kallikrein Activity But Not Intrinsic Coagulation

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Background: Binding to negatively charged surfaces activates contact coagulation factor (F)XII, which can trigger the inflammatory kallikrein-kinin system and intrinsic coagulation pathway via its substrates prekallikrein (PK) and factor (F)XI, respectively. Misfolded proteins have been recognized as contact system activators; however, the underlying mechanism remains to be established.

Aims: To assess effects and mechanisms of misfolded proteins on contact system activation.

Methods: Serum albumin was misfolded via glycation (SA-AGE). Conformational changes of denatured albumins were analysed by circular dichroism. Chromogenic assays in plasma and purified proteins examined activation of contact system components FXII(a), (pre)kallikrein, and FXI(a). Clotting assays in normal and contact system component deficient plasma samples were employed to determine the ability of SA-AGE to trigger coagulation via contact activation. SA-AGE effects on thrombus formation under flow was analysed in whole blood. Protamine sulfate was employed to neutralise the negative charge of SA-AGE to examine this effect on clot formation.

Results: SA-AGE induced autoactivation of FXII in a negative charge dependent manner and led to PK but not FXI activation. Addition of the positively charged protein protamine sulfate neutralised the negative charge of SA-AGE and interfered with FXII contact activation in plasma. SA-AGE inhibited FXIa, but not PKa or FXIIa, by a non-competitive inhibition mechanism. SA-AGE demonstrated an anticoagulant effect in plasma, and whole blood under flow conditions. SA-AGE dose dependently prolonged FXII contact activator silica-stimulated plasma clotting time indicating that SA-AGE inhibits downstream FXIIa mechanisms. Counterintuitively, we found that SA-AGE enhanced the extrinsic pathway, with a mechanism yet to be identified.

Conclusions: Albumin misfolding via glycation results in a clustering of negative charge, which can contact activate FXII leading to PK activation, and the subsequent cleavage of high-molecular-weight kininogen. SA-AGE inhibits FXIa, offering an explanation why some misfolded protein surfaces do activate the kallikrein-kinin system but not coagulation.

PB0264 | In-vivo Evidence for Contact Activation after Implantation of a Left Ventricular Assist Device in Patients with End-stage Heart Failure

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Background: Left Ventricular Assist Devices (LVADs) are surgically implanted in patients with end-stage heart failure when pharmacological therapy is inadequate. Thrombus formation is a common cause of failure of these intracardiac surgical implants, despite adequate anticoagulation with vitamin K antagonists (VKA) and platelet inhibitors. It is likely that activation of the contact system by artificial surfaces of LVADs contributes to the prothrombotic effects of these devices. Clinical evidence to support this theory is lacking.

Aims: To evaluate contact system activation in patients before and after LVAD implantation.

Methods: Plasma levels of the contact system activation marker cleaved H-kininogen (cHK) were determined at 1 month, 6 months and 12 months after LVAD implantation in 40 patients. Measurements before LVAD implantation were used as reference.

Results: In 40 patients a LVAD was implantated between September 2011 and December 2017 as a bridge to heart transplantation. Two different centrifugal flow pumps (Heartmate III (N=13) and Heartware (N=8)) and a continuous-flow pump (Heartmate II N=19) were implantated in these patients. Plasma levels of cHK remained stable at 1 month after LVAD implantation (mean, 21.25% and interquartile range, 3.54–34.13%) compared with baseline levels (mean, 20.89% and interquartile range, 3.83–29.35%). At 6 months after LVAD implantation, plasma cHK levels (mean, 29.19% and interquartile range, 5.70–48.82%) were increased compared with baseline levels. At 12 months after LVAD implantation cHK levels (mean, 28.35% and interquartile range 4.25–44.92%) were similar to baseline again. Pump thrombosis occurred in 4 patients and was associated with a 1.8–4.4 fold increase in plasma cHK levels in comparison with baseline levels in each patient.

Conclusions: This study provides the first in vivo evidence of contact activation in patients after LVAD implantation.

PB0265 | Relevance of Thrombin in the Activation of the Fluid Contact Phase in Patients with Hereditary Angioedema Carrying the *F12* p.Thr309Lys Variant

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Background: Hereditary angioedema(HAE) is a rare dominant disease resulting from an increased generation of bradykinin upon activation of the plasma contact pathway. HAE-FXII is caused by mutations in F12; the most prevalent, c.1032C>A p.Thr309Lys, disrupts an O-glycosylation resulting in a smaller FXII with increased sensitivity to the fluid phase activation by poorly understood mechanisms.

Aims: To study the functionality of the aberrant FXII p.Thr309Lys in plasma.

Methods: Thirty-three HAE-FXII^{T309K} patients, 25 healthy controls (NHP) and 53 patients with congenital disorder of glycosylation (CDG) were included. FXII and (pre)kallikrein (PK) were assessed by western-blot in basal conditions, and after treatment with neuraminidase, PNGaseF, plasmin, thrombin, dextran sulfate and silica. FXII amidolytic activity was measured by chromogenic methods. The generation and spontaneous or tPA-induced degradation of fibrin on FXII was evaluated. mRNA from peripheral mononuclear cells was sequenced.

Results: Aberrant FXII from all HAE-FXII^{T309K} had similar electrophoretic mobility (reducing SDS-PAGE) than FXII lacking an N-glycan

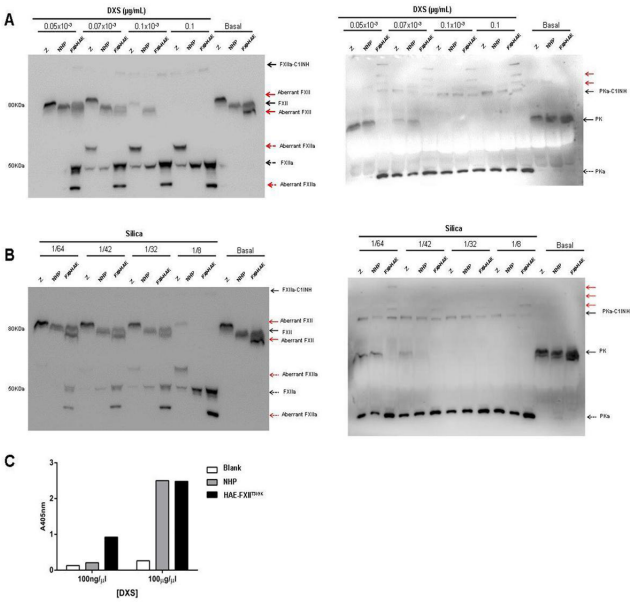

FIGURE 1 Activation of plasma FXII (left panels) or PK (right panels) with different doses of dextran sulphate (DXS) and silica, analysis by western blotting a

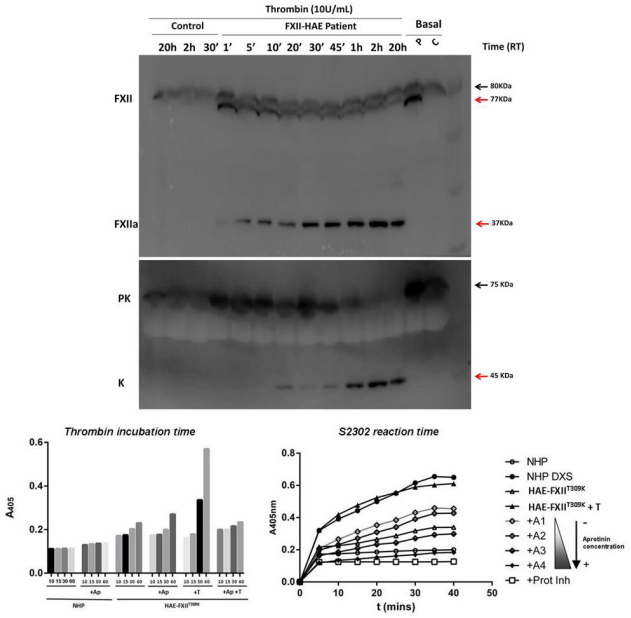

FIGURE 2 Effect of thrombin incubation on FXII and PK activation

from CDG patients and asialo-FXII generated by neuraminidase. Under non-reducing conditions or after PNGaseF this aberrant FXII was even faster. In silico predictions and F12 mRNA sequencing ruled out aberrant splicing. FXII^{309K} had increased sensitivity to activation upon low-dose triggers, generating an additional smaller band of approximately 37KDa. Plasmin activated similarly FXII^{309K} and FXII^{T309}. However, exogenous or endogenous thrombin cleaved only FXII^{309K}, releasing a 37KDa fragment that enhanced its further activation of the fluid phase.

Conclusions: Aberrant FXII from HAE-FXII^{T309K} has unexpected electrophoretic mobility, that might be explained by a less compact structure. It would facilitate a sequential proteolytic activation process firstly involving thrombin priming of FXII^{309K} followed by kallikrein cleavage, generating the active β-FXIIa. This and the fact that angioedema episodes occur during hyperocagulable situations suggest a key role for thrombin. The benefit of an antithrombotic prophylaxis should be further evaluated. CIBERER (ACCI18-04; ER19P5AC765/2019) SETH: grupos emergentes.

PB0266 | Corn Trypsin Inhibitor in the Preanalytical Phase of Contact Activation Assays

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Background: Corn Trypsin Inhibitor (CTI) is a known inhibitor of coagulation factor (F) XIIa. It is used - for example - to limit contact activation (CA) in thrombin generation tests when analyzing the tissue

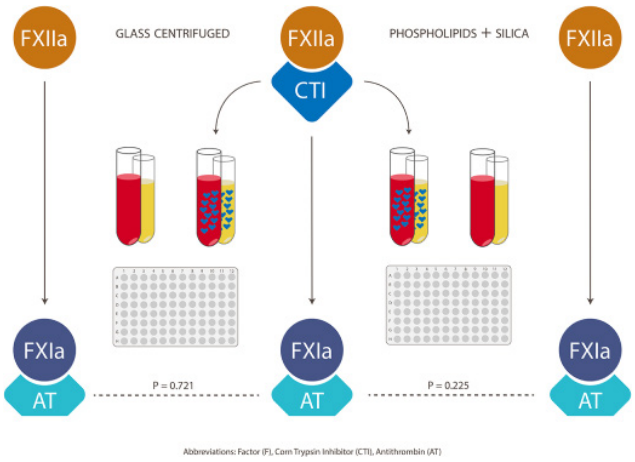

FIGURE 1 The role of Corn Trypsin Inhibitor in FXIa-AT ELISA

factor pathway. There is some discussion if CTI could inhibit FXIa and the thrombin feedback loop at particular concentrations.

Aims: With the rising interest in CA assays, one has to question the need for CTI to limit CA due to blood sample processing. We aimed to investigate the role of CTI in FXIa-Antithrombin (AT) complex ELISA in different conditions to optimize the preanalytical phase of CA assays.

Methods: We performed an in-house ELISA using anti-FXI and biotinylated anti-AT goat antibodies to analyze normal human plasma. Samples with or without CTI (50 μg mL^{-1}) were activated in centrifuged glass tubes (N=10) or by adding different concentrations of commercially available synthetic phospholipids and silica (SynthASil) in recalcified citrated plasma (N=5). Blood sampling conditions were identical with attention for undesired CA; e.g. no extra tubing, regular needles, and immediate processing. The difference in absorbances was statistically analyzed at a 0.05 significance level using Wilcoxon Signed Ranks test as the sample size is currently still small and normal distribution could not be assumed.

Results: If one compares samples activated in centrifuged glass tubes with or without CTI, there is no significant difference in FXIa-AT complex formation (P-value 0.721). The presence of CTI in citrated plasma, activated with SynthASil and calcium chloride seems to have no significant impact on FXIa-AT complex formation compared to solely citrated sample collection (P-value 0.225).

Conclusions: These preliminary results suggest that with careful sample collection, the role for CTI in FXIa-AT complex ELISA is rather small. CTI, therefore, could potentially be omitted in favor of simplified sample collections within clinical trials, for example.

CRITICAL CARE AND PERIOPERATIVE

PB0268 | "Plasma Free" Perioperative Approach during Lung Transplantation Does Not Seem to Impair Secondary Hemostasis Measured by ROTEM

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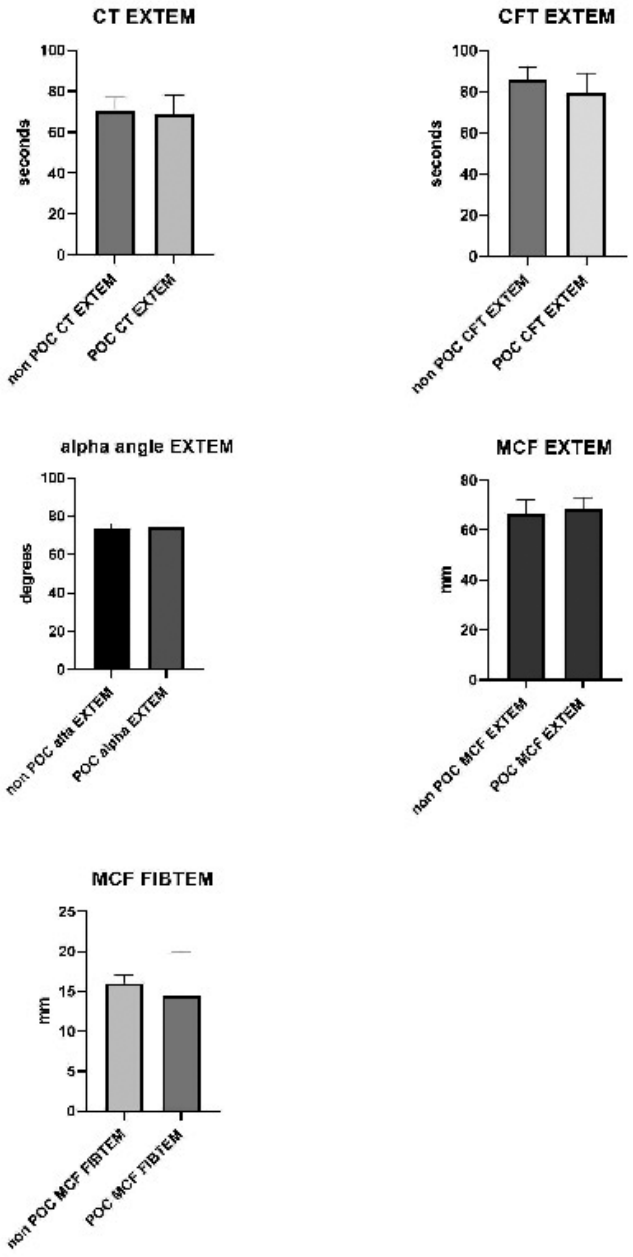

FIGURE 1

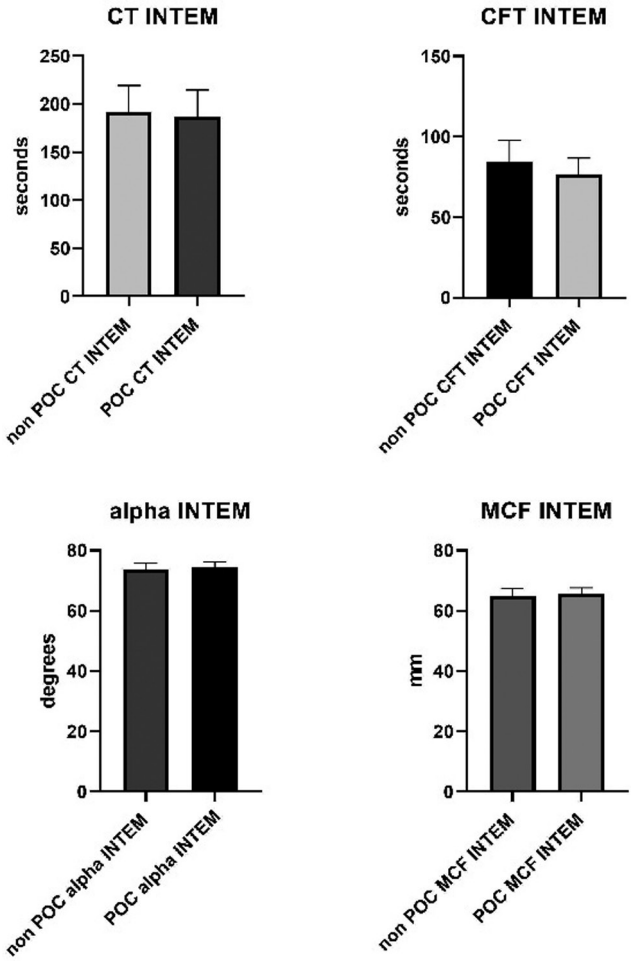

FIGURE 2

Background: Lung transplantation surgery is often performed on extracorporeal membrane oxygenation (ECMO) which is generally associated with coagulopathy and bleeding. Fresh frozen plasma (FFP) might be a source of clotting factors to support coagulation but on the other hand it may be associated with higher morbidity and mortality of patients in general.

Aims: The aim of this study was to find out whether point of care (POC) approach to diagnosis of perioperative coagulopathy may decrease consumption of FFP and whether it will impair secondary hemostasis measured by ROTEM.

Methods: In this randomized study, 40 patients undergoing lung transplantation (most of them done on ECMO) were analyzed. Twenty patients were treated using standard approach based on administering of FFP during bleeding (non POC group) and 20 patients were treated on the base of POC tests (POC group) such as ROTEM (administering prothrombin complex concentrate, platelets, fibrinogen). ROTEM (EXTEM, FIBTEM and INTEM) exam was performed before and after surgery in both groups.

Results: In POC group no FFP was administered during lung transplantation surgery. This approach actually enabled "plasma free" perioperative management. There was no significant difference ($p < 0.05$), shown on Fig 1 and 2, between both groups after surgery in results of ROTEM.

Conclusions: "Plasma free" perioperative approach to bleeding during lung transplantation surgery does not seem to cause secondary hemostasis pathology measured by ROTEM.

Acknowledgement: The study was supported by Supported by Ministry of Health, Czech Republic - conceptual development of research organization, Motol Univesity Hospital, Prague, Czech Republic 00064203.

PB0269 | "Plasma Free" Perioperative Approach during Lung Transplantation Seems to Decrease FXIII Level which Is Not Clinically Significant

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Background: Lung transplantation surgery is often performed on extracorporeal membrane oxygenation (ECMO) which is generally associated with coagulopathy and bleeding. Fresh frozen plasma (FFP) might be a source of clotting factors to support coagulation but on the other hand it may be associated with higher morbidity and mortality of patients in general.

Aims: The aim of this study was to find out whether point of care (POC) approach to diagnosis of perioperative coagulopathy may decrease consumption of FFP and whether it will impair level of FXIII, secondary hemostasis measured by ROTEM and blood loss.

Methods: In this randomized study, 28 patients undergoing lung transplantation (most of them done on ECMO) were analyzed. Fourteen patients were treated using standard approach based on administering of FFP during bleeding (non POC group) and 14 patients were treated on the base of POC tests (POC group) such as ROTEM (administering prothrombin complex concentrate, platelets, fibrinogen). ROTEM (EXTEM and FIBTEM) exam and level of FXIII was measured before and after surgery in both groups and perioperative blood loss was evaluated, as well.

Results: In POC group no FFP was administered during lung transplantation surgery. This approach actually enabled "plasma free" perioperative management. There was significantly decreased level of FXIII in POC group after surgery ($p < 0.05$) but blood loss was paradoxically lower in the same group. MCF of EXTEM and FIBTEM was not different between both groups. Data are shown on Figure 1.

Conclusions: "Plasma free" perioperative management of lung transplantation surgery, based on POC results, decreases FXIII level which is not clinically significant.

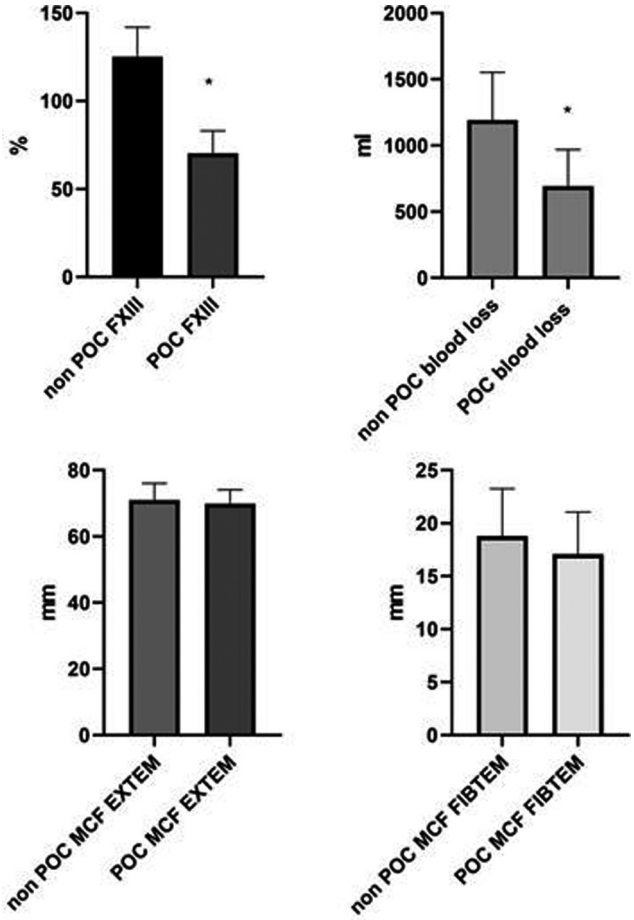

FIGURE 1 Measurements of MCF (maximum clot firmness) of EXTEM and FIBTEM, level of FXIII and blood loss

Acknowledgement: The study was supported by Supported by Ministry of Health, Czech Republic - conceptual development of research organization, Motol Univesity Hospital, Prague, Czech Republic 00064203.

PB0270 | Hemostasis Critical Values in a Laboratory without Hospitalization from CABA, Argentine

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Rossi Laboratory, CABA, Argentina

Background: the concept of identifying laboratory values requiring both immediate laboratory and clinician intervention was originally proposed by George Lundberg, in 1972. The identification and effective communication of so-called "critical values" are vital to good laboratory practice.

Aims: for the purpose of this work, we identified as "critical value" a test result that is clinically significantly outside the normal range, and may represent serious morbidity of life-threatening values.

TABLE 1 Critical values taken into account

RIN	>5
Prothrombin Time	<12%
APTT	>80 seg
D-Dimer	> 1 mg/ml
Anti-XA	> 1.2 U/dl

TABLE 2 Total data reported per year

Year	Data reported
2018	1.2%
2019	1.5%

Methods: A systematic review of the literature was performed to identify papers that reported critical risk result alert thresholds for clinical hemostasis; and compare the date with those obtained in our laboratory for two consecutive years.

Results: The results taken account correspond to the total % of patients who attended the hemostasis sector.

Conclusions: The available data for critical values were as follows: 1.5% for INRs anticoagulated patients; 1.5% for APTT test for anticoagulanted patients; 1.5% for LMWH anti-Xa heparin levels; 1.5% for D-Dimer (patients with thromboembolism risk). We found our data are according international consensus. Also we recognize that the reporting of critical values for commonly performed coagulation tests generates a significant workload, but it is vitally achieve communication in a timely manner.

PB0272 | Indiscriminate Coagulation Testing for Bleeding Risk Assessment Prior to Orthopedic Surgery. Is it a Rational Approach?

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[1]*Regional Blood Center, Karachi, Pakistan;* [2]*Jinnah Medical and Dental College, Pathology, Karachi, Pakistan*

Background: Indiscriminate preoperative coagulation testing to identify patients at risk of bleeding is commonly practiced. However, this practice is no longer supported by current guidelines.

Aims: We conducted this study with an aim to evaluate the advantage of doing screening testing for coagulation and hemostasis in unselected patients prior to elective orthopedic surgeries in our setting.

Methods: It was retrospective cross sectional study carried out in Medicare Cardiac and General Hospital, Jinnah Medical and Dental College, Karachi. All the patients admitted for elective orthopedic bilateral total Knee replacement and Hip replacement surgeries from March 2016 to December 2018 were included in the study. Electronic data of patients' pre-operative test results for

Prothrombin time, Activated Partial Thromboplastin time was retrieved. Frequency of patients with deranged coagulation screening was calculated.

Results: A total of 367 patients underwent presurgical coagulation testing. Male to female ratio was 1:3. Median age of patients was 61 ± 5 years. A total of 2.9% (n=11) patients were found to have deranged coagulation profile. Out of which 3 patients did not undergo surgery and were loss to follow up. 0.54% (n=2) patients had both prolonged PT and APTT. 1.6% (n=6) patients had prolonged APTT. 0.3% (n=1) patient experienced intraoperative bleeding and received one unit of packed cell transfusion. Only 0.3% (n=1) patient experienced post-operative bleeding and dropped her Hemoglobin requiring transfusion. Combined cost of these tests is 1110 PKR (8 USD). The total amount spent on these patients was 407,500 PKR (2915 USD).

Conclusions: Overall results of the study suggest that indiscriminate coagulation screening does not provide any additional information. It is suggested that routine screening tests should not be performed unless a medical history is indicative of bleeding tendency. Wasting of financial resources with very negligible benefits would be a major concern for developing countries and this irrational testing should be curtailed.

PB0273 | Hematologic Laboratory Parameters and Hemostatic Thresholds for the Safe Administration of Neuraxial Anesthesia in Adults with Pre-Existing Bleeding Disorders: A Scoping Review

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Background: Neuraxial anesthesia including spinal, epidural and combined spinal-epidural blocks are anesthetic techniques commonly used for acute pain control in surgery and obstetrics. One of the most feared complications of neuraxial anesthesia is spinal or epidural hematoma which can lead to permanent neurologic injury. The risk of spinal hematoma is generally accepted to be higher in patients with bleeding diatheses compared with the general population, however this conclusion is largely based on anecdotal evidence.

Aims: To describe the hematologic laboratory parameters where neuraxial anesthesia has been administered with and without spinal or epidural hematoma in patients with single pre-existing bleeding disorders/tendencies.

Methods: This scoping review was conducted according to the PRISMA-ScR guidelines. A detailed search strategy was developed in collaboration with a medical librarian and performed in Ovid MEDLINE, CINAHL, Embase, Scopus and Web of Science. There were no limits placed on date of publication or study type. Titles and abstracts were screened by two independent reviewers according to the inclusion and exclusion criteria found in Table 1. Reference lists of all publications included in full text review were screened for additional sources. Finally, data were extracted by two reviewers independently using a pre-piloted data extraction form with conflicts resolved by consensus.

Results: The literature search yielded 1362 studies after duplicates were removed. An additional 54 studies were discovered during reference list review and a total of 98 studies were ultimately included in the scoping review. Data analysis is pending with final results expected by April 2020. The final results will be presented at the conference.

Conclusions: Our findings are expected to highlight the paucity of data in the existing literature surrounding the safe use of neuraxial anesthesia in patients with single pre-existing bleeding disorders/tendencies. This represents an important knowledge gap that requires expert consensus-based recommendations and additional research to help guide clinical care.

TABLE 1 Scoping review inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Patients with current diagnosis of a single bleeding disorder known prior to administration of neuraxial anesthetic including: von Willebrand disease, immune thrombocytopenia, gestational thrombocytopenia, thrombocytopenia associated with hypertensive disorders of pregnancy, platelet function disorders, factor VII deficiency, hemophilia A and B including carriers, factor XI deficiency, factor XIII deficiency and fibrinogen disorders	Presence of a secondary bleeding disorder or co-morbidity that predisposes to bleeding (eg. liver or renal dysfunction, spinal cord arteriovenous malformation)
Patient received neuraxial anesthetic	Peripheral nerve blocks, lumbar puncture
Spinal or epidural hematoma, if present, was confirmed with imaging	Article in a language other than English

PB0274 | External Quality Assurance (EQA) for the Thromboelastometry Devices: UK NEQAS BC Programme Update

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UK NEQAS for Blood Coagulation, Sheffield, United Kingdom

Background: Thromboelastography and Thromboelastometry are Point of Care testing (POC) technologies for global haemostasis tests which measure the elasticity of a developing clot. Thromboelastometry is performed using Rotem® devices, the Rotem delta device requires reagents to be pipetted whereas the newer Sigma device uses cartridge based technology with no pipetting required. The TEG devices are used to perform Thromboelastography and can be either TEG5000 which requires reagents to be pipetted or TEG6 which is cartridge based. The majority of devices are in theatres and specialist wards and usually operated by staff without laboratory training.
Aims: UKNEQAS BC has been providing EQA samples for the Rotem Delta and the TEG 5000 for 5 years and have recently introduced EQA for the cartridge based technology.
Methods: One lyophilised sample plus a pre measured diluent and a disposable pipette is distributed 3 times per year. Users are allowed 24 days to test and return results. Median values are calculated and the R time for TEG parameters and CT time for Rotem parameters are performance assessed. Peer group assessment is undertaken so that each device is only compared to other of that type. Samples ranged from obtained from normal, spiked with heparin, rivaroxaban, apixaban and a sample designed to mimic excessive blood loss.
Results: Results are shown in table 1 for Rotem Delta and TEG 5000 (both pipette based devices) and for Rotem Sigma and TEG6 in table 2 (both cartridge based technology. Results show CVs ranged from 7.2%–73.2% for Rotem delta and 17.4%–59.5% for TEG5000. CVs for cartridge technology ranged from 3.9%–22.9% for Rotem Sigma and 13.6%–30.7% for TEG6, (data from first 2 surveys).
Conclusions: EQA is essential for this technology and that preliminary data on precision of cartridge based technology has shown an improvement compared to traditional testing.

PB0275 | Thrombohemorragic Events and Management of Haemostasis in Adult Patients under Berlin Heart Excor Devices

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[1]*Hospital General Universitario Gregorio Marañón, Haematology, Madrid, Spain*; [2]*Hospital General Universitario Gregorio Marañón, Cardiology, Madrid, Spain*

Background: Berlin-Heart EXCOR (BHE) is a ventricular assist device (VAD) used as bridge to cardiac transplantation. Thrombohaemorragic events (THE) are the most life-threatening complications, so close monitorization is needed.
Aims: Our aim was describing THE and antithrombotic monitoring results in our experience.
Methods: 15 adult patients undergoing BHE between 2009–2019 were identified. Median age was 57 years old (27–70) and 93% were male. 422 haemostatic controls (blood count, basic and special coagulation tests) based in our clinical protocol (Figure 1) were included: every 24h first week, every 3–7 days from then and at the time and in previous 48h before any THE.

TABLE 1 Results for Rotem delta and TEG 5000

Rotem Delta CT seconds						TEG 5000 R times minutes			
InTEM	%CV	ExTEM	%CV	HepTEM	%CV	Plain Cup	%CV	Hep Cup	%CV
240	15.1	153	22.3	263	10.5	17.1	26.9	19.7	23.9
308	17.1	96	73.2	283	10.2	16.0	25.3	18.5	20.9
195	12.3	92	20.5	214	18.3	10.3	17.4	12.1	25.2
223	14.9	57	20.9	154	8.5	Flatline	Not Applicable	16.9	59.5
167	7.2	55.5	18.5	175	8.8	8.1	27.8	8.7	22.7
310	18.4	200.5	69.0	308	19.5	Flatline	Not Applicable	Flatline	Not Applicable

TABLE 2 Resutls for Rotem Sigma and TEG 6

Rotem Sigma CT seconds						TEG 6 R times minutes			
InTEM	%CV	ExTEM	%CV	HepTEM	%CV	CK (Plain)	%CV	CKH (Hep)	%CV
261	3.9	111	13.9	258	Not Applicable	10.8	13.6	10.6	30.7
499	10.2	307	22.9	484	Not Applicable	Flatline	Not Applicable	Flatline	Not Applicable

TABLE 1 Haemostatic tests results

ANTICOAGULANT	UNH	LMWH	WARFARIN
Days from BHE implantation	1 (1–3)	42 (10–102)	32 (2–99)
Duration (days)	24.5 (7–59)	50.8 (6–147)	31 (2–99)
Days to first test on range	4 (2–31)	9.5 (1–35)	5 (2–15)
Normal range values	rate APTT 2–2.5 Anti-Xa 0.35–0.6	Anti-Xa 0.6–1	INR 3–3.5
Tests on range	29.6%	60.3%	62.1%
ANTIPLATELETS THERAPY	ASPIRIN	CLOPIDOGREL	DIPYRIDAMOLE
Normal multiplate range	ASPI 10–20 UI	ADP 20–35 UI	.
tests on range	52.8%	71.4%	.

Median, ranges and percentages are used. Med = median, UNH = Unfractioned heparin, LMWH = Low molecular weight heparin.

Results: Anticoagulation based on low molecular weight heparin (LMWH) and warfarin showed lower rate of anaemia (p< 0.001) and thrombocytopenia (p < 0.001) and better rate of anticoagulation (p< 0.001), antiplatelets (p< 0.001) and therapy stability (p< 0.001) (Table 1) than with unfractioned heparin (UFH). Antithrombotic stability (p< 0.001) was higher under LMWH than warfarin. 35.8% Multiplate® antiaggregation controls under dipyridamole, aspirin and clopidogrel were on range. No statistical differences on THE number were found between any anticoagulation (p=0293) nor antiplatelets (p=0.94) therapy.

56 THE occurred with a median of 4 THE (1–7) per patient. Bleeding was the most frequent (46.4%), followed by BHE thrombosis (39.3%). Clinical thrombosis was the less frequent (14.3%); all of them were ischemic stroke and 42.8% of haemostatic controls in previous 48 hours were in infratherapeutic range.

Median duration of BHE support was 105 days (18–194); 80% patients were successfully bridged to cardiac transplantation. 3 patients died while on a device: 1 because sepsis and 2 because neurological bleeding (both had severe coagulopathy previously diagnosed before BHE implantation).

Conclusions: Protocolized anticoagulation and antiplatelets monitoring allows focused management of these patients and could have diminished life-threating THE. LWMH could offer better therapeutical stability, but not less THE.

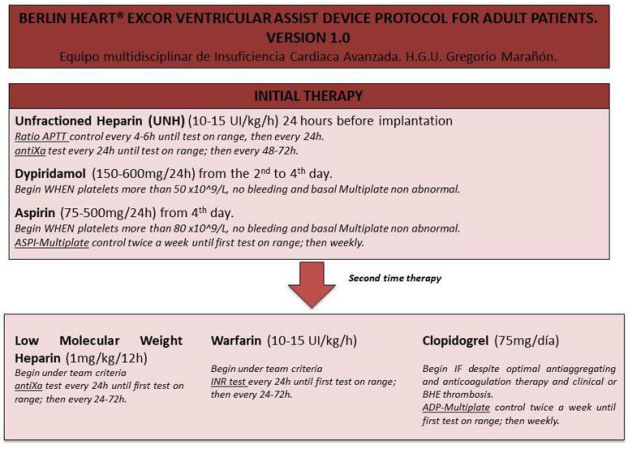

FIGURE 1 Berlin Heart EXCOR protocol for adult patients from our centre

PB0277 | The Association of Prothrombin Complex Concentrates with Transfusion Requirement and Postoperative Outcomes in Cardiac Surgery: A Post-Hoc Analysis of the FIBRES Randomized Controlled Trial

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Background: Coagulopathy after cardiopulmonary bypass (CPB) is common and associated with bleeding and adverse outcomes. The mainstay of therapy for coagulation factor deficiency is frozen plasma (FP), however prothrombin complex concentrates (PCCs) may be simpler to administer and more effective.

Aims: To describe the association of comparable PCC or FP doses with transfusion requirements and adverse outcomes after cardiac surgery.

Methods: This was a post-hoc analysis of the FIBRES randomized controlled trial (Effect of Fibrinogen Concentrate vs. Cryoprecipitate on Blood Component Transfusion after Cardiac Surgery) with ethics approval. The primary outcome was RBC transfusion within 24 hours of CPB. Secondary outcomes included platelet transfusion within 24 hours of CPB and adverse outcomes. Associations were examined with the use of multivariable models adjusted for creatinine clearance, hemoglobin, BMI, age, sex, heart failure, study arm, critical status, surgical complexity and urgency.

Results: Of the 735 patients in FIBRES, 416 received PCC (n=72, 17%) or FFP (n=344, 83%) (Table 1). A total of 66 (16%) experienced thromboembolic events and 54 (13%) fluid overload or respiratory

TABLE 1 Characteristics of the study population at baseline

Variable	Frozen Plasma Only (n=344)	Prothrombin Complex Concentrates Only (n=72)	P-Value
Age, Median (IQR), y	66 (58, 73)	65 (42, 72)	0.03*
Body mass index, Median (IQR)	23 (20, 26)	21 (18, 24)	<0.01*
Creatinine Clearance, Median (IQR), ml/min	71 (45, 97)	71 (48, 104)	0.71
Hemoglobin, Median (IQR), g/L	132 (116, 144)	132 (113, 146)	0.94
International Normalized Ratio, Median (IQR)	1.0 (1.0, 1.2)	1.1 (1.0, 1.3)	0.30
Left ventricular ejection fraction < 21%	13 (4%)	4 (6%)	0.37
Pulmonary hypertension (%)	65 (25%)	17 (24%)	0.83
Cerebrovascular Disease (%)	40 (12%)	12 (17%)	0.24
Critical Pre-Operative Status (%)	77 (22%)	16 (22%)	0.98

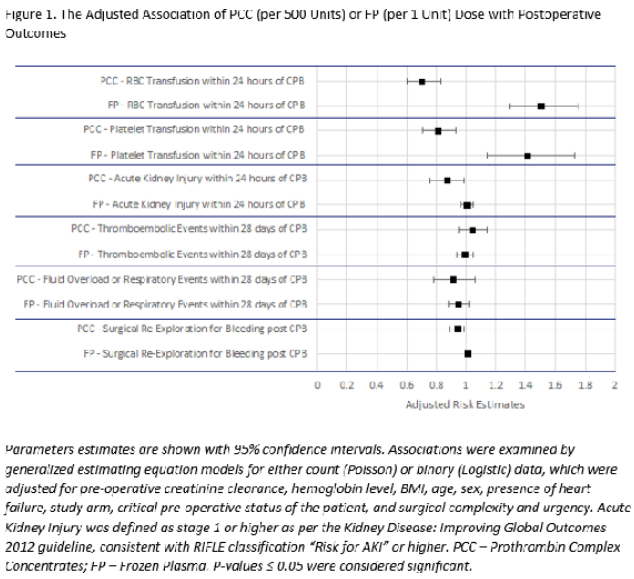

FIGURE 1 The Adjusted Association of PCC (per 500 Units) or FP (per 1 Unit) Dose with Postoperative Outcomes

events within 28 days; 145 (35%) renal events within 7 days; 78 (19%) surgical re-exploration. Each unit of FP was associated with increased adjusted odds (aOR) of RBC transfusion [aOR 1.50 (95% CI, 1.29, 1.75), p< 0.01], while each 500 units of PCC was protective [aOR 0.70 (95% CI, 0.60, 0.83), p< 0.01]. Each unit of FP was associated with an increased adjusted odds of platelet transfusion [aOR 1.41 (95% CI 1.14, 1.73), p< 0.01], while each 500 units of PCC was protective [aOR 0.81 (95% CI, 0.71, 0.93), p< 0.01]. PCC use was not associated with an increase in adverse events (Figure 1).

Conclusions: PCC use was associated with transfusion avoidance, without increases in adverse events. PCCs appear to confer hemostatic advantages to FP in this setting, however randomized trials are needed.

PB0278 | Delay in Time to Hip Fracture Repair Surgery among Elderly Patients Treated with Anti Platelet Therapy

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Background: Hip fractures are a major cause of morbidity and mortality in elderly population with one-year mortality rates up to 20%. Delayed surgery (beyond 48 hours) is a major predictor of the many factors that are associated with worse prognosis following hip fracture surgery (HFS). The use of anti-platelet therapy (APT) may potentially lead to excessive bleeding and is associated with surgical delay and in one large hip fracture patient cohort APT was the direct cause of HFS delay in 17% of patients.

Aims: To study the association between use of pre-surgery treatment with APT on time to HFS in elderly patients.

Methods: A retrospective study on consecutive patients with hip fractures aged ≥ 65 years of age admitted and operated at the Ortho-Geriatric department at Sheba Medical Center between 2014–2018. Admission and surgery times as well as demographic, co-morbidity, mortality and adverse event data were extracted from electronic patient files and time to surgery (TTS) and rates of delayed surgery were compared between APT treated vs. control patients (no APT or anticoagulant treatment).

Results: One thousand and thirty patients were studied, of which 370 (35.9%) patients were taking APT (mainly single APT, SAPT) while 36 were taking dual APT (DAPT). Most patients took low dose aspirin (326) while Plavix was used in 80 patients. Most patients (325/327) continued aspirin treatment whereas Plavix was stopped after admission in all patients. Median TTS was not different between groups (23.5 hours, 26.4 hours and 28.9 hours in controls and in SAPT and DAPT patients, respectively, P= 0.624). Similarly % patients operated beyond 48 hours was not different (14.9%, 13.9% and 20.0% in controls and in SAPT and DAPT patients, respectively, P= 0.101).

Conclusions: Elderly APT treated patients suffering a hip fracture underwent HFS at a similar time frame and similar TTS compared to non-treated control patients.

PB0279 | Assessment of Coagulation Factor Levels in LVAD Patients Following Temporary Warfarin Reversal with 4F-PCC (KVAD Study)

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Background: Left ventricular assist device (LVAD) patients require interruptions of warfarin for invasive procedures, which frequently requiring bridging with IV anticoagulation. Prothrombin complex concentrates (4F-PCC) can facilitate temporary reversal of anticoagulation and allow for continued warfarin.

Aims: To evaluate temporary reversal of warfarin with 4F-PCC in LVAD patients receiving continuous warfarin therapy, correlation of coagulation factor levels with INR, and return time to therapeutic INR.

Methods: A single center prospective analysis of LVAD patients was performed from March 2017 to December 2019. Baseline INR and subsequent INR with coagulation factor levels 2, 7, 9, 10, protein C/S were collected within 1-hour post 4F-PCC, and at 6, 12, 18, 24, 36, and 48-hours post infusion. 4F-PCC dose was based on baseline INR and target INR for the procedure. Informed consent was obtained. Clinical Trials.gov Identifier: NCT03473132

Results: Nine LVAD patients received 4F-PCC to temporarily reverse warfarin, with a median baseline INR of 3.1 (IQR: 2.3–3.2). Target INR goal for procedures ranged from < 2.0 to ≤ 1.4. Median dose of

TABLE 1 Details and Outcomes of LVAD Patients with Warfarin Continued Prior to Procedure and Temporarily Reversed for Procedure

Patient	Device & Strategy	Procedure	Baseline INR; Goal INR for procedure Achieved INR goal: achieved/not achieved	4F-PCC Dose units, units/kg	IV/SQ AC Bridge	Days to therapeutic INR (+/- 0.2)	Bleeding event intra-operative, 7 days, 30 days	Thrombotic event
1	HM3 LVAD DT	Robotic prostatectomy	3.2 Goal: ≤ 1.4 Achieved	2302 units 29 u/kg	No	1	Minor-expected hematuria; None None	None
2	HW LVAD DT	Colonoscopy w/ polypectomy	4.9 Goal: < 1.8 Achieved	4406 units 42 u/kg	No	3	Major: 2 U PRBC for Hgb drop 11.1 to 9.4 on POD1 and placed 2 hemostatic clips at active bleeding site POD2. INR at time of event was 1.6; None; None	None
3	HW BiVAD DT	Image guided right thoracentesis	3.2 Goal: ≤ 1.8 Achieved	1091 units 16 u/kg	No	2	None None None	None
4	HMII LVAD DT	Endoscopy and biopsy	3.5 Goal: < 2.0 Achieved	1094 units 10 u/kg	Yes - IV heparin POD 1	7	None None None	None
5	HM3 LVAD DT	Arthroplasty and unicondylar knee replacement	3.2 Goal: < 1.4 Achieved	2068 units 25 u/kg	No	1	None None None	None
6	HM3 LVAD DT	Colonoscopy w/ polyp clips	2.2 Goal: < 1.4 Achieved	2182 units 27 u/kg	No	4	None None None	None
7	HM3 LVAD DT	Left parotidectomy	2.9 Goal: ≤ 2.0 Achieved	1091 units 10 u/kg	Yes - SQ Enoxaparin POD4	1	None None None	None
8	HW LVAD DT	Laparoscopic sleeve gastrectomy	2.1 Goal: < 1.5 Achieved	1606 units 14 u/kg	No	1	None None None	None
9	HM3 DT	Colonoscopy	1.9 Goal: ≤ 1.5 Achieved	533 units 6 u/kg	No	1	None None None	None

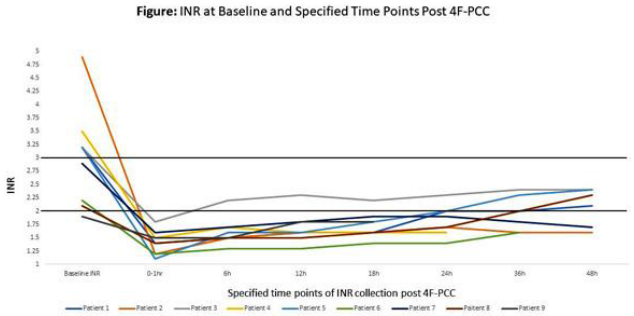

FIGURE 1 INR at Baseline and Specified Time Points Post 4F-PCC

4F-PCC was 1104 units (IQR: 1091–1952) or 12 units/kg (IQR: 10–22). The median INR at T0 was 1.5 (IQR: 1.3–1.6). Time to return to therapeutic INR of 2.0–3.0 (± 0.2) was 1 day (range 1–7 days; Figure). Two patients required bridging with an IV/SQ anticoagulant due to conservative warfarin dosing. One GI bleed occurred day 1 following polypectomy, requiring transfusion and intervention, INR was 1.6. At 7 and 30 days, no other bleeding or thrombotic events occurred. Corresponding factor levels and INR results will be reported at completion of the analysis.

Conclusions: In LVAD patients undergoing invasive procedures and receiving continued warfarin, 4F-PCC allowed for temporary warfarin reversal avoiding the need for IV AC bridging in most patients. One bleeding event occurred despite achieving target INR for the procedure. No thrombotic events were observed.

PB0280 | Low Preoperative Antithrombin Activity Is Associated with Increased Red Cell and Plasma Transfusion during Neonatal and Infant Cardiac Surgery

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Background: Heparin is most commonly used in cardiac surgery; however, neonates and infants have physiologically low antithrombin (AT) level. Low AT could increase needs for transfusion of plasma due to inadequate anticoagulation on cardiopulmonary bypass (CPB).

Aims: This study is to examine the influence of AT level on the transfusion requirements and coagulation markers of neonates and infants undergoing open heart surgery.

Methods: This is a prospective, observational study, approved by IRB. Neonates and infants < 6 months of age undergoing cardiac surgery with CPB were enrolled. Demographic, intraoperative, and transfusion data were collected. Preoperative AT and thrombin antithrombin complex (TAT) levels were measured after induction of anesthesia. Prior to separation from CPB, AT, TAT, D-dimer, fibrin monomers (FM), and anti-Xa were measured. Data are presented as median and interquartile ranges (IQR). Linear regression analyses were performed to examine preoperative AT
(pre-AT) levels and postoperative transfusion requirements and coagulation markers. Stata 15 was used for statistical analysis.

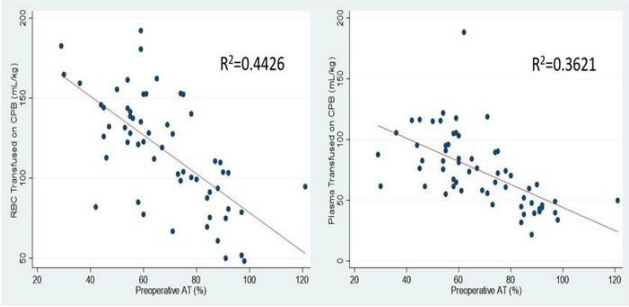

FIGURE 1 Correlation between antithrombin level and red cells or plasma

Results: A total of 60 patients were included. The age at surgery was 23.5 days (8.5, 99), weight 3.92 kg (3.25, 5.25) and CPB time was 161 min (123, 205). Pre-AT was associated with age (r=0.75; p< 0.001) and body weight,
(r=0.73; p< 0.001). Mean pre-AT level was 65% (55, 84) and increased to 81% (73, 93), p< 0.001 postoperatively. Using linear regression, lower pre-AT level was significantly associated with increased transfusion of red blood cells (RBC), coefficient −1.21 (95% CI, −1.57 to −0.85; p< 0.001) and plasma, coefficient −0.95 (95% CI, 1.28 to −0.62; p< 0.001) during CPB (Figure). Anti-Xa level was significantly associated with postoperative AT (post-AT) level, however, there were no associations between TAT, D-dimer, FM and pre- or post-AT levels.

Conclusions: Low pre-AT level is significantly associated with increased RBC and plasma transfusion on CPB in neonates and infants undergoing congenital heart surgery.

PB0281 | ROTEM Parameters As Bleeding Predictors In Pediatric Cardiac Surgery With Cardiopulmonary Bypass

D. Truong-Pham[1]; T. Nguyen[1]; S. Beaupha[1,2]; T. Tran[1]; T. Lai[1,2]; T. Nguyen[1]; P. Le-Thanh[3]; L.-X. Pham-Thi[3]; V. Le-Thanh[4]; P. Sa[1]; D. Nguyen[1]; T.-T. Nguyen[1]

[1]*Cho Ray Hospital, Department of Hematology, Ho Chi Minh City, Viet Nam;* [2]*University of Medicine and Pharmacy, Department of Hematology, Ho Chi Minh City, Viet Nam;* [3]*Cho Ray Hospital, Department of Anesthesiology- cardiac surgery, Ho Chi Minh City, Viet Nam;* [4]*Cho Ray Hospital, Department of Pediatric Cardiac surgery, Ho Chi Minh City, Viet Nam*

Background: ROTEM has been proven to be useful for bleeding prediction in adult patients undergoing cardiac surgeries, especially

in cases with cardiopulmonary bypass (CPB). However, evidence is still lacking for efficacy of ROTEM assessment in pediatric cardiac surgery.

Aims: The aim of this study was to investigate the correlation between perioperative ROTEM parameters and amount of postoperative blood loss in children undergoing heart surgeries.

Methods: We performed a case-series, prospective study on pediatric patients undergoing heart surgeries with CPB at Cho Ray Hospital from February to July 2019. We monitored the patients from the start of operations until 12 hours postoperatively. Activated partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen level and ROTEM assays (INTEM, EXTEM, FibTEM, HepTEM and APTEM) were performed simultaneously at the end of CPB. Evaluation of postoperative blood loss was determined by the total amount of blood in the chest drainage system. A blood loss of more than 10 ml/kg was considered clinically significant.

Results: In total, 40 patients were admitted to the study. 12 patients had clinically significant bleeding. ROTEM parameters including INTEM CFT, INTEM ALPHA, EXTEM CT, EXTEM CFT, EXTEM ALPHA, FIBTEM CT, FIBTEM A5, FIBTEM A20, FIBTEM A30, FIBTEM MCF, APTEM CT, and APTEM ALPHA were strongly correlated ($|r| > 0.5$) with blood loss 12 hours after surgery, while APTT (R), platelet count were only moderately correlated ($0.3 \leq |r| \leq 0.5$). Several ROTEM parameters showed good predicting value for postoperative bleeding, including INTEM CT (sensitivity 75.00%, specificity 71.43%), INTEM CFT (sensitivity 90.90%, specificity 46.40%), EXTEM CT (sensitivity 54.50%, specificity 92.86%), EXTEM CFT (sensitivity 90.90%, specificity 57.14%), FIBTEM CT (sensitivity 75.00%, specificity 92.80%), APTEM CT (sensitivity 75.00%, specificity 58.57%).

Conclusions: ROTEM may be useful for predicting postoperative hemorrhage in pediatric cardiac surgery. Future studies with larger samples are warranted in order to develop ROTEM-based transfusion guidelines in pediatric cardiac surgery.

PB0282 | Implementation of the Management of Anticoagulation in the Periprocedural Period (MAPPP) App into an Electronic Health Record (EHR): A Prospective Cohort Study

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Background: The perioperative management of patients receiving chronic oral anticoagulants (OACs) represents an important dilemma with potentially severe consequences (bleeding or thrombosis) in the postoperative period.

Aims: We hypothesized that newer health informatics technologies (HIT) can augment evidence-based perioperative anticoagulation management and result in fewer postoperative emergency department (ED) visits. We prospectively evaluated the effect of the MAPPP app as a clinical decision tool, integrated into electronic health record (EHR) workflow, in the management of patients on chronic OAC undergoing elective surgical procedures.

Methods: Patients age > 18 years old, with creatinine clearance ≥ 15 ml/min and on chronic OAC (warfarin or DOACs) were eligible. MAPPP app was integrated into the EHR via REDCap and the end-user was free to accept or reject guidance recommendations. Descriptive analysis compared patients' demographics, anticoagulant/antiplatelet medications, procedural bleeding and patient thromboembolic risk. Our primary study outcome was the incidence of ED visits within 30 days post-procedure. Logistic regression was performed to investigate the association between baseline

TABLE 1 Intervention group characteristics and outcomes, N = 642 (Mean (SD) or n (%))

Patient Characteristics	Acceptance (n=353)	Rejection (n=289)	p-Value		Acceptance (n=353)	Rejection (n=289)	p-Value
Age (y)	71.4 (13.1)	74.1 (11.8)	0.0068	Procedural bleeding risk			0.0068
Male Gender	201 (54.5%)	168 (45.5%)	0.7614	Minimal	14 (34.1%)	27 (65.9%)	
Weight (kg)	88.9 (25.4)	84 (22.2)	0.0118	Low	142 (53%)	126 (47%)	
Creatinine clearance (mL/min)	80.8 (46.5)	71.0 (36.8)	0.0040	High	197 (59.2%)	136 (40.8%)	
Anticoagulation medication			<.0001	Patient's thromboembolic risk			0.8586
Warfarin	96 (41.4%)	136 (58.6%)		Low	155 (56.2%)	121 (43.8%)	
Dabigatran	18 (51.4%)	17 (48.6%)		Medium	142 (54.4%)	119 (45.6%)	
Rivaroxaban	92 (59.0%)	64 (41.0%)		High	56 (53.3%)	49 (46.7%)	
Apixaban	147 (67.1%)	72 (32.9%)					

TABLE 2 Patient characteristics vs ED Visit in 30 days, N = 642

Logistic regression	Maximum Likelihood Estimate (MLE)	OR (95% CI)	p-Value
Intervention acceptance	−0.699	0.497 (0.249–0.992)	0.0473
Age 80+	0.750	2.116 (1.083–4.137)	0.0284
Female gender	−0.914	0.401 (0.185–0.869)	0.0206
High bleeding risk	−0.050	0.951 (0.481–1.883)	0.8861
High thromboembolic risk	0.144	1.155 (0.477–2.793)	0.7499
Aspirin	0.109	1.115 (0.486–2.555)	0.7972
C-index = 0.681			

parameters, intervention acceptance and primary outcome. The study was approved by our institutional review board.

Results: Between January 1, 2018 to January 31, 2019, 642 patients undergoing elective procedures or surgeries were included. Acceptance was more common in younger patients, patients on aspirin treatment or OAC other than warfarin, and in patients undergoing high bleeding risk procedures

(Table 1). Acceptance of MAPPP app recommendation was significantly associated with fewer ED visits (4.0% vs Rejection group: 8.3%, p=0.0205). Logistic regression (Table 2) showed that intervention acceptance and female gender were significantly associated with fewer -while age ≥ 80 with more - 30-day ED visits.

Conclusions: Integration of the MAPPP app into EHR of surgical patients on chronic OAC significantly decreased 30-day ED visits. The MAPPP app as part of HIT is a clinical decision tool with potential to decrease anticoagulation related adverse drug events.

PB0283 | Coagulation Research in Paediatric ECMO: Delayed Consent to Improve Enrolment

S. Van Den Helm[1]; J.G.F. Drop[2,3]; E.W. Wildschut[3]; R. Barton[1,4,5]; H.P. Yaw[1,4]; N. Letunica[1]; F. Newall[1,4,5]; A. Johansen[6,7]; D. Best[6,7]; J. McKittrick[6]; G. MacLaren[1,6,8]; R. Chiletti[6,7]; W. Butt[4,6,7]; Y. d'Udekem[4,9]; C.H. van Ommen[2]; V. Ignjatovic[1,4]; P. Monagle[1,4,5]

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Background: Extracorporeal membrane oxygenation (ECMO) involves complex coagulation management. Current anticoagulation strategies have been developed empirically due to the lack of systematic research. Understanding coagulation at ECMO commencement is essential to improve or predict ECMO outcome. Research in paediatric ECMO is hampered by a significant barrier of obtaining informed consent. Children who require ECMO are critically ill and this is a time of great anxiety for caregivers and seeking consent for research is difficult. Delayed consent is utilised in time critical studies in adult intensive care. We trialled delayed consent in similar ECMO studies across two countries to measure the impact on consent rates.

Aims: To make recommendations to improve enrolment in studies of coagulation in paediatric ECMO using delayed consent.

Methods: Two observational studies of coagulation parameters during ECMO in children are ongoing in Melbourne, Australia (Royal Children's Hospital) and Rotterdam, The Netherlands (Sophia Children's Hospital). Both studies have ethics approval to use delayed consent, enabling samples and data to be collected from children immediately prior to ECMO commencement and in the immediate hours after ECMO commenced. Parents are approached for consent within 48 hours (Melbourne) and 24 hours (Rotterdam) of ECMO initiation. If consent is obtained, ongoing samples and data are collected as per study protocol. If consent is denied, no further samples or data are collected, and any already obtained samples or data are destroyed.

Results: An enrolment rate of 65–67% was achieved at both sites (Table 1). In Rotterdam, enrolment improved after involvement of intensivist in the consent process from 1 out of the first 4 patients (25%) to 7 out of 8 patients (88%).

Conclusions: Delayed consent enabled high consent rates in a difficult situation. Improved systems to reduce reasons for not approaching parents will further improve consent rates. Observational studies of coagulation in ECMO are feasible with this approach.

TABLE 1 Enrolment rates at Royal Children's Hospital, Melbourne and Sophia Children's Hospital, Rotterdam

	Total number eligible ECMO patients	Number (%) eligible patients approached*	Number (%) approached patients successfully enrolled	Number (%)total eligible patients enrolled
Melbourne	109	83 (76%)	71 (86%)	71 (65%)
Rotterdam	12	12 (100%)	8 (67%)	8 (67%)

**Reasons for not approaching include ECMO run < 24 hours, no staff available; parents not available; social circumstances not appropriate.*

PB0284 | The Comparison of Critical Care Instrument CoaguChek® Pro II with Coagulation Analyzers Sysmex CS-5100 and Cobas t 511

M. Pulcer[1,2]; J. Gumulec[2]; L. Recova[2]; S. Kolarova[2]; Z. Svagera[1]; D. Stejskal[1,2]

[1]*University of Ostrava, Faculty of Medicine, Department of Biomedical Sciences, Ostrava, Czech Republic;* [2]*University Hospital Ostrava, Institute of Laboratory Diagnostics, Department of Clinical Hematology, Ostrava, Czech Republic*

Background: Point-of-care testing (POCT) devices are created for laboratory testing in the immediate vicinity of the patient in emergency medicine, general practitioners and in patients to monitor treatment at home. A significant benefit of POCT is the response time, which is shorter than the response time of the laboratory. The measurements are mainly performed by non-laboratory staff or even patients. For this reason the POCT technologies must be simple and able to ensure precission and accurate measurements.

Aims: Comprehensive evaluation of PT and aPTT determinations using CoaguChek® Pro II (Roche) with two coagulation analyzers CS-5100 (Sysmex) and cobas t 511 (Roche).

Methods: The investigated group contained samples ranging from physiological to very pathological (anticoagulant therapy). Venous blood was collected in Sarstedt tubes with 3.2% sodium citrate and tubes without anti-clotting reagent for CoaguCheck. Plasma was prepared by centrifugation for 7 minutes at 2500 g. Graphic and statistical evaluation was performed using MedCalc 18.2.1 (Passing-Bablok regression analysis, Bland-Altman difference diagram).

Results: The results of PT-INR (n = 62) confirmed by high correlation coefficients approaching 1.0 (0.945 and 0.947). The results of aPTT (n = 56) correlate less than the PT-INR. This is also evidenced by the lower values of correlation coefficients (0.881 and 0.901). The repeatability of measurements in series (n=6) were significantly higher for CoaguChek ($CV_{(PT)}$ = 6.74%, $CV_{(aPTT)}$ = 5.86%) in comparison with both analyzers (CV less 1%). The extended combined uncertainties of measurements were higher for CoaguChek® Pro II.

Conclusions: Statistical and graphical evaluation show a high correlation between PT-INR results, even in the areas of very pathological values. A lower level of comparability was found between aPTT results. It is caused by the different composition of reagents and the range of measurements, but also by comparing aPTT in seconds.

PB0285 | Coagulation Research in Paediatric ECMO: The Need for a Common Languagecoagulation Research in Paediatric ECMO: The Need for a Common Language

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Background: Extracorporeal membrane oxygenation (ECMO) involves complex coagulation management. Multicentre research is hampered by the fact that there are multiple variations to how each centre performs ECMO. Very few single centre publications include sufficient information about such variations to enable adequate comparison of results across studies. Hence, the applicability of any ECMO related research to other centres remains unclear. There is an urgent need to develop a common template of all ECMO and coagulation related variables to be documented clearly so that inter-study findings can be compared. The Extracorporeal Life Support Organization recently defined a mechanism for detailing exact cannulation techniques in a standard format. However, this is only one factor that impacts on coagulation research in ECMO.

Aims: To develop a common template of variables that are involved in coagulation management in paediatric ECMO patients so that studies can be compared across centres.

Methods: Two similar observational studies of coagulation parameters during ECMO in children are ongoing in Melbourne Australia (Royal Children's Hospital) and Rotterdam, Netherlands

(Sophia Children's Hospital). Each centre has independently developed their ECMO circuit set up and protocols. Sharing of data collection forms and database set up strategies, identified multiple discrepancies between circuit set up, management protocols, and definitions being used as research outcomes. An iterative process resolved many differences and agreed upon documentation techniques that enabled clear identification of remaining differences in analysis.

Results: Clinical parameters that required clear identification and research parameters that required consistency were identified and listed in Table 1.

Conclusions: ECMO research requires multicentre involvement to improve generalisability and enhance outcomes. This pilot demonstrates the approach that can be used to collaborate and improve comparability of research and results in the setting of ECMO.

TABLE 1 Dabigatran concentrations and other laboratory results in intoxicated patients

		Case 1	Case 2	Case 3
age (years)		**85**	**85**	**89**
Dabigatran (ng/mL)	**PeakTrough**	**373–476237–292**	**1269**	**968**
PT (%)	PeakTrough	19–2534–40	< 8	< 8
INR	PeakTrough	2.4–3.31.6–1.9	> 5.3	> 5.3
aPTT (s)	PeakTrough	68–7858–63	83	88
TT (s)	PeakTrough	> 150> 150	/	/
Hemoglobin (g/L)		163	65	101
creatinine (μmol/L)(CKD-EPI (mL/min/1.73m^2))		60(80)	149(27)	136(39)

PB0287 | Presentation of Three Patients Treated with Dabigatran that Strongly Confirm the Importance of Quantitative Measurement of Dabigatran Concentration in Selected Clinical Situations: Case Reports

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Background: Although clinical application of dabigatran does not require routine monitoring, there are special clinical situations in which laboratory measurement of drug concentration in plasma should be performed. In literature, there are increasing evidence of case reports of serious bleeding on dabigatran in the elderly, especially in those who develop acute renal failure.

Aims: To present three patients treated with dabigatran in which quantitative measurement of dabigatran concentrations in plasma have confirmed as very effective approach in clinical decision making and treatment.

Methods: Case 1: 85 years old female presented at consultant neurologist office with multiple skin hematomas.

Case 2: 85 years old female presented at Emergency Department (ED), referred from general practitioner with severe normocytic anemia and clinical symptoms of melena/hematochezia and chronic renal insufficiency in anamnesis.

Case 3: 89 years old male presented at ED in soporous state, with severe hypotension as a result of afterwards diagnosed aortic dissection.

Dabigatran was prescribed by cardiologist to all patients, in 110 mg twice daily regimen. Concentrations of dabigatran were measured by Innovance DTI assay on BCSXP analyzer (Siemens Healthineers, Germany). The study was funded by the Croatian Science Foundation as a part of the research project IP-2016-06-8208.

Results: Case 1: Peak concentrations of dabigatran were 473, 373 and 446 ng/mL, while trough concentrations were 257, 237 and 292 ng/mL, respectively.

Case 2: Dabigatran concentration in random sample was 1269 ng/mL.

Case 3: Dabigatran concentration in random sample was 968 ng/mL.

Results of screening coagulation assays and dabigatran concentrations are presented in Table 1.

Conclusions: In all three cases, measurement of dabigatran concentration directed further clinical decision making and treatment. In all three cases dabigatran treatment was immediately discontinued. In Cases 1 and 2 treatment was continued with apixaban, while in Case 3 surgical treatment was not done due to high risk of bleeding.

PB0288 | Clinical Outcome of Using Three-Factor Prothrombin Complex Concentrate in Patients with Major Bleeding Associated with Factor Xa Inhibitor

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Background: Since 2012, factor Xa inhibitors (FXai) have been widely used in Thailand, however, no specific reversal agent including four-factor prothrombin complex concentrate is available in Thailand so far. Therefore, three-factor prothrombin complex concentrate (3F-PCC) has been used to reverse the action of FXai. Nevertheless, the

clinical efficacy of 3F-PCC to reverse FXai had been reported in a few small case series.

Aims: To assess the effectiveness of 3F-PCC in major bleeding associated with FXai as primary outcome. And secondary outcome is to determine the use of blood component, duration of hospitalization, mortality and thrombotic event within 30 days after 3F-PCC administration.

Methods: We conducted a retrospective review of using 3F-PCC in patients with major bleeding associated with factor Xa Inhibitor at Siriraj hospital from January 2012 to December 2019.

Results: Ten patients (median age 73 years, range 60 to 83; 5 men) who were treated with 3F-PCC for reversal of FXai (7 rivaroxaban, 3 apixaban) were identified. The indications for using 3F-PCC included gastrointestinal bleeding (7 cases), intraperitoneal bleeding (one case), trauma-related bleeding (one case) and emergency surgery (one case). 3F-PCC were mostly given at emergency department (60%). Dose of 3F-PCC were 50 units/kg (70%), 75 units/kg (20%), and 25 units/kg (10%). There were 8/10 (80%) patients receiving fresh frozen plasma. According to ISTH criteria, the outcome of all patients was rated as effective and no thrombotic event or death occurred during 30 days.

Conclusions: In Thailand, 3F-PCC might be an effective and safe nonspecific reversal agent for major bleeding associated with factor Xa Inhibitor especially in gastrointestinal bleeding.

PB0289 | Comparison of a New Point-of-Care Hemostasis Analyzer with Laboratory Parameters in Patients with Veno-venous Extracorporeal Membrane Oxygenation

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Background: Bleeding and thrombotic events are the most common complications associated with veno-venous Extracorporeal Membrane Oxygenation (vv-ECMO) support. In patients with vv-ECMO, changes in the hemostatic balance including consumption of clotting factors, acquired von Willebrand defect, platelet dysfunction and altered fibrinolysis may result in both bleeding and thrombotic events, but their onset are difficult to predict. Data on the use of coagulation point-of-care tests in patients with vv-ECMO are scarce.

Aims: To compare the Quantra™ Point-of-Care Hemostasis Analyzer with laboratory parameters.

Methods: We conducted a prospective monocentric study at the Intensive Care Unit (ICU) of the Pitié-Salpêtrière Hospital. All adult patients admitted to ICU between November 2019 and January 2020 undergoing vv-ECMO were included in the study. Blood samples from patients were collected at baseline, then at days 1, 3, 5 and 7 after ECMO implantation. Quantra™ sonorheometric-based measurements included Clot Time (CT), Heparinase Clot Time (CTH), Clot Time Ratio (CTR), Clot Stiffness (CS), Fibrinogen (FCS) and Platelet (PCS) Contributions to clot stiffness. Laboratories parameters included platelet count, Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT), and Fibrinogen levels.

Results: Six patients were included in the study (n=30 samples). Significant correlations were observed between CT and APTT (r=0.624, p< 0.001), CTH and APTT (r=0.747, p< 0.0001), FCS and fibrinogen levels (r=0.798, p< 0.0001), CS and fibrinogen levels (r=0.818, p< 0.0001), and PCS and platelet count (r=0.849, p< 0.0001). All patients receiving unfractionated heparin had a Patient APPT / control APTT ratio < 1.2 and a CTR < 1.5. A 100% agreement between the Patient APTT / control APTT ratio and the CTR was observed (weighted kappa=1; p< 0.0001). Two patients presented a thrombotic event; CS and PCS dropped prior the onset of the thrombotic event in both patients.

Conclusions: The Quantra™ CT, FCS and PCS well-reflected APTT, fibrinogen-dependent clot stiffness properties, and platelet count.

PB0290 | Haemostatic Markers in Cardiopulmonary Bypass Surgery

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Background: Unfractionated heparin (UFH) can inhibit the von Willebrand Factor (VWF)-GPIbα interaction in vitro, an antithrombotic effect distinct from its anticoagulant role via antithrombin, which may in part explain why unpredictable bleeding complications occur during cardiopulmonary bypass surgery (CPB), when high doses of UFH are used.

Aims: To characterise the changes in VWF antigen (VWF:Ag) activity, function and platelet interaction, that occur pre and post UFH in patients undergoing CPB.

Methods: Following ethics approval and informed consent, samples were taken at baseline, 30 minutes post intravenous UFH, before reversal of UFH with protamine sulphate (PS), 30 minutes after reversal of UFH with PS, 12hrs and 24hrs postoperatively, in a prospective, single centre study, from 30 patients (>18yrs), undergoing first time surgery for the correction of atrial septal defects or tissue mitral valve replacement over a 6 month period.

Results: The mean age of the study group was 58.9yrs (SD±16.1). The VWF:Ag rose significantly by the end of surgery, p< 0.0001. Despite a significant rise in VWF:CBA by the end of surgery (p=0.0133) and 24hrs post-op (p< 0.0001), the VWF:CBA/VWF:Ag fell significantly (p=0.0015) suggesting loss of VWF high molecular weight multimers, presumed due to shear induced proteolysis by the bypass circuit.

The VWF Ristocetin Cofactor (VWF:RCo) and VWF:RCo/VWF:Ag both fell but not significantly following UFH (p=0.0588 and 0.0556 respectively). Although the absolute VWF:RCo recovered after UFH reversal with PS (p=0.0003), the VWF:RCo/VWF:Ag did not (p=0.5885). This may reflect reduction in multimer size or the reported inhibitory effect of PS on the VWF-GPIα interaction.

Conclusions: Both heparin and PS affect many components of haemostasis including platelet function and coagulation. The complex interplay between components of haemostasis, well-characterised consumptive and dilutional coagulopathy during CPB as well as these off-target effects of both heparin and PS, make it difficult to predict bleeding reliably in CPB.

PB0291 | Use of Recombinant von Willebrand Factor Alone during Surgeries in Type 2 von Willebrand Patients: Case Reports

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Background: Vonicog alpha is a recombinant Von Willebrand Factor (rVWF) used in prevention of surgical bleeding in the Von Willebrand (VW) disease. The monitoring of its activity in clinical practice by the usual biological tests is not yet well established because of the presence of high molecular weight multimers. Moreover, most studies reporting the use of rVWF had combined the use of Factor VIII (FVIII).

Aims: To report our experience of rVWF use alone during surgeries in type 2 VW patients.

Methods: We report six cases of surgeries or delivery using rVWF: bilateral breast lumpectomy, colic polyp excision, carpal tunnel, circumcision, hip arthroscan and delivery. There was no use of FVIII and therefore, no hospitalization the day before the surgery. We administrated between 39 and 57 UI/kg of rVWF one hour before the interventions. We monitored the Factor VIII level, platelet closure time (PCT), VWF antigen (VWF:Ag) and VWF activity measured by ristocetin activity (VWF:RCo) one hour after the rVWF infusions. Except for the delivery where a single injection was administered, patients received an other injection of 20 to 45 UI/kg of rVWF between 12 and 24 hours after the surgery, depending of the hemorrhagic risk associated with the surgery. Injections were then continued or interrupted depending of the clinical condition and biological results. At the outside, one patient received 7 injections of rVWF for a total duration treatment of 10 days. Patients were hospitalized between 24 and 48 hours.

Results: We observed a correction of PCT with VWF :Ag and VWF: RCo > 100% one hour after the first rVWF infusions. No bleeding complications or thromboembolic events have been observed after surgeries. There was no adverse effects with the rVWF infusions.

Conclusions: rVWF effectively corrects hemostasis tests and reduces the number of injections compared to what was usually done with other VWF concentrates.

PB0293 | Prospective Study of Haemostatic Activation Markers and Correlation with Bleeding and Thrombotic Complications in Patients Receiving Veno-Venous Extra-Corporeal Membrane Oxygenation (VV-ECMO) - HAE Study

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Background: Bleeding and thrombosis are common during Veno-Venous Extra-Corporeal Membrane Oxygenation (VV-ECMO) but their etiology remains unclear.

Aims: To determine the changes in haemostasis during VV-ECMO and correlation with major bleeding (MB) and thrombosis.

Methods: The study was approved by an external ethics committee. Samples were collected into citrate +/- corn trypsin inhibitor at commencement of VV-ECMO (prior to bolus dose unfractionated heparin [UFH]) (day 0) and after 24hrs (D1) and 5-day intervals (D5, D10 etc) until completion of VV-ECMO from 22 consecutive patients between September 2018 to Jan 2019. Samples were also collected 1hr and 24hrs after decannulation. All assays were performed after neutralising UFH with hepzyme.

Results: Mean age was 45.2 ±11.4yrs and 60% were male. Median (range) on VV-ECMO was 9.5days (5–29). Factor(F) II, V, VIII, IX, X, XI, XII and von Willebrand antigen (VWF:Ag)/VWF Ristocetin Cofactor (VWF:RCo) ratio fell with nadirs at D25 which was the longest VV-ECMO duration sampled. Post-decannulation and 24hrs post VV-ECMO, these factors returned to day0 levels or above. Endogenous thrombin potential [ETP] and peak thrombin fell and lag time and time to peak thrombin increased at D25. 27%(6/22), 54%(12/22) and 32%(7/22) patients respectively had circuit change, thrombosis and MB. Circuit changes and MB associated with longer ECMO (21.5 vs 9.6,p=0.0001 and 18.4 vs 10.3,p=0.012; days respectively) but with thrombosis (14.5 vs 10.9 days,p=0.27). Time to event analysis linked increased FV and FVIII to reduced circuit changes and increased FV, FVIII, FIX and ETP to increased thrombosis. Increased D-dimer predicted circuit change. Bleeding linked only to ETP where educed risk of MB associated with increased ETP.

Conclusions: MB and thrombosis occurred in 32% and 54% patients respectively. Intrinsic coagulation factors reduced gradually during VV-ECMO but increased factor V, VIII and ETP associated with development of thrombosis whilst ETP was a predictor of both bleeding and thrombosis.

PB0294 | PTT a Surrogate for Quantification of Factor VIII Inhibitor

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Background: Cardiovascular surgery (CVS) accounts for fifteen percent of the annual blood supply use.

As transfusion is associated with sepsis, stroke, immune modulation and mortality, perioperative optimization is critical component of CVS. Particularly, in cases with complex coagulation challenges.

Aims: We describe a case of acquired factor VIII inhibitor in the setting of an aortic valve replacement (AVR); highlight the diagnostic challenges of acquired factor VIII inhibitors; and describe the utility of using PTT as a surrogate for quantification of the Factor VIII inhibitor.

Methods: 53-year-old female with rheumatoid arthritis on infliximab, presenting with anemia and left arm and neck swelling. An ultrasound identified a left brachial DVT. Two units of pRBCs were transfused and IV heparin was started.

A CT chest showed mediastinal lymphadenopathy and severe stenosis of the SVC. The echocardiogram demonstrated an aortic valve vegetation and severe regurgitation, which necessitated surgical management. A prolonged PTT in the setting of line associated bleeding, and diffuse hematomas prompted discontinuation of heparin and a complex coagulation workup.

FIGURE 1 Factor VIII Inhibitor Correlation

Results: Normal thrombin time excluded heparin contamination. A mixing study suggested a factor inhibitor. Factor VIII was undetectable, the inhibitor was quantified at 17.60 Bethesda units and the corresponding PTT was 97.8. As the turnaround time for quantification of the Factor VIII and FVIII inhibitor is not readily available at our institution, we used the PTT as a surrogate marker. Before immunomodulation, a trial run of recombinant porcine FVIII (rPFVIII) was given and the PTT declined to 62 secs. In the setting of immunodulation, an overall retrospective correlation was noted between the PTT level and the factor VIII inhibitor trend. In the perioperative period, the patient was supported with rPFVIII and required minimal exposure to allogenic blood products.

Conclusions: PTT can be used as a surrogate marker of activity for Factor VIII inhibitor in perioperative period.

PB0295 | A Multicenter Experience with Idarucizumab "In Real World" as Reversal Anticoagulation of Dabigatran

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Background: Idarucizumab is the first reversal agent approved for dabigatran and works by binding to the drug compound to neutralize its effect in major bleeding and emergency surgeries. However there is not patients reported outcomes of using idarucizumab "in real world".

Aims: Analyze the use of idarucizumab as reversal agent of dabigatran in current clinical practice in the hospitals of Aragón (Spain) from 2016 up to now.

Methods: From January-16 to date, 23 patients were registered in this multicenter retrospective study. We analyzed clinical variables and laboratory tests; efficacy, safety and thrombogenic capacity of the idarucizumab and related mortality with its use.

Results: The indications of idarucizumab were emergency surgeries in 16 pts, major bleeding in 6 pts and 1 pt with severe renal failure requiring dialysis received the drug off-label. Surgeries requiring reversal anticoagulation were 1 splenic rupture, 2 aortic dissections, 1 cardiac transplant, 1 cholecystectomy, 1 stroke fibrinolysis, 1 nephrectomy, 3 lower limb thrombectomies, 1 stroke thrombectomy, 1 ankle osteosynthesis, 1 femur fracture, 1 left nephrostomy, 1 strangulated umbilical hernia and 1 drainage subdural hematoma. The use of the drug in major bleeding was 4 pts with intracerebral hemorrhage, 1 with retroperitoneal hemorrhage, 1 lower gastrointestinal bleeding and 1 hematuria. Activated partial thromboplastin time (aPTT) and prothrombin time (PT) were prolonged in 21 patients prior to idarucizumab infusion. Neither adverse effects nor thrombotic events were identified. Four patients died in relation with intraventricular hemorrhage, transplant complications, septic shock and multi-organic failure respectively.

Conclusions: Our series includes 23 patients treated with idarucizumab to reverse the anticoagulation effect of dabigatran in hospitals of Aragon. In our experience "in real world" idarucizumab reversed immediately, completely and safely the anticoagulation with dabigatran. The implementation of multidisciplinary protocols would optimize the cost / effectiveness ratio of idarucizumab in the approved indications.

PB0296 | Whole Blood Coagulation Profiles at the Time of Emergency Unit Admission in Patients with Major Trauma

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Background: Trauma-Induced Coagulopathy (TIC) affects nearly 1 in 4 patients after major trauma. Post-traumatic bleeding is still the leading cause of death among trauma patients. The appropriate strategy for the management of trauma-induced coagulopathy is still a matter of debate.
Aims: The main purpose of the study was to characterise traditional screening coagulation tests and Point-of-Care (POC) profiles of each patient at time of admission to the Emergency Department of Padua University Hospital for major trauma.
Methods: Adult trauma patients (≥16 years) with "major trauma" according to Padua triage scoring system admitted to the Emergency Department of Padua University Hospital were considered eligible for the study. For each patient the characteristics of anamnesis and triage were collected at admission. Vital signs, Focus Assessment with Sonography for Trauma (EcoFAST), arterial blood gas, routine screening coagulation tests and ROTEM profile were also evaluated and trauma scores were calculated.
Results: 33 patients, predominantly males (73%), with a mean age of 49 years old were enrolled. Thirty-day mortality rate was 12%. ROTEM profiles described a hypocoagulable state in patients with ISS ≥ 16: CFT_{INTEM} was significantly prolonged (p=0.025) and MCF_{EXTEM} significantly reduced (p=0.020). We also found a negative correlation between CT_{EXTEM} and PT (r= −0.65, p< 0.001); a direct correlation between CT_{INTEM} and aPTT (r= 0.80, p< 0.001); a direct correlation between A10 EXTEM and PT (r=0.49, p=0.007); and finally a negative correlation between A10 INTEM and aPTT (r= −0.85, p< 0.001).
Conclusions: Our data show a hypocoagulable trend in both patients with the most severe trauma and those who died within 30 day. The present study, although preliminary, proves that POC methods may provide rapid (10–15 minutes) and consistent information about the patient's coagulation state. Larger multicentre studies are needed to confirm our results.

PB0297 | Postoperative Heparin Monitoring by Anti-Xa Is More Accurate Than by aPTT After Pulmonary Endarterectomy - Role of Increased Systemic and Endothelial Production of Factor VIII

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Background: Pulmonary endarterectomy (PEA) is curative for suitable chronic thromboembolic pulmonary hypertension (CTEPH) patients. Careful anticoagulation prevents postoperative bleeding and thromboembolic recurrence. Post-operative heparin monitoring is usually guided by aPTT which may be shortened by postoperative elevation in Factor VIII - an acute phase reactant. Anti-Xa-guided anticoagulation on the other hand is not affected by elevated Factor VIII.
Aims: We investigated the correlation between aPTT and Anti-Xa for monitoring unfractionated heparin after PEA and the impact of coagulation factors.
Methods: 55 paired perioperative aPTT and Anti-Xa samples from 14 PEA patients were analyzed. aPTT monitoring was used for post-operative unfractionated heparin titration. Samples were considered concordant if aPTT (target 45–65 sec) and Anti-Xa (target 0.3–0.5 IU/ml) were in the same category of subtherapeutic, therapeutic or supratherapeutic as defined by our local aPTT and Anti-Xa nomograms for monitoring heparin. Antithrombin activity, Factor VIII and fibrinogen were concomitantly measured. Single-cell RNA sequencing of PEA specimens (n=5) was used to screen for coagulation factor transcription.
Results: aPTT and Anti-Xa were concordant in 64% of the postoperative sample pairs. Most common discordant pattern was subtherapeutic aPTT with therapeutic (15.6%) or supratherapeutic (11.1%) Anti-Xa. Compared to pre-operative levels, plasma Factor VIII increased on day 1 (p=0.04) and day 3 (p=0.0003), and fibrinogen on

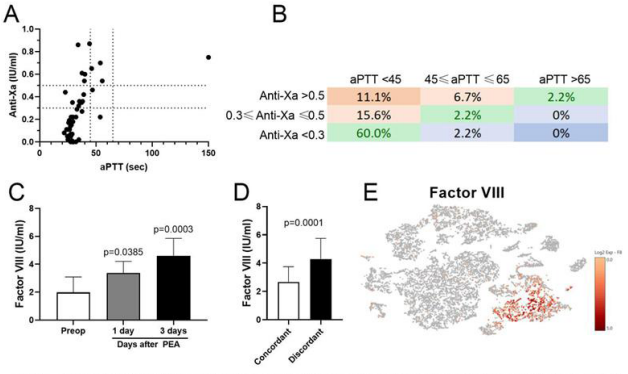

Figure. A) Postoperative paired sample aPTT and Anti-Xa measurements. Therapeutic ranges with dashed lines. B) Concordance of aPTT and Anti-Xa measurements according to target levels. C) Systemic Factor VIII levels over time. D) Systemic Factor VIII levels stratified by aPTT and Anti-Xa concordance status. E) Single-cell RNA sequencing of pulmonary endarterectomy specimens. Factor VIII mRNA was expressed in endothelial cells. Data mean±SD, analyzed by C) 1-way ANOVA with Dunnett correction comparing post-operative values to preoperative values or D) T-test.

FIGURE 1 Correlation between aPTT and Anti-Xa for heparin monitoring after PEA and effect of Factor VIII

day 3 after PEA (p=0.008). Discordant aPTT and Anti-Xa samples had higher antithrombin (p< 0.0001), Factor VIII (p=0.0001) and fibrinogen (p=0.0002) levels than concordant samples. Single-cell RNA sequencing showed endothelial Factor VIII transcription in the PEA specimen.

Conclusions: aPTT monitoring underestimates anticoagulation after PEA compared to Anti-Xa and is possibly influenced by elevated postoperative Factor VIII levels. Local Factor VIII production by thrombus endothelium may contribute to CTEPH pathogenesis.

PB0298 | Predictors of Residual Preprocedural DOAC Levels

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Background: In patients taking a direct oral anticoagulants (DOAC) who require elective surgery, there is uncertainty about the timing of DOAC interruption that results in a minimal-to-no residual anticoagulant effect at time of surgery. Factors associated with elevated residual DOAC levels and whether these factors are modifiable are also unknown.

Aims: We accessed the PAUSE study database to investigate the effect of DOAC interruption duration on residual DOAC levels at surgery.

Methods: Logistic regression was performed to identify factors associated with residual DOAC levels ≥30 ng/mL and ≥50 ng/mL.

Results: The proportions of patients with residual DOAC levels ≥30 ng/mL and ≥50 ng/mL according to interruption interval are shown in Figure 1. For patients having low-bleed-risk procedures, age ≥75 years, female sex, creatinine clearance (CrCl) < 50 mL/min, and interruption interval < 36 hours were associated with DOAC levels ≥30 ng/mL; age ≥75 years, female sex, CrCl < 50 mL/min, and standard DOAC dosing (compared with reduced dosing) were associated with DOAC levels ≥50 ng/mL. Apixaban users were more likely to have DOAC levels ≥30 ng/mL than dabigatran users and more likely to have levels ≥50 ng/mL than rivaroxaban users. For patients having high-bleed-risk procedures, weight < 70 kg, CrCl < 50 mL/min and standard DOAC dosing were associated with DOAC levels ≥30 ng/mL; female sex was associated with levels ≥50 ng/mL. Apixaban users were more likely to have DOAC levels ≥30 ng/mL than dabigatran users; rivaroxaban users were more likely to have levels ≥30 ng/mL than apixaban users.

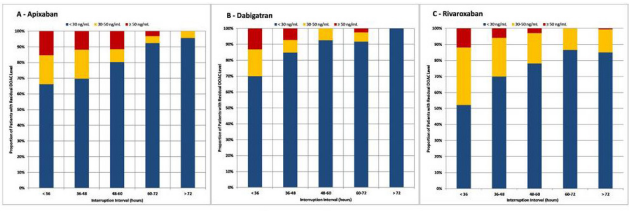

FIGURE 1 Proportion of Patients with Residual DOAC Levels ≥ 30 ng/mL and ≥ 50 ng/mL According to Interruption Interval

Conclusions: Determinants of residual DOAC levels prior to a surgery/procedure, comprising patient age ≥75 years, female sex, reduced CrCl, and DOAC dose regimen, do not appear to be modifiable. Further research is needed to determine if adjusting the DOAC dose regimen and timing of DOAC interruption will affect residual DOAC levels at surgery.

PB0299 | Microstructural Characterisation of in vivo Paediatric ECMO Circuit Binding and Blood Clot Structure

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Background: Extracorporeal Membrane Oxygenation (ECMO) is a form of circulatory support for children with severe respiratory or cardiac failure. Circuit thrombosis increases mortality of children on ECMO. However, protein and cellular adsorption to the surface of the ECMO circuit, as an initiating factor for circuit thrombosis, has largely been overlooked.

Aims: To characterise the microstructure of the blood clot and blood components adsorbed onto an ECMO circuit obtained from a paediatric patient.

Methods: This study was approved by the Royal Children's Hospital ethics committee. An ECMO circuit was collected from a one-year-old boy after he was successfully decannulated. A thrombus found post-oxygenator, as well as circuit samples from three different sites (Fig. 1) were studied using the Phenom XL desktop scanning electron microscope (SEM) (ATA Scientific, Australia).

Results: SEM images of the thrombus and circuit sites are shown in figure 2. The clot largely consists of erythrocytes trapped by fibrin (Fig. 2a). Circuit binding was markedly different between the three sites. Cells at site 1 show minimal aggregation (Fig. 2b), while site 2 contains a thin fibrin network and deposition surrounding leukocytes which is hypothesised to be the protein adsorption layer

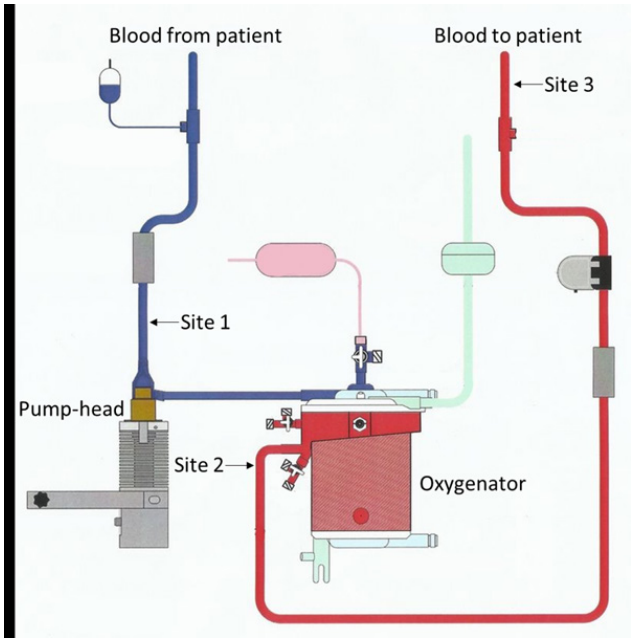

FIGURE 1 The RCH ECMO circuit. Circuit samples were collected from Site 1: before pump-head; Site 2: following the oxygenator; and Site 3: prior to patient

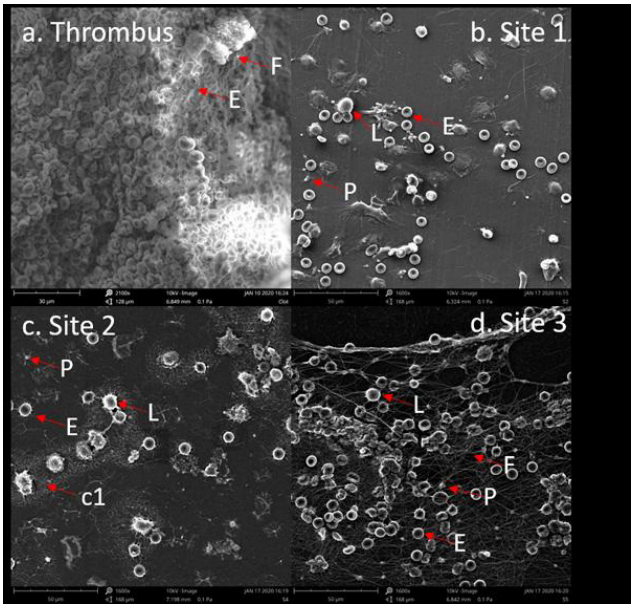

FIGURE 2 SEM images. Scale rule:(a)30μm;(b,c,d)50μm;Arrows: E-Erythrocytes;L-Leukocytes;P-Platelets;F-Fibrin Network; c1-protein adsorption

(Arrow c1). Site 3 shows significant binding to the circuit consisting of activated platelets, leukocytes, and red blood cells embedded in a thick fibrin network.

Conclusions: This is the first demonstration of ECMO circuit binding and site-specific differences in a circuit obtained from a paediatric patient. This is the first step towards characterising the blood components involved in circuit adsorption, showing the topography of the circuit binding. By identifying proteins/cells involved in circuit adsorption we will establish strategies to prevent this adsorption from taking place.

Acknowledgements: The authors would like to acknowledge the SEM imaging contributions of Peter Davis, Theano Stafidas and David Myint from ATA Scientific (Australia).

PB0300 | Investigation of the Coagulation Potential of PAS-E Resuspended Platelets

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Background: Platelet (PLT) transfusion is used to restore hemostatic capacity to bleeding patients with quantitative or qualitative defect of the PLT function. Nowadays, in Belgium, platelet concentrates are pathogen reduced and stored in a mixture of donor plasma (1/3) and platelet additive solution (PAS-E) (2/3). PLT transfusion might represent a source of coagulation factors and fibrinogen to the transfused patients.

Aims: In this study, we evaluated the coagulation potential of the mixture PAS-Plasma. Thrombin generation potential and fibrinogen concentration have been measured during 14 days in the PAS-Plasma supernatant of treated (T) (i.e. pathogen reduced) and non-treated (NT) PLTs.

Methods: A two-arm pool-and-split study was carried out using buffy coat-derived PLTs: no treatment vs INTERCEPT treatment. Fibrinogen concentration was measured using a coagulometric method (QFA Thrombin, Werfen). Endogenous Thrombin Potential (ETP) was evaluated using a Calibrated Automated Thrombogram (CAT, Thrombinoscope). Statistical analyses were performed through paired T-tests.

Results: Despite the PAS-dilution effect, the coagulation potential of T and NT PLT supernatants is well preserved compared to normal value of patients (600–1800 nM*min) (ETP, Table 1). In the NT arm, ETP values start to decrease from day 3 whereas in the T arm, values are stable until day 14. INTERCEPT treatment clearly affects the potential of the PAS-Plasma supernatant to generate fibrin, as fibrinogen concentration was statistically different between NT and T arms at each tested day (Table 1).

Conclusions: PLT transfusion helps bleeding patients to restore their PLT function. Our data show that transfusion of PLT preserved in a PAS-Plasma mixture can improve the coagulation potential of patients despite the dilution effect, with or without INTERCEPT treatment. In addition, our results also demonstrate that INTERCEPT treatment induces a two-fold decrease of fibrin generation. Further studies are needed to evaluate the clinical consequences of our findings.

TABLE 1 ETP ratio and Clauss fibrinogen results (mean±SD) *=significant difference (p<0.05) relative to day 1 †=<0.05 between T and NT at each test day

		Day 1	Day 3	Day 5	Day 7	Day 14
ETP ratio (nM*min)	NT	1.48 ± 0.09	1.39 ± 0.09*	1.40 ± 0.13	1.48 ± 0.15	1.35 ± 0.20
	T	1.48 ± 0.09	1.50± 0.08γ	1.54 ± 0.18γ	1.60 ± 0.11*	1.47 ± 0.11
Clauss fibrinogen (mg/dL)	NT	92.82 ± 5.98	96.55 ± 10.38	109.09 ± 20.84*	105.45 ± 16.87*	107.00 ± 12.06*
	T	51.09 ± 4.44γ	51.82 ± 4.19γ	51.73 ± 4.08γ	50.45 ± 3.91γ	50.90 ± 5.11γ

PB0301 | Use of Prothrombin Complex Concentrate for Reversal of Oral Anticoagulants in the Clinical Practice of a Portuguese Tertiary Hospital

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Background: Prothrombin complex concentrate (PCC) is recommended for reversal of oral anticoagulation (OAC), either vitamin K antagonists (VKAs) or direct inhibitors of Factor Xa (IFXa). A comparison of clinical characteristics, reversal indications and outcomes between OACs may allow a consensus for PCC usage.

Aims: Evaluate the use of PCC to reverse VKAs or IFXa (apixaban, rivaroxaban, edoxaban), in our hospital, in a 2-year period, and compare the two types of OACs.

Methods: We performed a retrospective analysis of PCC use in 323 patients, from January 2018 to December 2019, for reversal of VKAs (n=214) or IFXa (n=109).

Variables are presented as appropriate. A p value of 0.05 indicates statistical significance. Analyses were performed with SPSS software.

TABLE 1 Demographics of the study population (IQR - Interquartile range, AF - atrial fibrillation, VTE - venous thromboembolism)

Characteristic		VKAs	IFXa	Total	p-value
N (%)		**214 (66.3%)**	**109 (33.7%)**	**323**	
Age, median (IQR)		**77 (15)**	**80 (12)**	**78 (14)**	**p<0.05**
Male sex, n (%)		**131 (61.5%)**	**51 (46.8%)**	**182 (55.4%)**	**p<0.05**
OAC main indications	AF alone (%)	141 (65.9%)	95 (87.2%)	236 (73.1%)	p<0.05
	Prosthetic valves +- AF (%)	34 (15.9%)	2 (1.8%)	36 (11.1%)	
	VTE (%)	24 (11.2%)	6 (5.5%)	30 (9.3%)	

TABLE 2 Portrays the clinical context presented when PCC was used and its outcome. There was not an association between any type of OAC and PCC indication, or with the outcome

Characteristic		VKAs	IFXa	Total	p-value
PCC total dose, mean (SD)		**1635.51 (630.113)**	**2146.79 (761.646)**	**1808.05 (718.217)**	**p<0.05**
Emergency department (%)		**162 (75.7%)**	**101 (92.7%)**	**263 (81.4%)**	**p<0.05**
PCC main indication (%)	**IC Bleeding (%)**	**75 (35%)**	**39 (35.8%)**	**114 (35.3%)**	**p=0.807**
	GI Bleeding (%)	24 (11.2%)	14 (12.8%	38 (11.8%)	
	Other bleeding (%)	25 (11.8%)	8 (7.4%)	33 (10.3%	
	Urgent surgery/ Urgent Procedures (%)	85 (39.7%)	47 (43.1%)	132 (40.9%)	
Outcomes (45-day period)	Mortality (%)	38 (17.8%)	20 (18.3%)	58 (18%)	p=0.961
	Bleeding events (%)	18 (8.4%)	9 (8.3%)	27 (8.4%)	
	Thrombotic events (%)	4 (1.9%)	3 (2.8%)	7 (2.2%)	

PCC reversal indications and outcomes (SD- standard deviation, IC - intracranial, GI - gastrointestinal)

Results: Simultaneous administration of blood components was more frequent in patients with major bleeding (72.6%, p< 0.05), with no difference between groups.

Among patients treated with IFXa, 63.5% (n=66) presented an abnormal increase of serum creatinine - more frequently in context of surgeries/ invasive procedures (76.1% versus 53.4% for bleeding, p< 0.05).

Over the two years, although not significant, we found an increase in the reversal of IFXa - 29.9% of all reversals in 2018 and 36.9% in 2019.

Conclusions: Indication for reversal with PCC does not differ between studied groups. In spite of IFXa demanding a superior dose of PCC, there was not an association with thrombotic events (or with bleeding events for the VKAs).

Despite the short term study, it is possible to foresee the increasing number of patients under IFXa.

PB0302 | Efficacy and Safety of Ciraparantag in Reversing Apixaban and Rivaroxaban as Measured by Whole Blood Clotting Time in Healthy Adults

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Background: Ciraparantag is in clinical development as a reversal agent for various anticoagulants including non-vitamin K oral anticoagulants (NOACs). Ciraparantag was previously shown to reverse the anticoagulant effects of edoxaban and enoxaparin, as measured by whole blood clotting time (WBCT).

Aims: Two Phase 2 studies evaluated the efficacy (measured by WBCT) and safety of ciraparantag for reversal of anticoagulation induced by apixaban or rivaroxaban in healthy adults.

Methods: Two randomized, placebo-controlled, dose-ranging studies were conducted in healthy subjects age 50–75 years. Study 1 subjects received apixaban 10 mg orally twice daily for 3.5 days. Study 2 subjects received rivaroxaban 20 mg orally once daily for 3 days. In both studies, subjects at steady-state anticoagulation were randomized 3:1 to a single IV dose of ciraparantag (Study 1: 30, 60, or 120 mg; Study 2: 30, 60, 120 or 180 mg) or placebo. Efficacy was based on WBCT at multiple timepoints over 24 hours. Subjects and technicians performing WBCT testing were blinded to treatment.

Results: Across both studies, preliminary data are available for 80 subjects (60 ciraparantag, 20 placebo). Among subjects anticoagulated to ≥120% of baseline WBCT, reversal of WBCT to ≤110% of baseline within 1 hour post-dose and sustained through 5 hours was observed in Study 1 (apixaban) in 83%, 100%, and 0% of subjects receiving ciraparantag 60mg, 120mg, or placebo, respectively; and in Study 2 (rivaroxaban) in 92%, 92%, 100%, and 0% of subjects receiving ciraparantag 60mg, 120mg, 180mg, or placebo, respectively. The most common adverse events related to ciraparantag were mild, transient sensations of warmth (reported as hot flashes or flushing). Final results from all dose cohorts will be presented.

Conclusions: Ciraparantag single IV doses ≥60 mg showed rapid and sustained reversal of anticoagulation in high proportions of subjects anticoagulated with apixaban or rivaroxaban, and was well tolerated.

PB0303 | Assessment of the Difference Between EXTEM and FIBTEM Amplitudes (PLTEM) and Their Correlation with Platelet Count (PLT), and the Effect of Fibrinogen and Hematocrit (HTC) in Cardiovascular Surgery (CVS)

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Background: Thromboelastometry ROTEM® is a technique used in CVS for hemostatic and transfusion monitoring, and a correct interpretation of its parameters on the contribution of platelets and fibrinogen to the blood clot is needed.

Aims: To evaluate PLTEM at 5–10 minutes (PLTEM5 and PLTEM10) and at maximum clot firmness (PLTEMMCF), their correlation with PLT, and probable effect of HTC and Fibrinogen as FIBTEM MCF (FIBTEMMCF) or factors as EXTEM CT (EXTEMCT).

Methods: A total of 651 samples corresponding to baseline and post-protamine stages from CVS patients (adults and pediatrics under 4 y.o., during 2015–2019 period).

Instrument: Thromboelastometer ROTEM delta® (Instrumentation Laboratory).

HTC and PLT: HemoCell CxH800 (Beckman Coulter): Statistics: SPSS 23 software.

Results: Results are shown in tables 1–2.

As expected, the PLTEMs are greater for PLT≤100x109/L than 50x109/L and increases over time (PLTEMMCF>PLTEM10>PLTEM5). The PLTEM-PLT Quadratic R2 (R2) are smaller for FIBTEMMCF>10mm than for ≤10 only in adults. Most pediatrics R2 are higher than adults regardless of FIBTEMMCF. The highest R2 is observed in pediatrics with HTC≤30% and FIBTEMMCF≤10mm.

Adults ROC-AUC are generally higher for HTC≤30% and FIBTEMMCF≤10mm, and greater for PLT≤50x109/L. Pediatrics′ ROC-AUC are higher than adults′, for both PLT≤50 and 100x109/L.

TABLE 1 Statistics for adult patients. AUC: Area under de curve MCF: maximum clot firmness

ADULTS		PLTEM5 (mm)		PLTEM10 (mm)		PLTEMMCF (mm)	
		HTC ≤30%	HTC >30%	HTC ≤30%	HTC >30%	HTC ≤30%	HTC >30%
	CUADRATIC R2 FIBTEMMCF ≤10	**0.503 (n=29)**	**0.695 (n=8)**	**0.567 (n=29)**	**0.669 (n=8)**	**0.540 (n=29)**	**0.701 (n=8)**
	CUADRATIC R2 FIBTEMMCF >10	0.422 (n=377)	0.222 (n=139)	0.361 (n=377)	0.227 (n=139)	0.230 (n=377)	0.156 (n=139)
ROC CURVE PLATELETS ≤50x109/L	AUC / CUT OFF VALUE (%Sensitivity/%Specificity) FIBTEMMCF≤10	0.946 / 14.5 (93/100)	-	1.000 / 20.5 (100/100)	-	0.929 / 31.0 (96/100)	-
ROC CURVE PLATELETS ≤50x109/L	AUC / CUT OFF VALUE (%Sensitivity/%Specificity) FIBTEMMCF>10	0.717 / 19.5 (83/47)	-	0.718 / 24.5 (95/33)	-	0.649 / 33.5 (90/27)	-
ROC CURVE PLATELETS ≤100x109/L	AUC / CUT OFF VALUE (%Sensitivity/%Specificity) FIBTEMMCF≤10	0.876 / 15.5 (100/47)	0.917 / 14.5 (100/50)	0.910 / 24.5 (100/53)	0.917 / 22.5 (100/50)	0.926 / 37.5 (100/80)	0.917 / 29.0 (100/50)
ROC CURVE PLATELETS ≤100x109/L	AUC / CUT OFF VALUE (%Sensitivity/%Specificity) FIBTEMMCF>10	0.823 / 20.5 (91/48)	0.866 / 19.5 (92/36)	0.813 / 28.5 (94/42)	0.863 / 29.5 (92/64)	0.769 / 35.5 (92/33)	0.818 / 37.5 (91/50)

TABLE 2 Statistics for pediatrics patients. AUC: Area under de curve MCF: maximum clot firmness

PEDIATRICS		PLTEM5 (mm)		PLTEM10 (mm)		PLTEMMCF (mm)	
		HTC ≤30%	HTC >30%	HTC ≤30%	HTC >30%	HTC ≤30%	HTC >30%
	CUADRATIC R2 FIBTEMMCF ≤10	**0.760 (n=8)**	**0.547 (n= 26)**	**0.709 (n=8)**	**0.515 (n=26)**	**0.807 (n=8)**	**0.469 (n=26)**
	CUADRATIC R2 FIBTEMMCF >10	0.726 (n=14)	0.597 (n=50)	0.664 (n=14)	0.546 (n=50)	0.619 (n=14)	0.410 (n=50)
ROC CURVE PLATELETS ≤50x109/L	AUC / CUT OFF VALUE (%Sensitivity/%Specificity) FIBTEMMCF≤10	1.000 / 16.0 (100/100)	0.850 / 11.0 (95/33)	1.000 / 24.0 (100/100)	0.854 / 20.5 (90/33)	1.000 / 32.5 (100/100)	0.867 / 30.5 (90/50)
ROC CURVE PLATELETS ≤50x109/L	AUC / CUT OFF VALUE (%Sensitivity/%Specificity) FIBTEMMCF>10	0.939 / 13.5 (91/67)	0.951 / 15.5 (96/50)	0.939 / 20.5 (91/100)	0.945 / 26.0 (94/75)	0.978 / 24.5 (100/33)	0.951 / 31.5 (98/75)
ROC CURVE PLATELETS ≤100x109/L	AUC / CUT OFF VALUE (%Sensitivity/%Specificity) FIBTEMMCF≤10	1.000 / 21.5 (100/100)	0.938 / 18.5 (97/67)	1.000 / 24.0 (100/100)	0.958 / 27.5 (100/71)	1.000 / 38.0 (100/100)	0.958 / 37.5 (100/94)
ROC CURVE PLATELETS ≤100x109/L	AUC / CUT OFF VALUE (%Sensitivity/%Specificity) FIBTEMMCF>10	1.000 / 19.0 (100/80)	0.938 / 18.5 (97/67)	0.896 / 26.0 (87/63)	0.911 / 28.5 (95/67)	0.875 / 33.5 (87/67)	0.888 / 32.5 (100/50)

PLTEM cut-off values (COV) were selected with good sensitivity and intermediate specificity. PLTEM-COV are higher in pediatrics than adults when HTC≤30% and FIBTEMMCF≤10mm. Pediatrics R2 are better than adults ones when EXTEMCT is < 90 sec. However, COV calculated at any running time did not show any effect of EXTEMCT on PLTEM (data not shown).

Conclusions: Concluding, PLTEM reflects PLT in TEMograms amplitudes, but with some variability according to patient conditions. It seems to be a more accurate tool when CVS patients present low HTC and FIBTEMMCF, particularly in pediatrics. This must be kept in mind when PLTs transfusion is considered to improve hemostasis.

PB0304 | Outcomes of Patients with Anti-Xa versus APTTr Monitoring for Unfractionated Heparin Infusions during Extracorporeal Membrane Oxygenation

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Background: Anti-Xa levels and activated partial thromboplastin time ratios (APTTr) are suggested as methods for monitoring unfractionated

TABLE 1 Characteristics of patients receiving ECMO according to unfractionated heparin monitoring

Characteristics	APTTr	Anti-Xa
Mean duration of ECMO (days)	14	9
Mean SOFA Score at presentation	11	14
Mean Platelet Count (x10^9/L)	133	128
Number of patients with each Anticoagulation Target (prophylactic, therapeutic, fixed rate, no heparin)	9, 2, 2, 1	7, 1, 6, 0

heparin infusions (UFH) during Extracorporeal Membrane Oxygenation (ECMO). It is unclear which method of monitoring is most appropriate to prevent systemic thromboembolism and circuit thrombosis.

Aims: To compare clinical and therapeutic outcomes of patients receiving APTTr and anti-Xa monitoring during ECMO.

Methods: We performed a prospective audit of patients receiving ECMO from 03/09/19 to 03/11/19 using APTTr and 04/11/19 to 04/11/20 using anti-Xa. APTTr targets of 1.5–2.0 or anti-Xa of 0.3–0.7 were used for those without thrombosis (prophylactic) and APTTr 2.0–2.5 or anti-Xa 0.7–1.0 with thrombosis (treatment). Low-dose fixed rate infusions were used in those with high bleeding risk. Values were excluded during fixed rates or no heparin. Targets were adjusted at clinicians' discretion. Statistical analysis was performed by two-tailed Mann-Whitney U test with significance level $p< 0.05$.

Results: 14 patients were identified in each group. Bacterial and viral pneumonias were the most common underlying diagnoses (n=8 vs 9) for APTTr and anti-Xa respectively. Other characteristics are shown in Table 1. The mean number of monitoring tests per patients' ECMO duration for APTTr was 31 (2.5/day) versus 13 for anti-Xa (1.5/day) (p=0.026). The mean number of UFH dose changes was 7 vs 5 and time-in-target range was 40.7% vs 60.8% (p=0.038). Blood product usage was similar between both groups. Arterial and venous thrombosis during ECMO occurred in 4 vs 1 and deep vein thrombosis after decannulation occurred in 5 vs 1 in APTTr vs anti-Xa. Major bleeding occurred in 4 vs 1 for APTTr vs anti-Xa respectively.

Conclusions: Initial data from a small cohort suggests anti-Xa may provide a lower frequency of testing, higher time-in-target ranges and lower rates of thrombosis during ECMO over APTTr. This study needs further replication in larger patient groups to confirms these findings.

PB0305 | Profile of Effect of Sugammadex on Coagulation and Fibrinolysis

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Background: Inhibition of sugammadex in coagulation tests have been reported but mechanisms are not revealed.

Aims: Establish the profile of inhibition of sugammadex.

Methods: We used a new *in vitro* pharmacodynamic plasma clot growth model (Thrombodynamics) and documented thrombin generation in this model. Lysis was studied with a plasma clot lysis method and in the Rotem. Sugammadex was evaluated in the range 0–600 μg/ml and spiked in normal plasma.

Results: Sugammadex did not have effects on growth rate of plasma clots, neither in normal nor in factor IX deficient plasma, and not on the size of clots formed after 30–60 minutes. Delay in starting was minor. In the thrombodynamics, thrombin generation in the layer close to immobilised tissue factor, but also further away in the clot, was significantly reduced, up to 50% for 600 μg/ml sugammadex.

The consequence suspected, was a reduced clot stabilisation by thrombomodulin-thrombin-TAFI. This was indeed documented in the plasma clot lysis test including thrombomodulin, but not in lysis without added thrombomodulin.

In the Rotem, there was a small increase in lag time, but no change in maximal clot firmness. Clot firmness is dependent upon participation of factor XIII related crosslinking, which was apparently not affected by sugammadex.

This profile of an anticoagulant is not reported before, preventing benchmarking with clinical effects of other coagulation inhibitors.

Conclusions: During treatment with sugammadex, clots will be formed normally, with a small delay in formation. Mainly clot quality is affected, which will persist after clearance of sugammadex. Fibrinolysis can be increased when thrombomodulin is present. This profile may be expected in vasculature with significant thrombomodulin such as the brain circulation.

Thus, sugammadex effects may increase bleeding risk via fibrinolysis in tissues with high fibrinolytic status and with expression of thrombomodulin, such as in the brain. This suggests rational intervention and targeting with fibrinolysis inhibitors.

PB0306 | Reversal of Heparin Anticoagulation by Andexanet Alfa (Andexanet) in Human Plasma and Whole Blood: Differential Effect of Andexanet on Antithrombin-Dependent Anticoagulants and Direct Thrombin Inhibitors (DTIs)

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Background: Andexanet is a modified human FXa that is a specific reversal agent approved in the US and EU for patients treated with apixaban or rivaroxaban with life-threatening bleeding. Andexanet also effectively reverses antithrombin-dependent anticoagulants, e.g. fondaparinux, enoxaparin and heparin (Lu, Nature Med 2013, ASH2015, Crowther, ISTH2014). In the present study, reversal of heparin anticoagulation was studied in vitro by tissue factor (TF)-initiated thrombin generation (TF-TG) in plasma and activated clotting time (ACT) in whole blood. Additionally, ACT was used to evaluate andexanet's potential (or lack of) interference on DTIs

(argatroban and bivalirudin) as alternative anticoagulants to heparin in clinical settings like cardiac surgery following reversal of direct FXa inhibitors by andexanet.

Aims: To characterize the andexanet interaction with antithrombin-dependent heparin and DTIs:

Methods: TF (5 pM)-TG was measured using the automated calibrated thrombogram in plasma containing heparin (0–1.0 IU/mL) and andexanet (0–4 μM). ACT was measured using the ACT+ cartridge in whole blood containing heparin (1–4.0 IU/mL) ± andexanet. ACT prolongation was similarly studied with argatroban (0–10 μg/mL) or bivalirudin (0–30 μg/mL) ± andexanet.

Results: In human plasma, heparin (≥0.75 IU/mL) completely inhibited TF-TG. Andexanet (1, 2 or 4 μM) dose-dependently reversed heparin (1.0 IU/mL)-induced inhibition of TF-TG parameters by >75%, 97% and 100%. In human whole blood, heparin doubled ACT at ~1.6 IU/mL. Andexanet (1, 2 or 4 μM) reversed heparin (1.0 IU/mL)-induced ACT prolongation by 75%, 95% and 100%. Under similar conditions, ACT was prolonged by ~2-fold with argatroban (~1.0 μg/mL) or bivalirudin (~2.5 μg/mL). In contrast, andexanet (up to 4 μM) had no effect on DTI-induced ACT prolongation.

Conclusions: Andexanet was able to completely reverse heparin-induced inhibition of TF-TG and ACT without effect on DTIs, which could represent alternative anticoagulants to heparin in clinical settings like cardiac surgery in the presence of andexanet.

PB0307 | Rapid Thaw Plasma Units Thaw Significantly Faster than Standard Plasma Units while Retaining Similar Coagulation Profile over 5 Days of Cold Storage

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Background: Timely plasma transfusion is a critical part of hemostatic resuscitation during hemorrhage. Rapid thaw plasma (RTP) is a unit of plasma collected by apheresis and transferred in its pre-frozen liquid state into a 2000mL transfer bag that is also approved for plasma storage (TerumoBCT, Lakewood, CO) versus standard 400mL storage bags (Figure 1). Placing plasma into this large bag and freezing in a horizontal position creates a "paper thin" unit with significantly increased surface area, allowing RTP units to thaw more quickly versus standard plasma units.

Aims: Compare thaw times and major coagulation tests between RTP and standard plasma [plasma frozen within 24 hours of collection (FP24)] on both the day of thaw (Day 1) and Day 5 post-thaw.

Methods: Group A apheresis ACDA plasma were collected as 5 units of RTP and 5 units of randomly selected FP24, frozen, and stored at −30°C until ready for thawing. On Day 1, all units were thawed using the Barkey plasmatherm thawer (Barkey GmbH and Co., Leopoldshöhe, Germany). All 10 units were subsequently refrigerated at 1–6°C. On both Days 1 and 5, aliquots were drawn from

FIGURE 1 (A) Rapid Thaw Plasma (RTP) (left) and Standard Plasma (FP24) (right) Units (B) Cross Sectional View (RTP)

Assay	RT Plasma Mean (SD)	Standard FP24 Mean (SD)	p-value
PT (sec) (Day 1)	12.7 (0.9)	13.0 (0.6)	0.64
PT (sec) (Day 5)	14.5 (0.9)	14.7 (0.7)	0.78
INR (Day 1)	1.08 (0.08)	1.10 (0.05)	0.68
INR (Day 5)	1.24 (0.07)	1.25 (0.06)	0.82
aPTT (sec) (Day 1)	28.5 (1.4)	29.9 (2.5)	0.30
aPTT (sec) (Day 5)	31.9 (1.1)	33.2 (2.2)	0.29
Fibrinogen (mg/dL) (Day 1)	299.6 (39.8)	289.6 (85.4)	0.82
Fibrinogen (mg/dL) (Day 5)	303.4 (47.9)	289.8 (80.7)	0.76
Factor V (%) (Day 1)	102.9 (16.2)	91.7 (22.6)	0.40
Factor V (%) (Day 5)	89.0 (17.1)	82.0 (20.5)	0.57
Factor VIII (%) (Day 1)	113.8 (27.4)	114.8 (49.4)	0.97
Factor VIII (%) (Day 5)	59.1 (12.4)	59.3 (23.7)	0.99

FIGURE 2 Coagulation Assays for Rapid Thaw Plasma and standard FP24 plasma (n=5 for each)

each unit into a polypropylene transfer tube, re-frozen, and stored at −30°C. After subsequent thawing at 37°C, ACL Top 750 instruments with associated reagents (Instrumentation Laboratory, Bedford, MA) were utilized to perform coagulation testing on samples from Days 1 and 5, including PT/INR, PTT, Fibrinogen, and Factors V and VIII.

Results: RTP thawed in < 5 minutes for all units versus 18–28 minutes for FP24 (p< 0.0005). Coagulation test results demonstrated no significant differences between the products on Day 1 or 5 (Figure 2).

Conclusions: RTP thaws in < 5 minutes and maintains a similar coagulation profile to FP24 over 5 days of refrigerated storage. These findings support RTP as a superior product for rapid delivery of pla sma.

PB0308 | Investigation of Several Four Factor PCCs to Restore Thrombin Generation Following Anticoagulation with Rivaroxaban: Ex vivo Study

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Background: Reversal of severe bleeding under factor Xa inhibitor therapy requires either therapy with the specific antidote andexanet alfa or prothrombin complex concentrate (PCC). All PCCs are standardised and administered according to the quantity of factor IX, but there are considerable differences between PCCs in the quantities of coagulation factors II, VII, and X.

Aims: This study aims to explore the impact of different 4F-PCCs ex vivo using human blood treated with FXa inhibitors, as prediction of these impact on coagulation status due to the various quantities of

coagulation factors and inhibitors in these products. Therefore, four different 4F-PCCs and activated PCC (aPCC) were be compared for their antagonizing effect.

Methods: Blood samples from 10 healthy volunteers were spiked with rivaroxaban (RIVA). Four different 4F-PCCs (Beriplex, CSL Behring; Octaplex, Octapharma; Cofact, Biotest; Prothomplex, Baxalta) and aPCCs (FEIBA, Baxter) were added at clinical recommended doses (25 and 50 U/kg body weight). Various laboratory assays including PT, aPTT, and a thrombin generation assay (CAT) were applied to measure the impact of PCC/aPCC.

Results: Increasing the concentration of RIVA, decreased significantly thrombin generation potential (ETP, peak high) and prolonged the lag time. At low concentrations of RIVA (37.5 ng/mL, 75 ng/ml) all PCCs and aPCC restored ETP, but the peak did not reach baseline (figure 1). The addition of PCCs at high RIVA concentrations (150 ng/ml, 300ng/ml) had only small effects on thrombin generation potential. The prolonged lag time was only shortened by aPCC.

Conclusions: Restoration of thrombin generation following anticoagulation with RIVA showed no significant difference between commercially available PCCs. Higher concentrations of RIVA (≥150 ng/ml) cannot be reversed using PCC. The impact on the lag time after aPCC substitution is probably related to FVIIa.

PB0309 | Clinical Study of Children's Intracranial Hemorrhage in Single Hemophilia Center in China

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Background: Although hemophilia comprehensive care was improved theses years in China. Intracranial hemorrhage (ICH) is still a serious and not rare complication in children with hemophilia.

Aims: This study was to retrospectively analyze the clinical data of childre's intracranial hemorrhage with hemophilia in Hemophilia Comprehensive Management Center in China in order to to sum up the characteristics for the further clinical diagnosis and treatment.

Methods: The clinical data of total 19 children with hemophilia A or hemophilia B diagnosed ICH in Nanfang Hospital from 2016.1 to 2019.6 were enrolled.

Results: Among children with ICH, 73.7% (14/19) were hemophilia A and 26.3% (5/19)were hemophilia B. Severe hemophilia children counted 52.6%(10/19), meanwhile the proportion of children with intermediate type was also reached to 9/19 (47.4%). The median age occurred ICH was 17 months. There was 63.2%(12/19) of children with spontaneous bleeding and 36.8% (7/19) occurred after trauma. After ICH, 52.6% (10/19) of children had recurrent ICH. Nervous system sequelae occurred in 31.6% (6/19) of patients, and 1 case died of brain stem hemorrhage 1/19 (5.3%). About 94.7% (18/19) of ICH children did not underwent surgical treatment. About 78.9% (15/19) of ICH occurred at more than one site in CT imaging. Seven (7/19, 36.8%) patients developed inhibitor after ICH, and all of them with high titers inhibitor. Before the onset of ICH, 57.9%(11/19) of the children was in on demand treatment, and 42.1% (8/19) of them was in prophylaxis.

Conclusions: Intermediate hemophilia children was also in risk of ICH as well as severe patients. Most of them are spontaneous intracranial hemorrhage, easy to occur in multiple sites in one bleeding episode, and with high risk of recurrence. Most of the ICH did not need surgical treatment. ICH patients need more monitor for inhibitor.

PB0310 | Bleeding and Thrombotic Complications in Patients Undergoing Major Cardiac Surgery on Single, Dual Antiplatelet Treatment Compared to Controls

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Background: Bleeding is a significant complication of cardiac surgery and antiplatelet (AP) can be a contributory factor. Current guidelines recommend aspirin be continued whilst P2Y12 inhibitors (P2Y12i) are stopped 5–7 days prior to major cardiac surgeries (MCS).

Aims: To assess the bleeding, blood products use, thrombosis (TE) and 30-day mortality, we compared the following in patients who underwent MCS:

1) Patients continued aspirin as a single antiplatelet, patients discontinued single agent P2Y12i for 5–7 days or patients on DAP but discontinued P2Y12i 5–7 days preop and continued with aspirin were each compared to patients not on any AP (control)
2) Continued aspirin vs discontinued aspirin 5–7 days pre-op
3) Stopped DAP < 5 days vs stopped DAP 5–7 days pre-op (continued aspirin)

Methods: Retrospective, single-centre, observational study from 01.03.2016 to 31.12.2018 with 3,559 patients: 2221 controls, 1166 single AP (1052 Aspirin, 114 P2Y12i) and 172 DAP.

Results: Group1. Baseline characteristics and indicators of bleeding are presented in Tables1 and 2. Aspirin continuing (P=0.001) and P2Y12i stopped (P=0.036) groups received fewer units of FFP than control. Patients on DAP received fewer units of FFP, cryoprecipitate, and platelets than control (P=< 0.001, 0.002 and 0.001). TE rates were 0.8% (control), 0.0% (aspirin), 1.6% (P2Y12i) and 1.4%

TABLE 1 Baseline Characteristics

Characteristics	Control N=2221	Aspirin N=368	P value	P2Y12i N=64	P value	DAP N= 141	P value
Male sex (%)	71.1	78.3	0.013	87.5	0.006	83	0.005
Age (yrs)	67 (18–89)	68 (34–86)	0.015	68 (47–84)	0.273	65 (36–86)	0.264
EuroSCORE 2	1.51 (0.5–64.35)	1.31 (0.5–26.3)	0.002	1.1 (0.5–5.88)	0.007	1.01 (0.5–18.59)	<0.001
Hb (g/L)	135 (62–188)	133.5 (66–166)	0.012	134 (87–158)	0.628	138 (71–167)	0.079
Platelet count (x109)	203 (65–627)	201.5 (18–863)	0.82	188 (100–373)	0.199	205 (71–430	0.224
Fibrinogen (g/L)	2.6 (0.6–7.0)	2.5 (0.6–7.7)	0.163	2.7 (1.2–5.7)	0.662	2.65 (1–5.9)	0.301
Creatine clearance (ml/min)	86 (8–261)	83.2 (13–192.1)	0.113	87.3 (10–156.8)	0.204	84.8 (21.3–183.4)	0.512
Operation duration (minutes)	255 (45–760)	240 (85–630)	<0.001	250 (130–425)	0.33	240 (110–565)	0.001
TXA use (%)	74.7	77.2	0.309	81.3	0.233	73	0.66

TABLE 2 Indicators of bleeding

Indicator of Bleeding	Control N=2221	Aspirin N=368	P value	P2Y12i N=64	P value	DAP N= 141	P value
Hb (g/L)	−35 (−106–31)	−34 (−92–22)	0.1866	−36.5 (−71 - −5)	0.11	−35 (−76–21)	0.686
Platelet count (x109)	−66 (−378–141)	−56 (−245–132)	0.004	−53.5 (−217–70)	0.273	−51 (−294–35)	0.029
Volume of red cells salvaged (ml)	1792 (0–17112)	1432.5 (0–5336)	<0.001	1494 (0–4455)	0.029	1205 (0–4994)	<0.001
24-hr Surgical drain volume (ml)	550 (0–3640)	575 (25–5180)	0.01	712.5 (175–3690)	0.01	600 (25–1450)	0.172

(DAP) respectively with no difference between any treatment group and control.

Group2. Patients continued aspirin (n=368) received more units of PRC and platelets (p=< 0.001 and 0.001) than patients stopped (n=684), no difference in bleeding or TE rate.

Group3. Patients stopped DAP < 5days (n=31) received more units of platelets/patient vs those stopped 5–7 days (n=141 (p=0.002), however, no difference in bleeding or TE rate.

Conclusions: There was no difference TE or 30-day mortality in any group. Patients continued aspirin or DAP received more blood products than those stopped aspirin or P2Y12i in respective groups but no difference in bleeding.

PB0311 | Thrombocytopenia in adult ICU patients with sepsis

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Background: The incidence of thrombocytopenia in critically ill patients varies considerably in different studies, likely depending on setting and definitions.[1,2]Previous studies suggest that ICU patients with thrombocytopenia are sicker, have longer ICU stays and worse outcome.[3,4]Patients with sepsis are at particular risk,[3,5]but there are few studies on thrombocytopenia in these patients.[3,5]

Aims: To describe prevalence of thrombocytopenia in critically ill patients with sepsis during the first 5 ICU days, and assess association with mortality.

Methods: Post-hoc analysis of a randomised trial in patients with sepsis needing fluid (6S trial, ClinicalTrials.gov number NCT00962156) allocating participants to hydroxyethyl starch (HES) 130/0.42 vs. Ringers acetate in 26 general ICUs in 13 university and 13 non-university hospitals in 4 countries.[6] Patients with life-threatening bleeding were excluded.

Platelet counts were collected within 24-hours prior to randomisation and during the following 5 days in ICU. The association between thrombocytopenia and mortality was analysed by Cox regression adjusting for age, haematological malignancy, shock at admission and allocation (HES vs. Ringers) with a follow-up time of 28 days. Log-rank test was used to compare the Kaplan-Meier curves.

Results: The 6S trial included 798 patients; median time in ICU before randomisation was 3.8 (1.3–12.3) hours. At baseline, 37% of the patients had thrombocytopenia, and by day 3 half of the patients

TABLE 1 Thrombocytopenia in patients with severe sepsis. Data are in median (IQR) or no. (%)

Age	**66 (56–75)**					
mSOFA score (baseline)	7 (5–9)					
Haematological malignancy	72 (9)					
	Baseline (N = 771)	*Day 1 (N=705)*	*Day 2 (N=732)*	*Day 3 (N=633)*	*Day 4 (N=538)*	*Day 5 (N=465)*
Platelet count (x 10^9/L	194 (110–275)	164 (91–247)	161 (84–239)	144 (82–230)	143 (78–220)	154 (78–224)
Patients with thrombocytopenia	283 (37)	315 (45)	349 (48)	326 (52)	284 (53)	221 (48)
Mild (100–149)	118 (15)	122 (17)	134 (18)	132 (21)	104 (19)	79 (17)
Moderate (50–99)	88 (11)	107 (15)	108 (15)	93 (15)	90 (17)	65 (14)
Severe (20–49)	40 (5)	55 (8)	70 (10)	57 (9)	56 (10)	47 (10)
Very severe (<20)	37 (5)	31 (4)	37 (5)	44 (7)	34 (6)	30 (6)

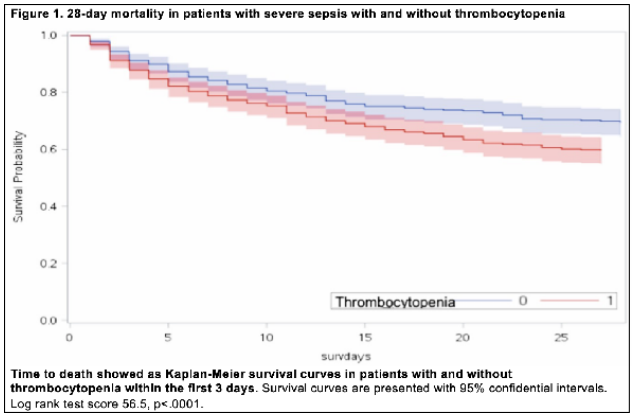

FIGURE 1 28-day mortality in patients with severe sepsis with and without thrombocytopenia within the first 3 days

had developed thrombocytopenia (platelet count < 150 x 10^9/litre), which was severe (< 50 x 10^9/litre) in 101/633 patients (16%) (Table 1). Patients with thrombocytopenia had higher 28-day mortality; adjusted hazard ratio 1.40 (95% CI 1.097–1.787) (Fig. 1)

Conclusions: These results are in line with previous studies showing that thrombocytopenia is common among ICU patients with sepsis and is associated with higher mortality.

References:

1 Vanderschueren *CCM* 2000
2 Venkata *J Intensive Care*. 2013
3 Sharma *Annals Intensive Care*2007

4. Williamson *Can J Anaesth*2013
5. Thiolliere *ICM* 2013
6. Perner *NEJM* 2012

PB0312 | A Strategy of Continued Antiplatelet Agents, Vitamin K Antagonists, and Direct Oral Anticoagulants throughout the Perioperative Period of Diabetic Foot Surgery in Patients Receiving Chronic Antithrombotic Therapy

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Background: Although continuing antithrombotic therapy is desirable to prevent perioperative cardiovascular and cerebrovascular diseases, perioperative blood loss remains a concern in patients undergoing diabetic foot surgery

Aims: Assess the impact of continuing chronic antithrombotic therapy on blood loss and major bleeding events.

Methods: We classified 54 consecutive patients undergoing diabetic foot surgery into 2 groups: (1) patients taking antiplatelet agents, vitamin K antagonists, and/or direct oral anticoagulants, referred to as the continuing antithrombotic therapy group (n = 22); and (2) patients not receiving these agents, referred to as the no antithrombotic therapy group (n = 32). During the study period, antithrombotic agents were continued perioperatively in all patients receiving antithrombotic therapy. Surgical procedures were performed without the use of a pneumatic tourniquet or drain. Screening for deep vein thrombosis was routinely performed before and after diabetic foot surgery.

Results: The perioperative blood loss after diabetic foot surgery did not differ significantly between the continuing antithrombotic therapy group and the no antithrombotic therapy group at 1 day postoperatively (412 ± 173 compared with 438 ± 311 mL [95% confidence interval (CI) of the difference, −162 to 74 mL]; p = 0.44), 3 days postoperatively

(798 ± 311 compared with 822 ± 301 mL [95% CI, −106 to 129 mL]; p = 0.79), and 7 days postoperatively (823 ± 313 compared with 850 ± 242 mL [95% CI, −121 to 104 mL]; p = 0.83). No patients in the continuing antithrombotic therapy group and 2 patients in the no antithrombotic therapy group had allogeneic blood transfusion (p = 1). No major bleeding events occurred in the continuing antithrombotic therapy group.

Conclusions: Perioperative blood loss in patients continuing chronic antithrombotic therapy during diabetic foot surgery was not significantly different from that in patients receiving no chronic antithrombotic therapy.

PB0313 | VMX-C001 Is a Reversal Agent for FXa-inhibiting DOACs and Displays a Favorable Pharmacokinetic and Pharmacodynamic Profile

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Background: Reversal of anticoagulation is important for safe usage of oral factor (F)Xa-inhibiting anticoagulants (DOACs), including rivaroxaban and apixaban. VMX-C001 is a modified human FX zymogen that is insensitive to DOACs. VMX-C001 may therefore prevent and stop bleeding associated with FXa-inhibiting DOACs.

Aims: To assess the pharmacokinetics, pharmacodynamics, and safety of zymogen VMX-C001 and potency of activated VMX-C001 in cynomolgus monkeys and rats.

Methods: Zymogen VMX-C001 was administered intravenously to male and female cynomolgus monkeys and male Sprague Dawley rats at 0.3, 1.0, or 3.0 mg/kg/day for 7-8 consecutive days. Blood was drawn at regular intervals to evaluate VMX-C001 levels and determine pharmacokinetics. Pharmacodynamics were examined employing the plasma endogenous thrombin potential (ETP). For safety assessment the plasma thrombin-antithrombin complexes (TAT) and D-dimer levels were determined.

Results: VMX-C001 was successfully manufactured in suspension CHO cells and purified in a scalable process. Analysis of pharmacokinetics revealed linear and dose-proportional kinetics of VMX-C001 and a plasma half-life of 7-9 hours in monkeys (Table 1) and 6 hours in rats. The distribution volume of VMX-C001 was approximately 90 mL/kg, which equals the estimated blood volume. Period or gender effects were not observed upon repeated dosing. Robust ex vivo and in vivo reversal of the effect of apixaban/rivaroxaban on the ETP was observed at the mid-dose level. Reversal was not impaired upon repeated VMX-C001 dosing. No dose-dependent increase in TAT and D-dimer was detected upon administration of zymogen VMX-C001, indicating that VMX-C001 does not have a procoagulant effect. In contrast, a rapid and dose-dependent increase in TAT and D-dimer levels was observed upon administration of 2 µg/kg activated VMX-C001, demonstrating its in vivo potency. Adverse events, drug related toxicity, or macroscopic deviations were not observed following administration of zymogen or activated VMX-C001.

PK parameters (mean ± SD)	Dose 0.3 mg/kg/day	Dose 1 mg/kg/day	Dose 3 mg/kg/day
n	10	10	10
C_0, µg/mL	5.2 ± 0.6	17.6 ± 1.5	53.5 ± 4.6
AUC_{24h}, µg.h/mL	34.2 ± 2.8	124.9 ± 15.8	388.1 ± 47.9
$AUC_{24h, DN}$, µg.h/mL	114.0 ± 9.2	124.9 ± 15.8	129.4 ± 16.0
$t_{1/2}$, h	7.6 ± 0.6	9.1 ± 2.5	9.2 ± 2.4
CL, mL/h/kg	8.9 ± 0.8	8.6 ± 0.7	8.4 ± 0.7
V_{ss}, mL/kg	90.5 ± 6.3	96.4 ± 15.6	91.4 ± 11.5
MRT, h	10.2 ± 0.7	11.2 ± 1.6	11.0 ± 1.1
Accumulation ratio	0.925 ± 0.068	0.988 ± 0.112	1.099 ± 0.076
Linearity index	1.021 ± 0.088	1.096 ± 0.130	1.210 ± 0.067

Legend: C_0: (concentration at time of dosing), AUC_{24h} (area under the concentration-time curve over a 24-hour dosing interval), DN (dose normalized), $t_{1/2}$ (elimination half-life), CL (clearance), V_{ss} (volume of distribution at steady state) and MRT (mean residence time).

TABLE 1 Pharmacokinetic parameters of VMX-C001 in cynomolgus monkeys

Conclusions: VMX-C001 is a potentially safe and broad anti-FXa DOAC reversal agent with favorable pharmacokinetics and pharmacodynamics.

PB0314 | Management of Intracranial Hemorrhage in Patients under Non-vitamin K Antagonist Oral Anticoagulants

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Background: Major bleeding, particularly intracranial hemorrhage (ICH), is the most feared adverse event observed in patients with atrial fibrillation under long-term anticoagulation. Multicenter observational studies concluded that reversal strategies of bleeding in patients under non-vitamin K antagonist oral anticoagulants (NOAC) differ largely and lack evidence of clinical efficacy.

Aims: Evaluate therapeutic options and clinical outcomes of patients with ICH under NOAC.

Methods: This is a retrospective observational study that includes patients under NOAC that were admitted to a tertiary hospital with ICH between 01/01/2017 and 13/12/2019. Data were analyzed using IBM®SPSS®Statistics 26.

Results: During this period, 34 NOAC concentrations were determined in the context of neurological alterations and/or cranioencephalic trauma, corresponding to 27 patients, 20 of whom with ICH, aging 53-92 years (µ=81.5; IQR=17), with a majority of men (85%, n=17). Of these events, 40% (n=8) were spontaneous and 60% (n=12) traumatic.

Although it was difficult to assess the hour of last NOAC intake, at least 10% (n=2) presented a concentration above therapeutic peak range.

Treatment was exclusively medical in 50% (n=10). Surgery was performed in 20% (n=4). Anticoagulation in 1 patient was reverted with idarucizumab; remaining 13 were empirically treated with prothrombin complex concentrates.

Of the 18 computed tomographies performed 24h after admission, 50% (n=9) revealed worsening of the bleeding; 35% (n=7) of the patients presented a worse modified Rankin Scale at discharge and 20% (n=4) a better one. Death occurred in 25% (n=5). The sample size doesn't permit to stablish an association between clinical evolution and performed treatment.

Conclusions: Lack of apparent correlation between clinical outcomes and ICH management emphasizes importance of primary prevention of these events, with correct initial and continuous assessment of individual bleeding risk. Application of tools as HAS-BLED score to evaluate modifiable and non-modifiable risk factors, together with implementation of institutional multidisciplinary protocols concerning bleeding management, assume crucial importance.

PB0315 | Routine Coagulation Assays for the Detection of Residual NOAC Levels in Patients Undergoing Elective Invasive Procedures

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Background: Each year, millions of patients treated with direct acting oral anticoagulants (DOAC) require invasive procedures. In PAUSE, a prospective cohort study of 3,007 DOAC-treated patients undergoing elective procedures, a standardized interruption strategy was associated with a low risk of bleeding and low residual DOAC levels in many, but not all patients. DOAC-specific assays are not widely available whereas routine coagulation tests, comprising prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT), are widely available.

Aims: To assess whether PT, aPTT and TT can identify patients with elevated residual DOAC levels after DOAC interruption for procedures.

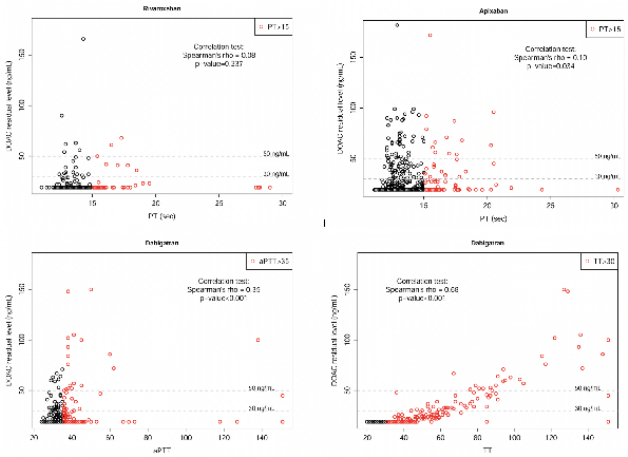

FIGURE 1 Distribution of residual DOAC levels by PT (rivaroxaban and apixaban) and aPTT and TT (dabigatran)

Methods: As a pre-specified sub-analysis of PAUSE, a blood sample was obtained immediately pre-procedure. We compared PT (Siemens Thromborel), aPTT (Siemens Dade Actin FS) and TT (Sigma-Aldrich) with DOAC levels assessed by dilute thrombin time (Hemoclot, Hyphen BioMed) for dabigatran, and anti-factor Xa levels (Hyphen BioMed) for rivaroxaban and apixaban. All tests were performed on a STAr-Evolution analyzer (Diagnostica Stago) in a central lab. In the absence of a validated threshold, DOAC levels were categorized as low (< 50ng/ml) or very low (< 30ng/ml).

Results: Following standardized interruption, the majority of patients had low residual DOAC levels. Distribution of residual DOAC levels by PT (rivaroxaban, apixaban), aPTT, and TT (dabigatran) are shown in Figure 1. Most patients with normal routine coagulation assays had low or very low residual DOAC levels: 88.6% for apixaban (PT), 96.4% for rivaroxaban (PT), and 98.1 and 100% for dabigatran (aPTT and TT). However, elevated routine assays were poor predictors of high residual activity (Table 1).

Conclusions: Use of a standardized DOAC interruption strategy led to low or very low residual DOAC levels in a majority of patients. Due to low specificity, routine coagulation tests are not helpful as a rapid screening test to detect pre-procedure residual DOAC levels.

TABLE 1 Very low, low, and elevated DOAC levels in patients with normal vs elevated routine coagulation assay results (N, %)

	Apixaban		Rivaroxaban		Dabigatran		Dabigatran	
Residual DOAC level (ng/mL)	PT≤15 (N=385)	PT>15 (N=86)	PT≤15 (N=194)	PT>15 (N=44)	aPTT≤35 (N=432)	aPTT>35 (N=100)	TT≤30 (N=254)	TT>30 (N=272)
<30	290 (75.32%)	60 (69.77%)	174 (89.69%)	36 (81.82%)	401 (92.82%)	69 (69.00%)	254 (100.00%)	211 (77.57%)
30-50	51 (13.25%)	12 (13.95%)	13 (6.70%)	4 (9.09%)	23 (5.32%)	13 (13.00%)	0 (0.00%)	35 (12.87%)
>50	44 (11.43%)	14 (16.28%)	6 (3.09%)	3 (6.82%)	8 (1.85%)	18 (18.00%)	0 (0.00%)	26 (9.56%)

PB0316 | Haemostatic Changes in Patients Undergoing Renal Transplantation

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Background: Chronic kidney disease results in altered haemostasis with increased risk of venous thromboembolism (VTE) and bleeding. Without thromboprophylaxis, the incidence of VTE post-transplant is estimated at 7.4% and major bleeding 2.1%. Reported incidence for renal allograft thrombosis is between 0.4-6%. Currently there is no consensus on optimum thromboprophylaxis strategies post renal transplant and protocols are largely based on guidelines in general surgery patients.
Aims: To evaluate and compare changes in haemostatic parameters in renal transplant recipients and donors at pre-determined time points before and after surgery. This study was approved by the medical ethics committee.
Methods: Single site observational cohort study. After informed consent was obtained, citrated blood samples were collected at admission, immediately post-operatively and at 24 hours for both recipient and donor. Samples at 72 hours and 5 days post-surgery were collected in addition for recipients. Assays performed included D-dimer, prothrombin fragments 1+2 (F1,2), anti-thrombin (AT), von Willebrand factor (vWF) activity and factor VIII activity.
Results: 34 patients recruited (17 recipients and 17 donors). Median age of recipients was 41 years (23-70 years) with a male preponderance (M:F 3:1). Median age of donors was 47 years (26-75 years) with a slight female preponderance (M:F 1:1.4). The average eGFR was 10ml/min in recipients compared to 74ml/min in donors. Improvement in eGFR was noted as early as 24 hours post-surgery with mean value of 77ml/min at 5 days. At admission, levels of all lab parameters assayed were higher in recipients compared to donors. In donors, levels began to taper toward baseline at 24 hours post-surgery but remained persistently elevated in recipients even at 5 days post-transplant. AT levels were lower in recipients compared to controls.
Conclusions: These results show that patients with underlying chronic kidney disease are pro-thrombotic and remain so early post-transplant even with recovery of renal function.

PB0317 | Reversal of Dabigatran by Idarucizumab in Heart Transplantation Surgery: A Case Report

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Background: Heart Transplantation (HT) is often an emergent cardiac surgery that needs optimal correction of hemostasis.
Many patients proposed to HT are under anticoagulation, mainly because of non-valvular atrial fibrillation (NV-AF). The prompt reversal of anticoagulation gains an extremely important role in the outcome of the surgery itself.
Idarucizumab is a monoclonal antibody fragment that efficiently neutralizes the activity of dabigatran, being approved for emergency surgery/urgent procedures or life-threatening or uncontrolled bleeding.
Aims: Case report
Methods: A 40-year-old male with hypertrophic cardiomyopathy/non-compaction cardiomyopathy with decrease of biventricular systolic function (class IV NYHA) and multiple comorbidities was included in an active list for HT. He was under dabigatran 150mg bid due to NV-AF, among other medications. Dabigatran was chosen due to the availability of idarucizumab in the eminence of HT surgery.
As soon as a compatible organ donor was available, patient was notified. Last dose of dabigatran was taken 4h before hospital admission. Idarucizumab 5g iv was administered before HT. Coagulation tests, including dabigatran concentration assessment, were performed before and after idarucizumab administration.
Results: At admission, activated partial thromboplastin time (aPTT) was 75.1 seconds (s) (N: 24.2-36.4s) and dabigatran concentration was 460 ng/mL. Fifteen minutes after idarucizumab administration, aPTT was 29.5 s and dabigatran concentration was 15 ng/mL..
Orthotopic heart transplantation requiring cardiopulmonary by-pass (CPB) was performed. During surgery, 4 FFP, 5 RBC and 1 pool of platelets were transfused.
The surgery was performed without major incidentes. Patient was discharged 30 days after surgery.
Conclusions: In this case, idarucizumab successfully neutralized dabigatran, enabling HT with CPB, without thrombotic ou bleeding complications.
The availability of a specific reversal agent against a direct oral anticoagulant is a valuable therapeutic weapon in patients needing emergent reversal of anticoagulantion. In our hospital, dabigatran is included in HT protocol when patients need anticoagulation and do not present contraindications for it.

PB0318 | Prevention of Thromboembolism Complications in Preoperative Patients

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Background: Postoperative thromboembolism is one of the main complications after operative interference in surgery and gynecology.
Aims: Aim of our research was prevention of thromboembolic complications in preoperative patients.
Methods: We investigated 50 preoperative patients (age 18-90 years), who had quite different reasons for operation (rhinoplasty, artificial

joint replacement, abdominal hernia, different types of benign or malignant tumor…). Patients with previous episodes of vein thrombosis were excluded from observation. All patients made electro- and echocardiography, common blood analysis, coagulation tests.

Results: Almost in all patients activated partial thromboplastin time (APPT), prothrombin time (PT)/ international normalized ratio (INR) were in the normal range. (APPT - 28-43 sec, INR - 0.96-1.3). Only 2 patients had INR-0.95. Unlike to these data prothrombin-time/ plasma fibrinogen concentration (PFC - 2-4 g/L, normal) level was quite in a wide range in these patients. Certainly, these data depended on the main diseases and comorbidities, but the main determinative was age. All patients under 30 had normal coagulation tests (15 patients). Above 30 years main determinative were main diseases and comorbidities. As far as our aim was evaluation of hypercoagulable states, we divided these patients according to the coagulation test levels. Patients with PFC in the range 3.5-5.5 g/L and INR≤1 (19 patients), received LMW heparin after operation 7-10 days longer, than was determined by the type of operation according to guideline. Patients with PFC >5.5 g/L (16 patients), received LMW heparin before operation for 3-5 days and after operation for 20-30 days according to objective data and normalization of coagulation tests. Neither of our patients had developed thromboembolic complications.

Conclusions: Though we held preliminary observational study, results of our investigation points to significance of coagulation tests values in the estimation of preoperative patients for the prevention of thromboembolic complications and appropriate treatment with LMW heparin after operation.

FVIII/IX

PB0319 | Comparative Pharmacokinetics of Two Recombinant FVIII Concentrates in Chinese Pediatric Patients with Severe Hemophilia A: A Single-Center Study

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Background: Recently, the treatment of patients with severe hemophilia A(SHA) in China has been required to switch from Kogenate FS to Kovaltry. However, no head-to-head comparison of these two recombinant FVIII pharmacokinetics (PK) concentrates were reported on the same pediatric patients.

FIGURE 1 Individual comparison between Kogenate FS and Kovaltry of the (A) trough FVIII level in daily prophy

Aims: To compare the PK profiles of Kogenate FS with Kovaltry on the same pediatric patient with severe hemophilia A.

Methods: This study enrolled 14 Chinese pediatric SHA patients (male, mean age 5.67 years, range 2.83-9.42y; 9 O blood type), who switched from Kogenate FS to Kovaltry for prophylaxis. PK study was performed on these two FVIII products for each patient separately. After a 3-day washout period, each patient received a single infusion of 50IU/Kg. Blood samples were collected at pre-dose, 1h, 9h, 24h and 48h post-infusion for pharmacokinetic assessment. FVIII activity was determined by one-stage assay and the PK parameters were calculated by WinNonlin software. Annual bleeding rate(ABR) and annual joint bleeding rate (AJBR) were collected by patients' daily record.

Results: Mean time between two PK tests were 9.64 months. Compared with Kogenate, Kovaltry had a longer $t_{1/2}$ (13.76 vs. 11.51 hours, P< 0.001), higher AUC (1745 vs. 1609 IU h/dL, P< 0.05)/ AUClast (1658 vs. 1478 IU h/dL,
P< 0.05), longer MRT (18.22 vs. 11.18 hours, P=0.001) and higher Vss (0.53 vs. 0.48 dL/kg, P< 0.01). No statistical difference was observed in CL and IVR. Patients with higher VWF:Ag level/blood type non-O had a longer augment of $t_{1/2}$ after the switch. The median trough FVIII level raised from 1 to 2.25 IU/dL. The ABR and AJBR decreased from 3.89 and 3.58 using Kogenate FS to 2.0 and 0.72 using Kovaltry.

Conclusions: Kovaltry showed better PK profiles and clinical outcomes comparing with Kogenate FS. Patients with higher VWF:Ag level or blood type non-O benefit more from the switch.

TABLE 1 PK parameters of Kogenate FS and Kovaltry

	Kogenate FS	Kovaltry	statistic	P value
T1/2 (h)	11.51±2.93	13.76±3.14	5.993	<0.001
CL (ml/kg/h)	3.79±1.47	3.35±1.12	1.178#	0.260
AUC (IU h/dL)	1609 (1065, 1739)	1745 (1359, 1952)	−2.411*	0.016
AUClast (IU h/dL)	1478 (1046, 1663)	1658 (1299, 1804)	−2.271*	0.023
MRT (h)	11.18 (10.53, 15.77)	18.22 (15.0, 20.22)	−3.296*	0.001
Vss (dL/kg)	0.48 (0.38, 0.53)	0.53 (0.50, 0.59)	−2.691*	0.007
IVR (kg/dL)	2.00 (1.62, 2.28)	1.72 (1.65, 1.89)	−1.695*	0.090

PB0320 | Monitoring and Pharmacodynamic Effect of Eftrenonacog-Alfa (Alprolix®) in Severe Hemophilia B Patients: A Real-Life Study

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Background: Eftrenonacog-alfa is a recombinant factor IX-Fc fusion protein (rFIX-Fc) increasingly prescribed in hemophilia B patients due to its extended half-life. A wide variation in FIX activity measurement has been reported in spiked samples.

Aims: Compare chromogenic (CSA) and one-stage clotting (OSA) assays to product-specific OSA for eftrenonacog-alfa monitoring and assess its pharmacodynamics (PD) in real-life setting.

Methods: Sixty samples from 15 severe hemophilia B treated patients were collected at different time points. FIX activity was measured using CK Prest OSA with a product-specific or a standard calibrator, Biophen FIX and Rox FIX. Thrombin generation assay (TGA) was triggered in 56 samples with 1 pM tissue factor. Five parameters were analyzed: lag time (LT), time to peak (TTP), peak height (PH), endogenous thrombin potential (ETP), and velocity. PD models were built to characterize their relationships with FIX activity level, using mixed effects models.

Results: Standard OSA significantly underestimated FIX (median difference −42% [min −79.5 - max 8.0]) compared to product-specific OSA throughout the measuring range (21.5, [1-86] IU/dL). Biophen FIX and Rox FIX allowed reliable measurements except in samples with FIX < 20 IU/dL (n = 30) in which FIX was underestimated (difference > 30%) in 43 and 37%, respectively. Bias correction using inverse linear regression functions increased the uncertainty of measurement making the corrected values unreliable. Velocity was the most sensitive TGA parameter to FIX level followed by PH, then ETP, TTP and finally LT. Pharmacokinetic models suggested that following the peak of FIX activity obtained after eftrenonacog-alfa injection, TGA velocity decreased first, followed by PH then ETP.

Conclusions: Both CSA and standard OSA failed to accurately measure FIX in severe hemophilia B patients treated with eftrenonacog-alfa throughout the measuring range in comparison to product-specific assay. TGA could be an additional valuable tool to evaluate hemostasis balance in treated patients.

PB0321 | Identification of a Novel "Laminin G" Domain Mutant of Protein S Defective in Binding and Inhibiting FIXa and Unaffected in APC/TFPI Cofactor Activities

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Background: Protein S (PS) is a unique vitamin K-dependent anticoagulant that functions in both hemostasis and inflammation. Several functions have been ascribed to PS, e.g., cofactor for activated protein C (APC) and cofactor for tissue factor pathway inhibitor (TFPI). We identified a novel function of PS in binding and inhibiting FIXa (Reference). We have identified residues in the FIXa heparin binding exosite (HBE) required for binding to PS, and we demonstrated that K126, K132, and R170 in the FIXa HBE form the PS binding pocket. However, no complementary information has been available about the residue(s) in PS responsible for binding to FIXa.

Aims: To identify a PS mutant unable to bind and inhibit FIXa but which retained binding and cofactor functions for TFPI/APC.

Methods: We expressed and purified GST fusions of the PS Laminin G domains, LG1 and LG2, together and separately (the domains do not need post translational modification). Protein purity was assessed by SDS-PAGE. The proteins were also subjected to immunoblotting with PS and GST antibodies to confirm purification. We used molecular docking to map the interface between FIXa and PS and selected 10 candidate amino acids in the LG domains to subject to site-directed mutation (Table 1).

Results: Using multiple in vitro and ex vivo assays (with plasma), we identified a double mutant of PS, E435AE437A, defective in binding and inhibiting FIXa. However, this mutant retained binding to, and cofactor activity for, APC/TFPI.

Conclusions: We determined the amino acid residues in the LG1 domain of PS that comprise the binding pocket for FIXa. Alteration of these residues did not interfere with binding to APC/TFPI.

TABLE 1

LG1 Cleft Interface	LG2 Cleft Interface	LG1 Distal Interface
D345	D496	T372
E435	E641	S394
E437	E653	E402
		D406

PB0322 | Bleeds and Imaging Scores in Relation to Pharmacokinetics of Coagulation Factor VIII in Chinese Pediatric Patients with Severe Hemophilia A

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Background: Relationship between PK parameters and imaging examination scores were not reported before.

Aims: To investigate the relationship between individual PK profiles and bleeds or imaging scores in Chinese boys with severe hemophilia A.
Methods: Thirty-five boys with severe hemophilia A were enrolled in Beijing Children's Hospital in 2018. After a wash-out period of 72 hours and a single FVIII infusion of 50IU/kg, blood samples were collected and tested at five time points for PK assessment. Trough level and time below 1IU/dL weekly were generated by WAPPS-Hemo. X-ray, ultrasound and MRI were used to check the both sides of knees, elbows and ankles in the form of Pettersson, HEAD-US and IPSG MRI scores. Baseline and bleeding details were collected.
Results: Twenty-one boys kept trough FVIII level above 1IU/dL in daily prophylaxis. Compared with those with lower trough level (< 1IU/dL), patients in higher trough FVIII level group (>1IU/dL) had fewer bleedings (ABR:3 vs 5, P< 0.05; AJBR: 1 vs 2, P< 0.05), lower Pettersson score (0 vs 2, P< 0.05), HEAD-US score (1.5 vs. 3, P< 0.05) and HJHS scores (1.5 vs. 4, P< 0.05). Time below 1IU/dL weekly was correlated with ABR (r=0.69, P< 0.01), AJBR (r=0.64, P< 0.01), MRI scores (r=0.54, P< 0.05) US scores (r=0.50, P< 0.05), X-ray scores (r-0.49, P< 0.05) and HJHS scores (r=0.47, P< 0.05). In addition, US score had a good relationship with X-ray scores
(r=0.78, P< 0.001), MRI scores (r=0.92, P< 0.001) and HJHS score (r=0.57, P< 0.001).
Conclusions: Patients with longer time spent below a trough FVIII level of 1IU/dL bleed more and had higher imaging and HJHS scores. The US has good relationship with X-ray, MRI and HJHS scores and has potential to be used in hemophilia patients' daily examinations.

PB0323 | Emicizumab Treatment in Pediatric Patients with Hemophilia A without Inhibitors: A Single-Institution Study

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Background: Emicizumab, a bispecific antibody mimicking activated factor VIII (FVIII), has been proven to be efficacious in a Japanese multicenter, open-label study in pediatric patients with hemophilia A without inhibitors (Hemophilia. 2019; 25 (6): 979-987).
Aims: To determine the efficacy and safety of emicizumab in clinical practice, we report a study using emicizumab at our institution.
Methods: We enrolled 12 pediatric patients with hemophilia A (10 severe and 2 moderate), who had started receiving emicizumab by January 2020. Baseline median age was 0.95 years, ranging from 6 days to 10 years. Before the start of the emicizumab regimen, 3 patients received FVIII prophylaxis, 5 received on-demand treatment, and 4 received no treatment. Emicizumab treatment was started to prevent severe bleeding early in life or switch from prophylaxis to high-risk intravenous injection. The median duration of emicizumab treatment was 31.9 weeks (range: 2.4-121).
Results: The median annualized bleeding rates before and during emicizumab treatment were, respectively, 1.0 and 1.6 for the prophylaxis group
8.6 and 1.1 for the on-demand group, and 0.0 and 0.0 for the untreated group. Except for one case of bleeding from a cervical lymphangioma, all patients had traumatic, mild, subcutaneous bleeding. Injection site reactions were noted in 2 cases. No neutralizing anti-emicizumab antibodies had been developed.
Conclusions: Although the observation period was short, safe and efficacious treatment for pediatric patients with hemophilia A without inhibitors, including 4 previously untreated patients, was observed at our institution. Emicizumab can prevent bleeding early in life, which has been difficult to achieve with FVIII prophylaxis, and may lead to a reduction in severe bleeding, including intracranial bleeding. Laboratory monitoring of the administration of emicizumab and the effect of emicizumab on preventing arthropathy remain to be further studied.

PB0324 | Comparative Analysis of the Coagulometric and Chromogenic Methods for the Determination of Factor VIII Activity in the Plasma of Patients Suspected with Blood Coagulation Disorders

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Background: Presence of lupus anticoagulant (LA) in the plasma sample may interfere in factor VIII (FVIII) level determination.
Aims: The aim was to compare the results of FVIII activity assay obtained by coagulometric and chromogenic methods. Furthermore, an attempt to demonstrate the impact of LA on the methods used was made.
Methods: A retrospective analysis of a group of 31 patients (median age 39; 19-89) with LA confirmed by the methods recommended

TABLE 1 Comparison of mean factor VIII activity level in study and control group using chromogenic and coagulometric assay

	Coagulometric	assay	Chromogenic	assay
	Control group (n=38)	Study group (n=31)	Control group (n=38)	Study group (n=31)
Mean factor VIII activity (%)	136.6	48.3	115.4	128.7
Standard deviation	37.5	9.2	36.5	35.7
P value	0.000001		0.099885	

by SSC/ISTH was performed. The control group consisted of 38 healthy volunteers (median age 39; 25-68). Measurements were conducted according to the manufacturer's instructions.

Results: The mean values of factor VIII activity obtained by I-stage clotting and chromogenic methods between the control and study group were compared. The mean FVIII clotting activity in the study group differed significantly from the control group where it was almost 3-fold higher (48.3±9.2 vs 136.6±37.5; p=0.00001). Results obtained using the chromogenic method did not show a statistically significant difference in the activity of FVIII between the compared groups (p< 0.05).

Conclusions: The FVIII level measured using I-stage clotting assay was decreased due to lupus anticoagulant, while using undiluted plasma of the patients. Serial dilution of the plasma caused a linear increase in the FVIII level. This phenomenon was not observed when using chromogenic assay - FVIII level was not decreased although LA was also present in plasma of the patients.

The more significant dependence between the presence of LA and FVIII activity obtained by the coagulation method than the chromogenic method may indicate that the coagulation method is more susceptible to interference from LA. It seems that the determination of FVIII activity in samples of patients with positive LA by chromogenic assay may help to avoid a false positive result. The chromogenic method may be helpful in deciding on therapeutic recommendations for patients with LA undergoing surgery.

PB0325 | Inhibitor Risks Estimation Associated with F9 Genotype Types in Patients with Severe Haemophilia B: Argentine vs EAHAD Database

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Background: Haemophilia B (HB), an X-linked coagulopathy affecting 1:30,000 males worldwide, is successfully treated by substitution of the deficient FIX. Development of inhibitory antibodies against therapeutic FIX represents a major clinical complication impacting patient's quality of life and the economy of National health systems. FIX-inhibitors typically associate with severe allergic and/or anaphylactic reactions.

Aims: We aimed to estimate the FIX inhibitor-risks associated with *F9*-genotypes in previously untreated patients (PUP) with severe-HB from Argentina (about 1/3 of all HB-cases countrywide) and from the International *F9*-variant database EAHAD (European Association for Haemophilia and Allied Disorders, http://f9-db.eahad.org/).

Methods: HB-causative variants were characterised by application of a cost-effective *F9*-analysis algorithm including 12 PCR-amplifications, primary detecting large-*F9*-deletions in hemizygous probands, small-variant screening by high-resolution CSGE and Sanger sequencing. Variant pathogenicity was evaluated following ACMG criteria (American College of Medical Genetics).

Results: Our Argentine case/control study, inhibitor[+]/inhibitor[-], included separated random samples of 13 cases and 67 controls. To correct the relative enrichment in inhibitor[+] cases (all inhibitor[+] cases with severe-HB were included) -as literature estimates 3-12% of FIX-inhibitor development in severe-HB, *F9*-genotype inhibitor-risks were estimated by ORs (CI 95%)(Fisher exact P-values) that normalise those case/control unbalance. Severe-HB patient series from EAHAD, in which inhibitor status was reported, present a similar enrichment in inhibitor[+] cases.

Argentine series of *F9*-missense variants showed the lowest FIX-inhibitor-risks, equal to EAHAD, while nonsense showed moderately augmented inhibitor-risks, similar to EAHAD. Argentina *vs* EAHAD figures were consistent indicating the highest inhibitor-risks classifying all types of *F9*-deletions together, which is even more important in cases with deletions of the entire-*F9*, and just doubling the inhibitor-risks but non-significantly in partial *F9*-deletions. Table.

Conclusions: Our findings reveal similar estimations of *F9*-genotype associated inhibitor-risks in severe-HB PUPs series from Argentina and EAHAD. Data of *F9*-variant-specific inhibitor-risks may provide haematologists with key evidence for designing therapeutics and follow-up regimes.

TABLE 1 F9-genotype inhibitor risks

F9-genotype	Argentine (n=80) [case/control]	EAHAD (n=210) [case/control]	FIX inhibitor-risks
F9-missense	[0/34] OR=0.036 (0.002-0.63) (P=0.0004***)	[1/85] OR=0.016 (0.002-0.12) (P<0.0001****)	extremely low (highly-significant)
All-F9-deletions	[5/2] OR=7.35 (1.92-28.1) (P=0.005**)	[21/1] OR=28.2 (9.11-87.3) (P<0.0001****)	extremely high (highly-significant)
Entire-F9-deletions	[6/7] OR=46 (7.08-298.8) (P=0.0001***)	[23/4] OR=99 (12.8-759.7) (P<0.0001****)	extremely high (highly-significant)
F9-nonsense	[5/13] OR=2.6 (0.73-9.26) (P=0.15)	[21/35] OR=2.2 (1.13-4.27) (P=0.02*)	high (non-significant)
Partial-F9-deletions	[1/5] OR=2.5 (0.25-24.4) (P=0.403)	[2/3] OR=1.96 (0.32-12.1) (P=0.605)	high (non-significant)

PB0326 | A New Test Set up to Dose Factor VIII Ability to Bind Exogenous VWF

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Background: High Factor VIII (FVIII) content is a major contributing factor to deep-vein thrombosis (DVT). On the other hand, Von Willebrand Factor (VWF), the FVIII carrier protein, is not associated with this condition. In thrombosis patients with high FVIII levels, DVT recurrence rates are higher than in patients without this coagulation abnormality. Although VWF could theoretically bind FVIII, an unbound FVIII plasma fraction remains. In venous thromboembolism, an altered unbound FVIII fraction could be indicative of dysfunctional FVIII regulation. The evaluation of the unbound component could better characterize high plasma FVIII levels and provide insight into the pathophysiology of this thrombophilic state.
Aims: To develop a method to determine the FVIII fraction able to bind VWF.
Methods: Five purified FVIII samples from Kedrion have been used after determination of FVIII:Ag and VWF:Ag levels with Asserachrom®-VIII:Ag and STA®-Liatest®-VWF. These concentrated samples have been diluted at 1:100 in 0.9% saline solution.
Assay optimization was performed using Asserachrom®-VWF:FVIIIB. Calibrations were carried out with lyophilized human plasma containing, after reconstitution, a known quantity of recombinant FVIII provided by Stago.
To dose the complex FVIII:VWF, the samples are immobilized directly in the wells, without dissociation of the complex.
To estimate the unbound FVIII capacity to bind exogenous VWF, the wells are first saturated with exogenous VWF from STA®-Immunodef-VIII. Then, samples are immobilized without dissociation of the complex.
Results: The addition of both assays results allows to dose the part of total FVIII:Ag able to bind VWF.
Conclusions: These new methods give the possibility to dose both the complex of FVIII:VWF and FVIII ability to bind exogenous VWF. This approach could be useful in the understanding of mechanisms associated with FVIII stabilization in DVT, as well as more generally to the blood derivatives industry.

PB0327 | Use of Thromboelastography to Monitor Emicizumab in a Patient with Severe Haemophilia A without Inhibitor

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Background: Emicizumab entered the market for the prophylaxis in patients with haemophilia A (HA) and inhibitor. Global tests, such as thromboelastography (TEG), seem to be more suitable than "classical" clotting ones for the monitoring of emicizumab. Recently, efficacy of emicizumab has been shown in patients without inhibitor. Here, we report TEG and clotting tests assessment in a patient with severe HA without inhibitor.
Aims: To determine the impact of emicizumab on TEG parameters.
Methods: The patient is a 14-years-old boy with a diagnosis of severe HA (factor VIII < 1%) without inhibitor. Annualised bleeding rate (ABR) was 3. When he was 3-years-old, he started factor VIII replacement prophylaxis (octocog alfa 2000 IU 3 times *per* week). At the beginning of December 2019, the patient switched to emicizumab and received a subcutaneously prophylaxis dose of 3 mg/kg body weight of emicizumab *per* week for 4 weeks followed by a current maintenance regimen of 1.5 mg/kg weekly. Factor VIII determinations were performed by means of one-stage clotting and chromogenic (bovine reagent) assays. TEG was performed on a TEG® 5000 Thrombelastograph® Hemostasis Analyzer (Haemonetics).
Results: Main laboratory findings obtained before starting emicizumab are summarised in table 1. The aPTT resulted to be prolonged and TEG assay showed reduction of enzymatic clotting (prolonged "R") and a hypocoagulable state (high "K" and low "angle" and "MA"). As expected, aPTT was shortened 30 min after the

TABLE 1 Summary of Stago assay results with Kedrion samples

(%)	VWF:Ag	FVIII:Ag	FVIII:VWF complex	Unbound FVIII capacity to bind exogenous VWF	Total FVIII capacity to bind VWF
Sample n°1	5750	7541	1891	901	37.0%
Sample n°2	6650	9051	2861	1511	48.3%
Sample n°3	4050	10077	1656	1727	33.6%
Sample n°4	7550	12606	5384	1376	53.6%
Sample n°5	6150	15375	4935	1490	41.8%

TABLE 1 Laboratory Findings

	aPTT ratio (0.8-1.2)	R (min) (2-8)	K (min) (1-3)	angle (degrees) (55-78)	MA (mm) (51-69)
1st infusion_Pre	2.50	16.40	5.50	34.20	49.00
1st infusion_Post (30 min)	1.70	13.20	4.20	44.10	53.80
2-months of prophylaxis	0.78	12.70	2.50	57.50	63.10

first administration of emicizumab. At the same time we observed changes in TEG parameters towards a normal profile. All tests confirmed this trend at 2-months of treatment (table 1).

Conclusions: TEG reflects trends toward normalization of haemostasis induced by emicizumab in a patient with HA without inhibitor. Present data could be helpful to measure globally the haemostatic effects during treatment with emicizumab. However, further data are warranted.

PB0328 | FVIII Gene Mutation Analysis and Evaluation of Clinical Data: One Center Result

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Background: Hemophilia A is a X-linked hereditary bleeding disorder. According to mutation, decrease in FVIII level cause severe to mild phenotype. Development of FVIII inhibitor is the most feared complication of treatment.

Aims: We evaluated our Hemophilia-A patients' mutations, factor levels and inhibitor status.

Methods: The data of 48 boys with Hemophilia A treated at Uludag University Pediatric Hematology Department were retrospectively screened. Patients' factor levels and inhibitor status were recorded and compared with FVIII mutation type.

Results: Thirty-nine out of 48 patients were severe Hemophilia A; 4 moderate and 5 mild Hemophilia A. In severe Hemophilia A group; intron 22 inversion (n: 24), inversion 1 (n:1), missense (n:3), nonsense (n:3), frame shift (n:3), deletion (n:5) mutations were detected. In moderate Hemophilia A group; 2 missense mutation were detected. In the rest (n:2) mutation analayses still continuing and MLPA test was planned. All of the mild Hemophilia A patients (n:5) had missense mutation. We found 3 novel missense mutations among our cases.

Of 48 patients, 8 (16.6%) had FVIII inhibitor. One of them had mild hemophilia A, inhibitor level was low responsive and became negative within one year. The remaining 7 patients had severe Hemophilia A (4 intron 22, 3 large deletion). Of the four cases with intron 22, two were siblings. In the other, there was no additional risk factor except intron 22. The remaining 3 patients with inhibitor had severe hemophilia with large deletion and they were close relatives.

Conclusions: In our study, the most common mutation was intron 22 inversion as noted in the literature. Only 4 (16.6%) of these cases had inhibitor. Three other patients with severe hemophilia A with inhibitor had large deletion. Three novel missense mutations found.

PB0329 | Identification of Zebrafish Orthologue to Human Factor IX Gene and its Age Dependent Regulation

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Background: Human coagulation Factor IX (FIX) plays a central role in coagulation. Deficiency of FIX causes hemophilia B. Modeling hemophilia B in zebrafish was not possible because zebrafish genome has three factor IX genes, *fixa, fixb, and fixl* and the orthologue to *FIX* is unknown.

Aims: To identify zebrafish orthologue to *FIX* and study its regulation.

Methods: kPT, kPTT, kRVVT kinetic assays, qRT-PCRs, laser-induced thrombosis, and piggyback knockdowns were performed according to published methods. One-way ANOVA was used for statistical analysis.

Results: To identify the zebrafish orthologue to *FIX*, we analyzed the Ensembl database and found *fixa, fixb, and fixl* genes. Synteny suggested that *fixa* is closer to *FIX* than *fixb*; *fixl* is not syntenic to *FIX*. A comparison of protein sequences suggested that zebrafish fixl is closer to fx than fix. We then checked for their expression using adult zebrafish RNA from brown-mass by RT-PCR and gel electrophoresis and found all the three genes were expressed. We then tested for their expression at various ages by qRT-PCR data, which showed that *fixa* and *fixl* expression levels in the zebrafish larvae are low; however, *fixb* is expressed abundantly. Similarly, we noted the expression of all genes increased with age. The knockdown of each of these genes in adult fishes resulted in a more extended kPTT lag period for *fixa* and *fixl* but not for *fixb*. *fixa* and *fixb* knockdowns did not affect kPT. *fixl* knockdown showed longer kRVVT and kPT lag period. Knockdown efficiency was confirmed by qRT-PCR. Knockdown of these genes in larvae showed longer venous thrombosis TTO for *fixa* and *fixl* but not for *fixb*.

Conclusions: Our data revealed *fixa* is orthologue to *FIX*. This finding will be useful in modeling hemophilia B and *fIX* gene regulation during aging. Because of this additional *fx*, it explains the *fX* knockouts are not embryonically lethal.

PB0330 | Difference Molecular Profiles of Type 1 and 2 Inhibitor Developed in Mild/Moderate Hemophilia A

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Background: Some patients with mild/moderate hemophilia A (MHA) develop anti-FVIII inhibitors in spite of the presence innate self FVIII. When inhibitor is developed in MHA, bleeding phenotype becomes severe and hemostatic treatment would be challenging. Genotypic risk of inhibitor development is revealed by INSIGHT study about MHA with inhibitor (MHA-inh), inhibitory mechanism on developed inhibitor was not fully understood, however.
Aims: To clarify the inhibitory mechanism on inhibitor developed in MHA.
Methods: We assessed hemostatic and immunologic properties of inhibitors in 10 MHA-inh. Peak inhibitor titers were 1.0-1,656 BU/mL. In all of them, inhibitor was developed following infusion of large amount of FVIII concentrates for hemostatic treatment or perioperative management, and severe bleeding occurred after inhibitor development.
Results: Inhibitors of 8 out of 10 patients (A group) inhibited both of FVIII from concentrates (non-self FVIII) and mutated self FVIII which existed in patient's plasma prior to inhibitor development or which was expressed by BHK cells. However, inhibitors of other 2 patients (B group) inhibited non-self FVIII only. Inhibitors of A group were type 1 antibodies and those of B group were type 2 antibodies. Inhibitors of A group inhibited interaction between FVIII and phospholipids (PL), and this mechanism seemed to cause the inhibition of FVIII activity. By contrast, inhibitors of B group didn't inhibit FVIII-PL interaction and one of them inhibited to FVIII cleavage by thrombin and FXa. Most mutations on F8 gene of the A group were identical or close site to reported in INSIGHT study, whilst those of B group weren't reported in that study (P1809L, E272K). In all cases, IgG subtypes of inhibitors were observed in IgG_1 and/or IgG_4 and inhibitor epitopes didn't show any tendency.
Conclusions: Inhibitors developed in MHA-inh had same immunereactivity depending on types of antibody, and all of type 1 inhibitors showed PL-dependent inhibitory mechanism.

PB0331 | Treatment and Joint Health in Nordic Moderate Haemophilia Patients - the Mohem Study

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Background: The prevalence of arthropathy in patients with moderate haemophilia is not well known.
Aims: We evaluated treatment and joint health in Nordic moderate haemophilia patients to explore if they receive optimal care.
Methods: The MoHem study is a cross-sectional, multicentre study covering patients with moderate haemophilia A (HA) and B (HB) in all ages from Sweden, Finland and Norway. Arthropathy was evaluated by ultrasound (HEAD-US) and Haemophilia Joint Health Score (HJHS). Data on treatment and medical history were self-reported supported by clinical records.
Results: We enrolled 145 patients with moderate haemophilia from Oslo, Stockholm, Malmö, Gothenburg and Helsinki. Median age was 28 years (IQR 13-52) and 89 (61%) had HA. Median baseline factor activity (lowest historical value) was 2.0 IU/dL (IQR 1.7-3.5): significantly lower for HB (2.0 IU/dL, IQR 1.4-2.0) than HA (2.8 IU/dL, IQR 2.0-4.0), $p< 0.01$. Fifty-five patients (38%) were receiving prophylactic treatment since median 10 years of age (IQR 4-24). 117 patients (81%) had a history of haemarthrosis: 76 (85%) among HA and 41 (73%) among HB, $p=0.07$. Median age at first joint bleed was 5 years (IQR 3-8) for the whole group: significantly lower for HA (5 years, IQR 3-7) than HB (7 years, IQR 5-12), $p=0.01$. Bleeding frequency was low: median number of joint bleeds and serious other bleeds during the last 12 months were both zero (IQR 0-1). Median total HEAD-US score for elbows, knees and ankles (n=120) was 0 points (IQR 0-2), while median total HJHS score (n=135) was 4 points (IQR 1-10), gait score excluded. Twenty-six patients (18%) had undergone joint surgery due to arthropathy. Correlation between HJHS and HEAD-US score was strong ($r=0.74$, $p< 0.001$).
Conclusions: Overall, the current joint health for Nordic moderate haemophilia patients was good. A subgroup of the patients had severe arthropathy. HEAD-US was strongly correlated with HJHS.

PB0334 | Screening of Hematuria in Children with Hemophilia: A Preliminary Report

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Background: Hemophilia is a rare bleeding disorder that the most common clinical manifestations are joint and soft tissue bleedings. However, adults with hemophilia have a higher prevalence of renal

disease and hypertension than the general population. There is limited data about prevalence of hematuria in children or its long-term impact on the renal system.

Aims: We retrospectively reviewed the data to identify the frequency of hematuria and evaluated associated risk factors in children with hemophilia A and B.

Methods: We reviewed retrospective records including age, type and severity of hemophilia, urinalysis, renal functions, and urinary imaging of all male patients with hemophilia. Haematuria was defined as ≥3 red blood cells (RBCs) per high power field.

Results: A total of 42 patients and 91 urinalysis were evaluated. Thirty-eight with Hemophilia A (3 mild, 10 moderate, 25 severe) and 3 with Hemophilia B (all of them moderate). Twelve (1 with hemophilia B) of the patients (28%) were identified as having hematuria (median RBCs 3, mean RBCs 72 per high power field). Except four patients, hematuria was identified on routine clinical screening. One of the patients with hematuria had urinary infection. One of the two patients with recurrent hematuria was diagnosed as Nutcracker Syndrome. Renal function tests and urinary ultrasonography were normal in other patients with hematuria. Age or severity of hemophilia were not associated with hematuria.

Conclusions: Our study showed that the prevalence of hematuria is not rare in children with hemophilia. Further prospective, multicenter studies are need to determine the prevalence of hematuria and its impact on renal function in children with hemophilia.

PB0335 | Evaluation of Structurally Altered Protein in Factor VIII Therapeutic Products Using Chromatography on Immobilized von Willebrand Factor

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Background: Therapeutic products with coagulation factor VIII (FVIII) have wide ranges of specific activity (IU/mg), indicating the presence of significant amounts of protein with altered structure and properties. In particular, the products with recombinant FVIII (rFVIII) contain a protein fraction (FVIII*) with reduced activity and inability to bind von Willebrand factor (vWF) (*Lin et al, 2004; Ofosu et al 2012*). As the ability to bind vWF is important for reduction of FVIII immunogenicity *(Hartholt et al, 2017)*, FVIII* may contribute to the development of FVIII inhibitors. Thus, it represents a product-related impurity and risk factor for product efficacy.

Aims: To develop a method to separate the FVIII* fraction from FVIII preparations and characterize this protein in various rFVIII products.

Methods: Chromatography of FVIII samples on immobilized vWF and testing the fractions by PAGE with Coomassie/silver-staining/Western blotting and chromogenic FVIII activity assay.

Results: The conditions of chromatography assay were elaborated and proven to be robust for 55 column cycles. Ten rFVIII products

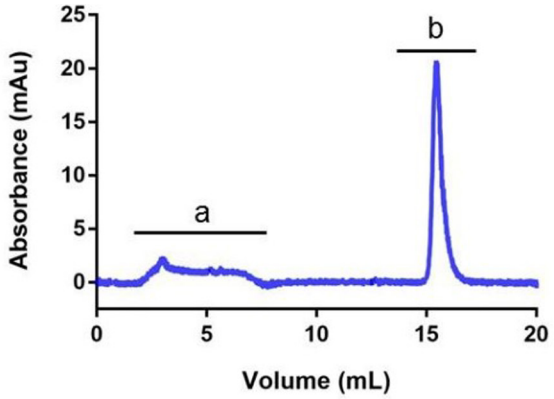

Figure 1. Chromatography of a FVIII product on a vWF-immobilized resin. The lyophilized product was reconstituted in provided diluent, diluted with buffer I (20 mM Tris-HCl, 150 mM NaCl, 5 mM $CaCl_2$, 0.02% Tween 80, pH 7.4), loaded on the column and the column was washed with the same buffer. Unbound protein (**a**) was collected in the flow-through, and bound protein (**b**) was eluted with buffer II (20 mM Tris-HCl, 600 mM NaCl, 350 mM $CaCl_2$, 0.02% Tween 80, pH 7.4).

FIGURE 1

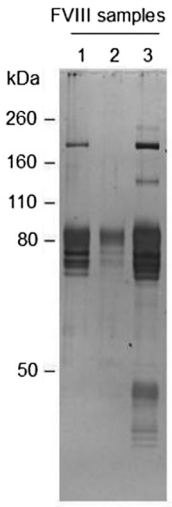

Figure 2. Characterization of a FVIII product fractions by PAGE/silver-staining. Lane 1, original product (a B-domain deleted FVIII); lane 2, protein in column flow-through (FVIII*); lane 3, protein eluted from column.

FIGURE 2

available in the U.S. including those with extended half-life were tested. The protein unable to bind vWF (FVIII*) was found in all products at level of 0.5-23% (Figure 1) and activity 0.4-17% compared to starting samples. In some products, the band pattern of FVIII* by PAGE differed from that of protein bound to the column (Figure 2).

Conclusions: We developed a robust method to separate the FVIII* fraction from FVIII samples. The applicability of the method was demonstrated by characterization of ten rFVIII products for the content and molecular weight distribution of FVIII* provided better knowledge of these products. The method can be useful to achieve higher purity and efficacy of FVIII products for the care of Hemophilia A.

The study was funded by FDA. This is an informal communication and it represents the authors' own best judgment. These comments do not bind or obligate FDA.

PB0336 | New Hyperactive Factor IX Variants Identified with a Novel Computational Analysis

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Background: The high-specific-activity factor IX (FIX) variant, FIX-Padua, is currently being used in all gene therapy trials for hemophilia B (HB). The increased potency of the vector afforded by the hyperactivity of FIX-Padua improves the safety and efficacy of the gene therapy product. Further enhancements in the vector potency may lead to additional clinically relevant improvements. Our previous studies of FIX-Padua indicate that the specific-activity of wild-type (WT) FIX is not optimized and therefore is amenable to bioengineering enhancements. We hypothesized that amino acid substitutions at positions that were 1) evolutionary conserved and 2) spared from HB missense mutations may increase the specific-activity of FIX. We refer to this computational approach as contraphenotypic analysis.
Aims: To develop a strategy to identify positions that can be substituted to enhance specific-activity.
Methods: We determined the specific-activity of 130 FIX variants with amino acids substituted at 20 positions identified by contraphenotypic analysis after transient transfection into mammalian cells (Figure 1).
Results: We observe that substitutions at 55% of these positions yielded a hyperactive variant with at least twofold increase specific-activity and 30% yielded a variant with at least threefold increase specific-activity relative to FIX-WT. However, at each position, different amino acid substitutions typically resulted in variants with a range of specific-activities. At 8 positions, the highest specific-activity variants was comparable to FIX-WT while at 1 position the highest specific-activity variant was lower than FIX-WT.
Conclusions: Our contraphenotypic approach efficiently identified positions in FIX where amino acid substitutions resulted in new hyperactive variants the majority of the time. Contraphenotypic analysis may be useful in bioengineering other therapeutic proteins beyond FIX.

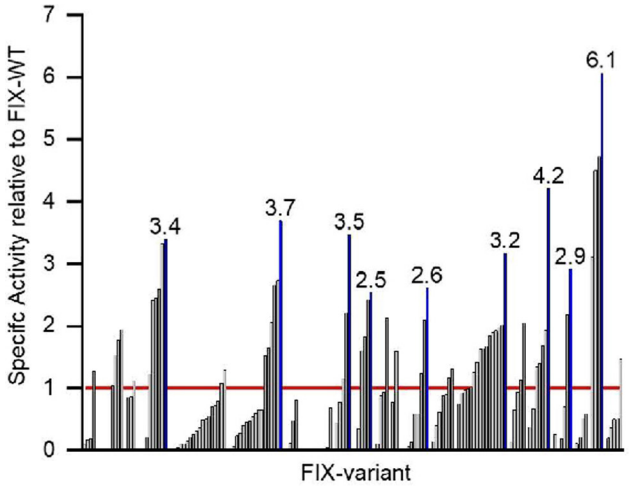

FIGURE 1 Specific activity of new factor IX (FIX) variants relative to wild-type FIX (WT)

PB0337 | Potential Role for Regulatory B-cells in the Control of Immune Tolerance to Factor VIII Treatment in Haemophilia A: *Evaluation of Involvement of Regulatory Cell Types and Markers in Immune Tolerance Induction (ITI) in Haemophilia A*

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Background: One of the most challenging complications in the treatment of haemophilia A is the development of neutralizing antibodies against factor VIII (FVIII). Treatment of these 'inhibitors' by Immune Tolerance Induction (ITI) is demanding and the working mechanism of ITI is still unresolved.
Aims: Improve the knowledge of the working mechanism of ITI, focusing on regulatory immune cells and markers.
Methods: Cross-sectional and longitudinal evaluation of the frequency of immuno-regulatory cells and markers in haemophilia A patients. Peripheral Blood Mononuclear Cells (PBMCs) were analyzed by flow cytometry using the following parameters: regulatory B-cells (Bregs; $CD19^{+}CD24^{high}CD38^{high}$), regulatory T-cells (Tregs; $CD4^{+}CD25^{+}$Fox-$P3^{+}$), myeloid derived suppressor cells (MDSCs; $Lineage(Lin)^{-}HLA\text{-}DR^{low}CD11^{+}CD33^{+}$)) and expression of regulatory markers on T-cells (PD1, ICOS, CTLA4) and monocytes (PD-L1). In the cross-sectional cohort, data were compared according to inhibitor status (current inhibitor (N=20), ex-inhibitor (N=17) and inhibitor-negative (N=28)).
For the longitudinal analysis the relative change in immunoregulatory response (expressed as ratio) was assessed in inhibitor patients during ITI (N=13) and compared with a inhibitor-negative haemophilia A cohort (N=36).
Results: In the cross-sectional cohort the frequency of Bregs was significantly lower in inhibitor patients (3.2%) compared to inhibitor-negative (5.9%) and ex-inhibitor patients (8.9%), $p < 0.01$, table 1. CTLA4 expression on T-cells was also significantly lower expressed

TABLE 1 Comparison of regulatory immune profile in patients with haemophilia A

		No inhibitor patients (N =28)	Inhibitor patients (N = 20)	Ex-inhibitor patients (N = 17)	P value (Kruskal-Wallis test)
Age (years)	median (interquartile range; IQR)	30.9 (11.8-52.8)	43.3 (1.6-62.8)	23.2 (9.0-47.8)	0.95
Inhibitor titer (BU)	median (IQR)	0.0 (0.0-0.0)	7.2 (3.3-18.4)	0.0 (0.0-0.0)	< 0.001
Regulatory B-cells (% of CD19+ B-cells)	median (IQR)	5.9 (4.12-10.40)	3.2 (1.6-6.6)	8.9 (4.6-15.6)	< 0.01
Regulatory T-cells (% of CD4+ T-cells)	median (IQR)	5.2 (4.1-6.2)	5.2 (3.8-7.4)	4.4 (4.0-6.1)	0.92
MDSCs (% of PBMCs)	median (IQR)	0.54 (0.41-0.91)	0.60 (0.15-1.30)	0.63 (0.4-1.1)	0.76
PD-L1 on monocytes (% of monocytes)	median (IQR)	78.4 (68.9-82.7)	71.8 (46.6-79.6)	80.2 (70.7-84.1)	0.18
PD1 (% on CD4+ T-cells)	median (IQR)	2.9 (2.0-4.1)	2.6 (1.7-3.9)	3.0 (1.5-4.5)	0.65
ICOS (% on CD4+ T-cells)	median (IQR)	1.5 (1.0-1.9)	1.0 (0.5-3.4)	1.8 (0.8-2.5)	0.30
CTLA4 (% on CD4+ T-cells)	median (IQR)	3.3 (2.0-6.3)	0.5 (0.3-2.6)	2.4 (0.4-3.8)	< 0.01

in current inhibitor patients compared to inhibitor-negative patients (0.5% versus 3.3%, p< 0.01).

In ITI patients the same parameters, Bregs and CTLA4, as well as PD1 and ICOS, significantly increased during inhibitor eradication when comparing to the control cohort, figure 1.

No significant differences were found in other regulatory immune cells.

Conclusions: Bregs and regulatory markers on T-cells were significantly reduced in inhibitor patients and increased during ITI, supporting that these parameters contribute to the loss and recovery of tolerance to FVIII.

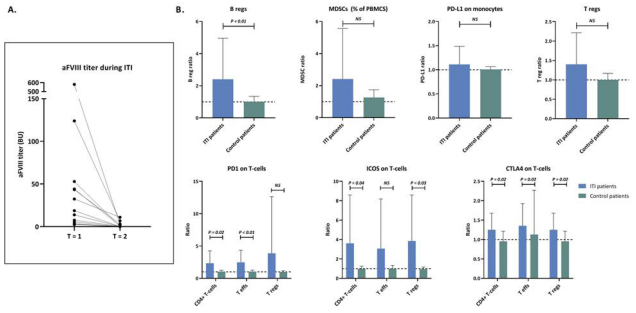

Figure 1: Change in immuno-regulatory cells and markers during ITI.
The relative change in frequency of immuno-regulatory cell types and markers was evaluated by flow cytometry during inhibitor eradication in 13 ITI patients and compared with 36 control patients (haemophilia A patients with no inhibitor).
A. Decrease in aFVIII titer during inhibitor eradication in the ITI patients.
B. Mean (+ SD) ratios of outcome parameters, expressing the relative change in frequency of regulatory immune cells or markers during ITI or during a 6-month interval without any relevant clinical changes for the control cohort. B regs and PD1, ICOS and CTLA4 on Tregs showed a significant increase during ITI compared to control patients. Analysis with Mann-Whitney U test. aFVIII: anti-FVIII; BU:Bethesda Units; ITI:Immune Tolerance Induction; B regs:regulatory B-cells; MDSCs:myeloid derived suppressor cells; PBMCs:T effs:Effector T-cells; T regs: regulatory T-cells.

FIGURE 1 Change in immuno-regulatory cells and markers during

PB0338 | Protease Domain Determinants of the Distribution, Clearance and in vivo Activity of Human Factor IX(a) in Hemophilic Mice

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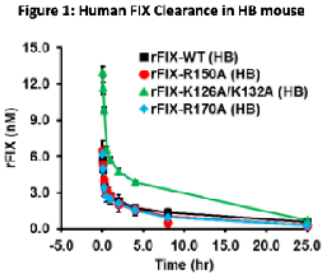
Background: Unique characteristics of factor IX (FIX) include substantial extravascular distribution and a poorly reactive protease with prolonged plasma half-life.

Aims: Identify protease domain determinants of distribution, clearance and in vivo activity of FIX and FIXa.

Methods: Recombinant human FIX wild type (rFIX WT) or variants with substitutions in the Padua residue (R170A), antithrombin- (R150A) and heparin-binding (K126A/K132A) exosites (chymotrypsinogen #) were purified from HEK293 cells. rFIX was activated with human FXIa. Following tail vein injection of 20 U/kg rFIX or equivalent FIXa, pharmacokinetics were determined for FIX in hemophilia B (HB) mice over 25 hr and FIXa in hemophilia A (HA) mice over 3 hr using a human FIX(a) specific ELISA. Data were fit to a 2-compartment model. Plasma FIXa activity was detected in the enhanced thrombin generation assay (eTGA).

Results: rFIX WT, R170A and R150A demonstrated similar recovery (~21-24%) and clearance in HB mice, while FIX K126A/K132A showed remarkably enhanced (2.2-fold) recovery (Fig 1). FIXa WT clearance demonstrated ~50% residual protease antigen at 25-30 min post-injection. Similar to zymogen, FIXa WT demonstrated ~23% recovery at 5 min



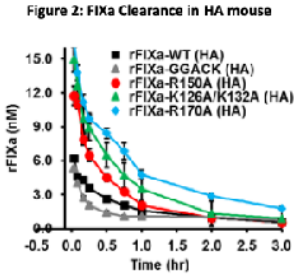


FIGURE 1 & 2

in HA mice and clearance suggested a 2-compartment model (Fig 2). GGACK-FIXa demonstrated similar recovery to FIXa WT, while FIXa R170A (2.7-fold), K126A/K132A (2.4-fold), and R150A (1.9-fold) demonstrated markedly enhanced recoveries (Fig 2). Residual protease activity was detected for FIXa R170A in the eTGA at 5 min and 2 hr post-injection.

Conclusions: Consistent with treatment of hemophilia B, rFIX WT recovery was poor in HB mice. Similar recovery and 2-phase elimination of rFIXa in HA mice suggested substantial protease extravascular binding. The heparin-binding exosite contributed to extravascular distribution of both FIX and FIXa, while the R170A residue and antithrombin-binding exosite contributed exclusively to FIXa extravascular distribution. GGACK inhibition does not affect FIXa recovery, but significantly modified both clearance phases, suggesting a protease conformational effect.

PB0339 | aPTT Reaction Curves Provide Important Additional Information in Hemophilia Patients

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Background: With modern coagulation analyzers, single activated partial thromboplastin time (aPTT) result is not just a number but a collection of photo-optical data in the form of a reaction curve.

Aims: To investigate if aPTT reaction curve provides important additional information and whether it allows to predict the degree of FVIII/FIX deficiency from reaction curve in hemophilia patients.

Methods: The study examined plasma samples from 25 healthy, 89 hemophilia A and 17 hemophilia B subjects. aPTT (Actin FS, Atellica COAG360, Siemens, Germany) was determined with two evaluation modes: fixed absorbance (onset of coagulation, FA) and drifting baseline (DB) proposed by manufacturer. Moreover, aPTT at 50% coagulation (PI) was manually determined. We calculated DELTA-1 (aPTT-DB minus aPTT-FA) and DELTA-2 (aPTT-PI minus aPTT-DB). FVIII and FIX activities were measured by one-stage clotting assay and inhibitor using Nijmegen-Bethesda assay. Hemophilia A patients were further divided into HA1 (FVIII< 1IU/dL, N=20), HA2 (FVIII=1-5IU/dL, N=22) and HA3 (FVIII>5-40IU/dL, N=47) groups, hemophilia B into HB1 (FIX=< 1IU/dL, N=8) and HB2 (FIX≥1UI/dL, N=9) groups, and all hemophilia patients into 2 groups according to the presence (I2, N=29) or absence (I1, N=77) of an inhibitor.

Results: Results of DELTA-1 and DELTA-2 in all studied groups are presented in Table 1, whereas results of correlation between DELTA-1/DELTA-2 and FVIII/FIX activities are presented in Table 2. According to Receiver Operating Characteristic (ROC) analysis DELTA-1 >1.30 s allowed distinguishing between healthy and hemophilia subjects (sensitivity:100%, specificity:100%) and >3.64 s between patients with and without inhibitors (sensitivity:79.3%, specificity:53.2%). DELTA-2 values >5.90 s and >13.70 s allowed distinguishing between healthy and hemophilia subjects (sensitivity:93.4%, specificity:100%) and between patients with and without inhibitors (sensitivity:75.9%, specificity:74.0%).

Conclusions: aPTT reaction curve provides important additional information in hemophilia patients. Parameters DELTA-1 and DELTA-2 can be reliable used to distinguish healthy from hemophilia subjects, as well as hemophilia patients with and without inhibitors.

TABLE 1 Results of DELTA-1 and DELTA-2 in all studied groups (medians with ranges)

			HA (N=89)			HB (N=17)		All hemophilia patients (N=106)	
Parameter	Healthy subjects (N=25)	All hemophilia patients (N=106)	HA1 (N=20)	HA2 (N=22)	HA3 (N=47)	HB1 (N=8)	HB2 (N=9)	I1 (N=77)	I2 (N=29)
DELTA-1 (s)	0.9 (0.3-1.3)	4.0 (1.7-10.6)	6.1 (4.4-10.6)	4.7 (1.8-10.1)	2.7 (1.7-6.8)	6.1 (3.4-7.3)	2.8 (1.9-5.1)	3.5 (1.7-10.6)	5.0 (1.8-8.3)
P	<0.001*		<0.001**			0.004*		0.023*	
DELTA-2 (s)	4.4 (3.7-5.9)	10.4 (3.9-47.7)	41.2 (22.0-45.9)	18.7 (8.0-45.4)	8.4 (4.7-36.3)	45.2 (7.0-47.7)	2.8 (1.9-5.1)	9.2 (3.9-45.9)	27.9 (6.7-47.7)
P	<0.001*		<0.001**			0.003*		<0.001*	

*Mann-Whitney test; **Kruskal-Wallis test

TABLE 2 Correlation of parameters DELTA-1/DELTA-2 with FVIII/FIX activities in all studied groups

PARAMETERS	HEMOPHILIA A (N=89)				HEMOPHILIA B (N=17)		
	HA group	HA1 group	HA2 group	HA3 group	HB group	HB1 group	HB2 group
	FVIII:C 6.0* (0.9-39.0) IU/dL	FVIII:C <1.0 IU/dL	FVIII:C 3.0* (1.0-5.0) IU/dL	FVIII:C 13.0* (6.0-39.0) IU/dL	FIX:C 1.0* (0.9-23.0) IU/dL	FIX:C <1.0 IU/dL	FIX:C 8.0* (1.0-23.0) IU/dL
DELTA-1 (s)	r=0.815 P<0.001	not applicable	r=0.667 P=0.768	r=0.655 P<0.001	r=0.836 P<0.001	not applicable	r=0.882 P=0.002
DELTA-2 (s)	r=0.849 P<0.001	not applicable	r=0.520 P=0.013	r=0.587 P<0.001	r=0.845 P<0.001	not applicable	r=0.840 P=0.005

* Median with range;r- Spearman's Rank coefficient.

PB0340 | Real-world Analysis of Hemophilia Patients in China: A Single Center's Experience

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Background: The management of hemophilia is critical to minimize risk of disability and reduce burden on China's health care system, and key initiatives have been taken in China to raise disease awareness and enhance treatment availability.

Aims: The present study based on a single center in Tianjin, China was conducted to understand the devolvement of real-world hemophilia care over the past 15 years.

Methods: This study retrospectively analyzed the key clinical characteristics, diagnosis, treatment, and medical expenditures of 428 patients with hemophilia A (HA) or hemophilia B (HB) in the Institute of Hematology & Blood Diseases Hospital (IHBDH) in Tianjin, China.

Results: Between January 2004 and December 2018, 357 patients with HA and 71 patients with HB seek care in the IHBDH. Over the years, the average gap between the diagnosis and the first bleeding decreased significantly from 13.3±5.1 years before 2004 to 0.4±0.4 year in 2014-2018 ($p< 0.05$). Among children and adults receiving prophylaxis treatment, the total factor consumption per year increased from the period between 2004-2008 to the period between 2009-2013 (168.8 vs 389.2 IU/kg in children, 120.7 vs 316.2 IU/kg in adults, respectively, both $p< 0.001$) and 2014-2018 (168.8 vs 1328.0 IU/kg in children, 120.7 vs 878.8 IU/kg in adults, respectively, both $p< 0.001$). Similar trends of total factor consumption were observed in patients receiving on-demand treatment (Figure 1). Number of patients receiving inhibitor tests regularly increased from 2004 (1.9%, 2/105) to 2018 (21.5%, 59/275), while rates of positive inhibitor remained unchanged. The seroprevalence of hepatitis C virus (HCV) was 33.8% across the years, while the incidence rates of HCV among patients with hemophilia dropped significantly (7.3% in 2008 to 0.4% in 2018). Meanwhile, the total annual medical insurance expenditure for hemophilia had increased steadily in the past 10 years (Figure 2).

Conclusions:

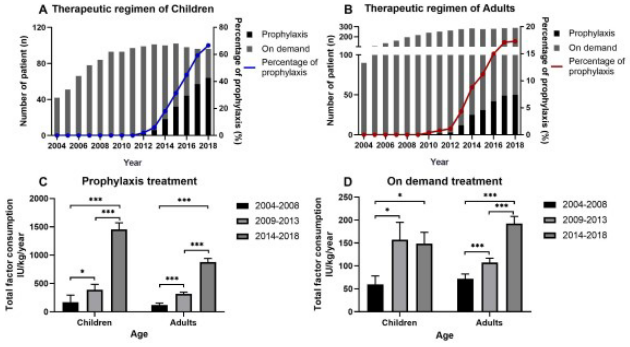

FIGURE 1 Change of treatment. A Percentage of prophylaxis in children. B Percentage of prophylaxis in adults. C Total dose of factor consumption of pa

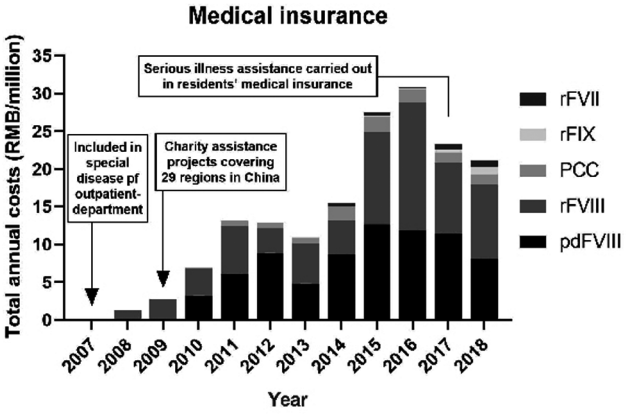

FIGURE 2 Changes of medical insurance costs for hemophilia in past 10 years. The costs were increasing every year

PB0341 | Evaluation of Coagulation FIX Binding to Collagen IV and the Impact on Thrombin Generation

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Background: Haemophilia B is a blood clotting disorder caused by deficiency in coagulation Factor IX (FIX). Patients are treated by infusion of FIX concentrates based on the plasma concentration, however, significant evidence points towards an extravascular compartment of FIX. FIX binds reversibly to collagen IV in the basal lamina of blood vessels and may be the site of an extravascular store.
Aims: To evaluate the impact of the collagen-FIX interaction on thrombin generation.
Methods: Flat-bottomed plates were coated with collagen IV overnight at 4°C, prior to application of recombinant FIX (BeneFIX; Pfizer) for 1 h at 37°C. Wells were washed with PBS in between steps to remove any unbound collagen or FIX. Thrombin generation (TG) was performed on platelet-poor plasma (PPP) or FIX deficient plasma (FIX-DP) and measured using the Calibrated Automated Thrombogram (CAT). In some cases, 2 x 10^8plt/mL platelets were incorporated. FIX was measured using a commercial chromogenic assay.
Results: FIX-DP ± platelets did not generate thrombin on uncoated or collagen coated surfaces. However, on surfaces with the FIX-collagen complex present, FIX-DP + platelets generated a peak thrombin concentration comparable to the healthy control; 67.07 ± 7.0 *vs.* 101.4 ± 28.2 nM, respectively. The lag time was significantly delayed in FIX-DP (41.3 ± 2.9 min) compared to PPP (25.3 ± 2.3 min, $p < 0.05$). FIX binding to collagen was confirmed by chromogenic assay and FIX activity was found to be 34% of normal circulating levels, approximately 1.7 μg/ml.
Conclusions: Our data suggests the FIX-collagen interaction is required for normal TG to occur. Collagen IV binding of FIX may provide a long-lasting extravascular reservoir of FIX at a haemostatic and functional location, such as the joints. Similar results were obtained in preliminary experiments with Factor VIII, suggesting the intrinsic tenase may be able to assemble on a collagen surface.

PB0342 | Evaluation of the Effect Emicizumab Neutralizing Antibodies on aPTT Clotting-based Tests Results in Patients Treated with Emicizumab

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Background: Emicizumab, a humanized bispecific antibody for haemophilia A (HA) treatment, is well known to affect the activated partial thromboplastin time (aPTT) making not reliable all the aPTT-based tests (one-stage assay and Bethesda assay for measuring FVIII inhibitor titre). The use of chromogenic assay with bovine regents (insensitive to Emicizumab) or preventive neutralization of the drug are mandatory in order to avoid misinterpretation of the results.
Aims: To evaluate the effect of Emicizumab Neutralizing Antibodies (anti-Emi) by comparing the results obtained with aPTT-based and chromogenic assays on samples from HA patients treated with Emicizumab.
Methods: Twenty-five plasma samples of HA patients without FVIII inhibitor were analysed for aPTT and FVIII:C activity; FVIII inhibitor titre was measured using Nijmegen-Bethesda assay on 10 samples of HA patients. FVIII:C activity and inhibitor titre were measured both with the one-stage (Actin FS - Siemens, Germany) and the chromogenic assay (with bovine regents, Siemens). Emicizumab concentration was measured by using a modified one-stage assay calibrated with a specific Calibrator (r2 Diagnostics Inc., US). All the tests were performed on Sysmex CS-2400 (Sysmex, Kobe, Japan) before and after the addition (1:20) of a mixture of two anti-idiotype monoclonal antibodies neutralising Emicizumab (Chugai Pharmceutical, Japan).
Results: Emicizumab concentration measured after the neutralization was < 5 μg/mL in all the samples.
The table reports the results [mean (range)] obtained on the 25 plasma samples without FVIII inhibitor
Inhibitor titre was < 0.5 UB in almost all the 10 samples when measured with the one-stage before Emicizumab neutralization. A good correlation (r=0.996) was found between inhibitor titre

TABLE 1 Results of patients without FVIII inhibitor

	Before neutralization	After neutralization
Emicizumb (μg/mL)	61.9 (36.4-99.2)	0.3 (0.3-1.1)
aPTT (sec)	20.25 (18.4-25.7)	> 70
one-stage FVIII:C (IU/dL)	> 480	0.25 (0.2-0.3)
chromogenic FVIII:C (IU/dL)	< 3.3	-

measured with chromogenic assay and with one-stage assay after neutralization.

Conclusions: These preliminary results showed the efficacy of the anti-Emi that could be used in the laboratory monitoring of HA patients treated with Emicizumab when the one-stage is the only available assay.

PB0343 | An Overview of Patients with Hemophilia A in China: Epidemiology, Disease Severity, and Treatment Strategies

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[1]*CAMS Key Laboratory of Gene Therapy for Blood Diseases, Tianjin, China;* [2]*Analysis Group, Inc., Beijing, China, Beijing, China*

Background: Hemophilia A (HA) is an X-chromosome linked inherited bleeding disorder characterized by decreasing coagulation factor VIII (FVIII), and is associated with significant disease burden. As a member of the World Federation of Hemophilia (WFH), China has made remarkable progress in the past 30 years to understand the current epidemiology and disease profile of HA patients through the Hemophilia Treatment Center Collaboration Network of China (HTCCNC).

Aims: To better understand the development of hemophilia management in China.

Methods: HTCCNC enabled collaborative web-based data collection of hemophilia patients encountered from the 166 member hospitals (2007-2019). Patient demographics, disease severity, and key clinical and treatment information were summarized using descriptive statistics.

Results: HTCCNC has identified 17,779 diagnosed patients with HA during the study period, and 13,146 patients had lab-measured disease severity. The number of patients categorized to be severe (< 1% of normal activity), moderate (1-5% of normal activity), and mild (5-40% of normal activity) was 6,519, 4,788 and 1,809, respectively. Among all patients, 5,038 (28.34%) patients had family history of HA. Based on 2,619 patients with treatment information, delayed diagnose and delayed treatment remain significant challenges in current care for HA in China. In average, patients had 8.38 ± 10.82 (mean ± SD) years' gap between the time of first bleed to the time of diagnosis. The delay from the first bleed to the original treatment was 8.64 ± 10.51 (mean ± SD) years. Near half (44.29%) of patients relied solely on on-demand treatments, and 16.2% received any prophylaxis treatments (e.g., intermittent/primary/secondary/tertiary).

Conclusions: Beyond systematic training and education, HTCCNC has made significant progress in data collection with the support from WFH. Results based on current data highlighted the unmet need and indicated that disease awareness and treatment accessibility are key challenges to improve patient outcomes in China.

PB0344 | Detection and Quantification of *F8* Inv1 and Inv22 Inversions Using Digital Droplet PCR and Mile-post Assays

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Background: Inversions involving intron 1 (Inv1) and intron 22 (Inv22) of *F8* are detected in almost 50% of severe hemophilia A (HA) patients. Diagnosis of Inv22 inversions is complicated by the ~9.5 kb *int22h* repeated sequence which generates these inversions. Methods such as long-range PCR and inverse-shifting PCR are used diagnostically but suffer from low PCR efficiencies and have been difficult to standardize.

Aims: To characterize the properties of mile-post analysis using digital droplet PCR (ddPCR) for detection and quantification of *F8* Inv1 and Inv22 inversion chromosomes and screen collections of patients and carrier mothers containing inversions to validate the assay performance.

Methods: Digital droplet PCR using mile-post assays was used to investigate fresh and archival DNA samples.

Results: Mile-post assays specific for wild type and Inv22 type 1 and 2 chromosomes were designed, optimized and validated. All assays

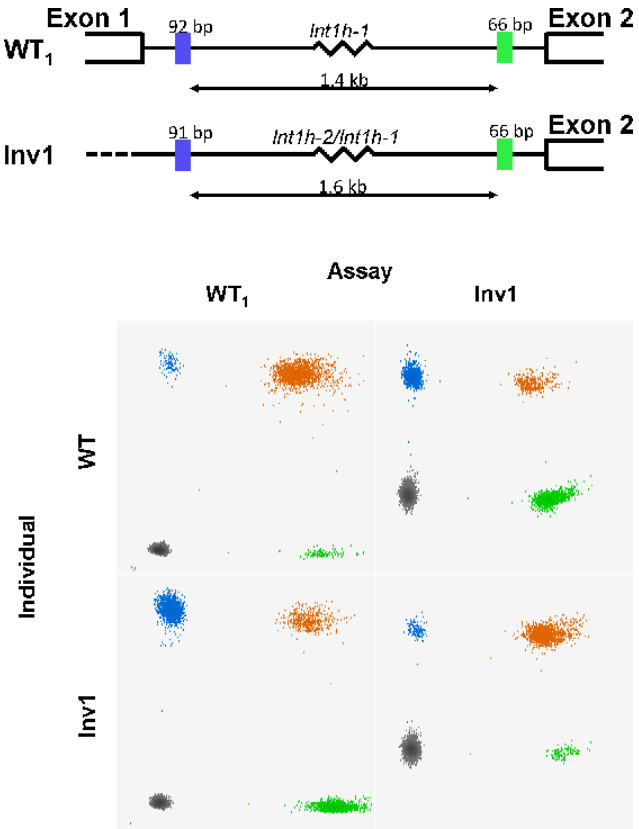

FIGURE 1 Location of Inv 1 mile-post systems and typical results of the assays

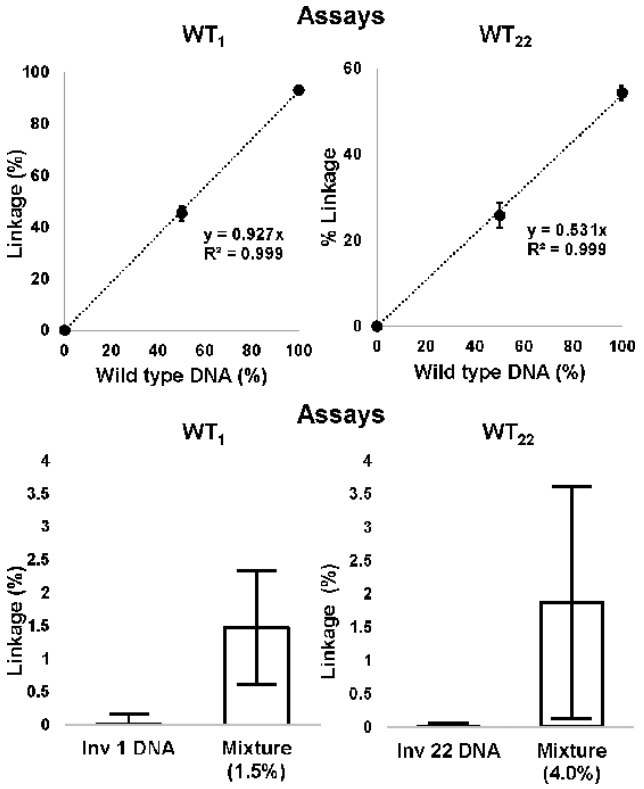

FIGURE 2 Inv 1 and Inv 22 mile-post analysis of artificial DNA mixtures showing quantitative response and detection levels

showed high specificities and sensitivities. Analysis of 106 patients and 20 carrier mothers with varying DNA concentrations demonstrated the robustness of the assays versus DNA concentration and quality. Mile-post assays specific for wild type and Inv1 chromosomes also showed high specificities and sensitivities (Figure 1). The quantitative properties of the Inv1 and Inv22 mile-post assays were investigated using artificial mixtures produced by mixing mutant and wild type DNA samples in varying proportions. Overall the assay systems showed a good correspondence between the observed relative linkage and the proportions of the different types of DNA (Figure 2). The limits of detection of these assays allow for sensitive detection of mosaics and high molecular weight DNA preparations increase the sensitivity of these assays.

Conclusions: Digital droplet PCR and mile-post assays can be used to detect *F8* Inv1 and Inv22 inversions in patients, carrier mothers and in mosaics. The assay systems are technically simple to perform, highly efficient, quantitative and very robust.

PB0345 | Prevalence and Incidence of Non-neutralizing Antibodies in Congenital Hemophilia - A Systematic Review and Meta-analysis

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Background: In hemophilia A, non-neutralizing antibodies (NNAs) against Factor VIII (FVIII) have been suggested to predict the development of neutralizing antibodies (inhibitors) and to accelerate the clearance of administered FVIII concentrates. Whereas the prevalence and incidence of inhibitors is well known, this is less precisely defined for NNAs.

Aims: This systematic review assesses NNA prevalence and incidence in inhibitor-negative patients with congenital hemophilia.

Methods: We conducted a literature search in MEDLINE, Embase, Web of Science and Cochrane. We included cross-sectional and longitudinal studies on NNAs in inhibitor-negative patients with congenital hemophilia. Data were extracted on: hemophilia type and severity, patient and treatment characteristics, NNA prevalence and incidence, NNA assays and inhibitor development. Study quality

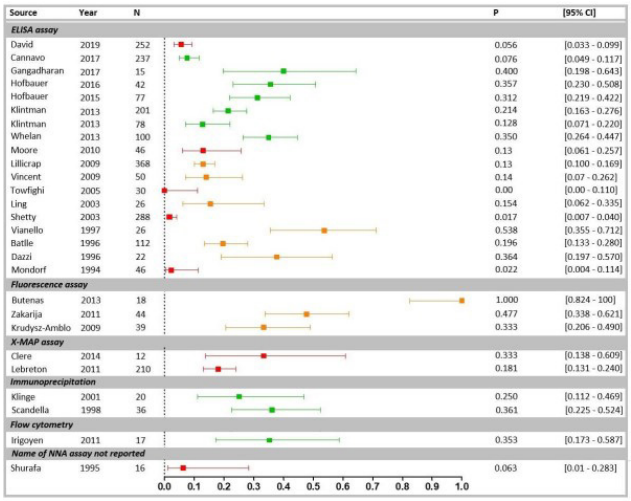

FIGURE 1 NNA prevalence in all studies. N, number of patients; P, proportion. Bar color = quality NNA assay

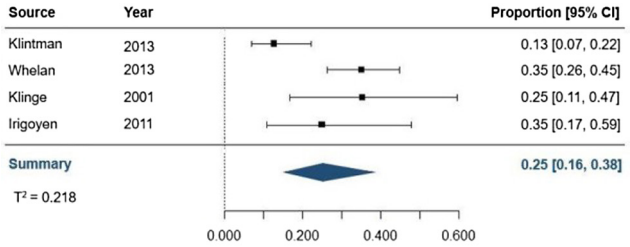

FIGURE 2 Meta-analysis of NNA prevalence in high-quality studies including previously treated patients with hemophilia A

was assessed using adapted criteria of the Joanna Briggs Institute. Study quality was classified as high when ≥5/9 criteria were met. NNA assay quality was classified as high when both of the following criteria were met: 1) use of positive controls and 2) competition with FVIII to establish FVIII-specificity. The pooled NNA prevalence was calculated for the selection of high-quality studies in previously treated patients, employing high-quality NNA assays.

Results: We included 28 studies involving 2723 inhibitor-negative patients with hemophilia A. Most studies (68%) were cross-sectional and none reported on hemophilia B. The quality assessment was high for 16 studies (57%) and 9 NNA assays (32%). Various NNA assays were used, predominantly ELISA (64%), with different cut-off values. The NNA prevalence in all studies ranged from 0-100%, with a straight unweighted average prevalence of 26% (95% CI, 18-33%) (Figure1). The pooled NNA prevalence was 25% (95% CI, 16-38%) (Figure 2). The NNA incidence was 0.01 new NNA/person-exposure day (reported in one study).

Conclusions: This systematic review provides a more accurate estimate of the prevalence of NNAs in previously treated inhibitor-negative patients with hemophilia A.

PB0346 | Pleiotropic Effects of PCSK9-inhibition on Hemostasis: Anti-PCSK9 Reduce FVIII Levels by Enhancing LRP1 Expression

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Background: Anti-PCSK9 reduce cholesterolemia enhancing the expression of LDL- receptors (LDLR) which mediate LDL-cholesterol clearance. Hepatic LRP1, a member of the LDLR family, is the most important receptor responsible for the hepatic clearance of FVIII. Previous in vitro and animal studies showed that statins increase LRP1 expression and recently statin intake was found to significantly reduce elevated plasma FVIII levels in patients with venous thromboembolism (VTE). No data instead are available on the influence of anti-PCSK9 on FVIII levels.

Aims: We aimed to evaluate the effect of lipid-lowering treatments on circulating FVIII levels and LRP1 expression in mice after an infusion-induced acute increase of circulating FVIII levels.

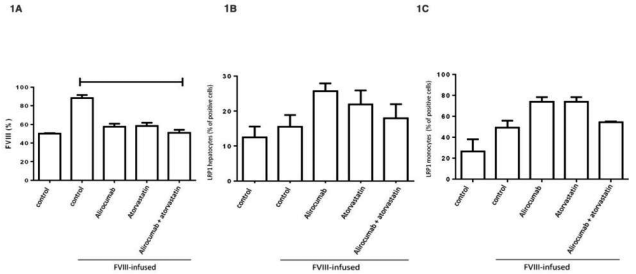

FIGURE 1

Methods: Male C57BL6 mice were randomized to treatment with atorvastatin 20 mg/kg o.i.d. by gavage, alirocumab 10 mg/kg o.i.d. by s.c. injection, alirocumab+atorvastatin o.i.d., or their veichles for five days, and on the last day i.v. injected with rhFVIII (50 IU/kg).
Plasma FVIII levels, liver and monocyte LRP1 expression were measured 90 min after infusion.

Results: Lipid lowering treatments reduced FVIII levels, significantly with the combination alirocumab + atorvastatin (88.3 ± 5.7 vs 50.9 ± 7.3% $p < 0.05$)(figure 1A). LRP1 expression by monocytes and hepatocytes was increased by lipid-lowering treatments compared with controls (figure 1B, 1C), especially by alirocumab (74 ±7.2 vs 49 ± 11.7% of positive cells).

Conclusions: Our data show that anti-PCSK9 reduce circulating FVIII levels by enhancing LRP1 expression. Given the role of elevated FVIII levels as cardiovascular risk factor, PCSK9 inhibitors, may exert an additional protective effect against cardiovascular events besides cholesterol lowering, by reducing FVIII. The effect of anti-PCSK9 on VTE incidence deserves to be assessed.

PB0347 | B Cell Cytokines in Italian Hemophilia A Patients with Inhibitors

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Background: Factor VIII (FVIII) inhibitors remain the most significant complication of therapy in hemophilia A (HA), increasing both morbidity and mortality. Identification of molecular biomarkers of inhibitor development and/or persistence could aid in determining optimal therapy for HA inhibitor patients. Previously, we demonstrated that levels of the transitional B cell and plasma cell survival cytokines, B lymphocyte stimulator (BLyS) and B cell maturation antigen (BCMA), respectively, were elevated in a cohort of U.S. pediatric HA inhibitor patients. BLyS has been associated with high risk for auto-immune disease in Italy, raising whether levels may be elevated in Italian FVIII inhibitor patients.

Aims: Here, we investigated whether BLyS and BCMA levels were elevated in a cohort of Italian adult and pediatric HA with inhibitors.

Methods: Fifty HA patients were enrolled from the Careggi University Hospital hemophilia treatment center. Inhibitor titers

were measured by Bethesda assay and BLyS and BCMA levels by a multiplex ELISA. BLyS and BCMA levels were compared by Mann-Whitney test. All studies were done according to Declaration of Helsinki and approved by Institutional Review Boards.

Results: Of 40 adult and 10 pediatric HA patients, 23 patients had no history of inhibitors, 5 had achieved FVIII tolerance, and 22 had an active inhibitor. The median age was 47.5 years (range 1-83) with no difference between those with and without inhibitors. Median BLyS levels were higher in inhibitor positive compared to inhibitor negative patients (1.13 vs 0.88 ng/ml, p=0.03, respectively) however BCMA levels were equal between the two cohorts (18.72 vs 19.34 ng/ml, p>0.05).

Conclusions: This study shows, for the first time, that elevated levels of BLyS are present in a cohort of Italian HA patients. As high BLyS has been described in auto- and allo-immune disease processes and targeted anti-BLyS therapy has been approved for lupus, these results further support anti-BLyS therapy for FVIII inhibitors.

PB0348 | Analytical Variability (CV) of One Stage Factor VIII Assay: A Quality Control (QC) Experience. Argentinian Group of Biochemists in Hemophilia

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Background: One Stage FVIII is the most frequently used assay in Argentinian labs taking care Haemophilia A (HA) patients. A novel QC program was created to improve the labs' performance. The novel QC program recruited at least one laboratory from each of the 24 provinces. Normal and abnormal lyophilized control plasmas were distributed within the participants. The results were statistically analyzed and then informe.

Aims: To evaluate the variability of one stage clotting Factor VIII assay between laboratories from Hemophilia Care Centers in Argentina in the first 7 month of program

Methods: Twenty eight laboratories from all over the country participated in the study. PT, APTT, FVIII (Chromogenic and one stage clotting assays), FIX, Fibrinogen and Thrombin Time, were perform, on one vial of control plasma (alternatively Normal or Abnormal). Only 17/28 laboratories used automated instruments (A): 7 used International Laboratory (IL), reagents and calibrators for FVIII assay, 3 used Stago supplies and equipment and 7 used Siemens Health Care. The remaining 11 labs used a combination of reagents, calibrators and controls according to their local availability, and perform the tests on semi-automated (SA) instruments (Wiener, Coatron, Amelung, KC1, STArt). In this report only FVIII by one stage clotting assay were analyze.

Results: see Tables 1 y 2.

Conclusions: The preliminary results of this local QC program showed no significant differences between automated and semi-automated FVIII quantification by one stage clotting assay. However, the CV% was higher with SA instruments particularly with abnormal control plasma. Another observation was that laboratory using IL® informed the lowest FVIII: C values and those using Stago® the highest, as was previously reported by ECAT in 2019. This experience is of great value for small centers around our large country that take care their

TABLE 1 Results

	FVIII (UI/dL) IL	CV%	FVIII (UI/dL) STAGO	CV%	FVIII (UI/dL) SIEMENS	CV%
May	81	8.9	105	10.9	93	10.8
June	25	15.1	30	22.9	34	25.0
July	28	20.0	32	22.8	31	21.3
August	28	11.5	42	23.3	31	9.4
September	81	14.4	109	11.4	94	7.4
October	45	21.5	39	5.1	35	7.9
November	89	13.4	118	19.0	92	21.0

TABLE 2 Results

	FVIII (UI/dL) SA	CV%	FVIII (UI/dL) A	CV%
May	93	20.5	90	13.7
June	31	40.7	30	24.4
July	30	15.3	30	20.5
August	25	45.8	31	27.5
September	88	25.2	91	15.6
October	41	29.0	38	14.6
November	105	23.1	96	20.6

local HA patients and do not have the opportunity to participate in External Intenational QC programs.

PB0349 | Real-life Inter-individual Variability of Pharmacokinetics - Reason for Individualization of Replacement Therapy in Haemophilia

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Background: Replacement therapy is mainstay of treatment in haemophilia (H). But, "one size doesn't fit all", making challenging the dosing of clotting factor concentrates (CFCs).
Aims: Evaluation of pharmacokinetics (PK) inter-individual variability of CF in the real-life.
Methods: This descriptive, non-interventional study was performed on 68 severe hemophilia patients: 52 HA and 16 HB, with 28.94±17.58 years of age. Using a Bayesian estimation of a population PK model constructed on 3 blood samples (prior, 1 and 48 hours post CFCs administration), following parameters were calculated: in vivo recovery (IVR), half life (T1/2) and peak level at 48 hours of diverse CFCs.
Results: We have noticed in HA following values: IVR - 1.65±0.68, T1/2 –13.93±4.95 and peak at 48 hours - 5.60±5.15, almost similar to the specific PK values of CFCs brands, with exception of IVR ($p< 0.001$). What concerns the HB we obtained: IVR 0.97±0.67, T1/2, 21.13±5.12 and peak at 48 hours 5.39±2.02, not different from the specific values of CFCs used ($p>0.1$). But, analyzing the distribution of data, it was a striking the difference between estimated and real values. In HA the dispersion of 48h peak value was very large: < 1IU/dl in 9.61%, 1-5IU/dl in 44.23%, 5-10IU/dl in 30.76%, >10IU/dl in 15.38% of cases, the real target value of 1-5% being attained in significantly lower proportion than estimated ($p< 0.001$). Differently, in HB the behavior of FIX peak values was more homogeneous, none of patients having values < 1 or > 10IU/dl. Our main source of bias was the high heterogeneity of CFCs; in order to overcome it, we used a percentage expression of patient′s and CF brand′s PK parameters. The results confirmed the same inter-individual variability of PK data.
Conclusions: Population PKs provide important information, suggesting a concrete modality of tailoring the replacement in accordance with patient's characteristics.

PB0350 | Development and Performance Characterization of an Automated Chromogenic Assay for Human Factor IX Activity

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Background: The one stage coagulation assay is widely used in diagnostic laboratories to monitor Factor IX levels in patients with Hemophilia B despite poor precision at low levels of Factor IX. We report here the performance characteristics of a newly developed chromogenic assay for Factor IX on an automated analyzer platform (Siemens BCS XP).
Aims: Summarize the assay format and performance of an automated chromogenic assay using a single calibration curve for the measurement of Factor IX in human plasma samples
Methods: The assay principle is based on the activation of Factor FIX in the sample, mediated by Factor XIa in the reagent. The activated Factor IX then potentiates the activation of Factor X in the presence of Factor VIIIa, Calcium and Phospholipids. A chromogenic substrate is used to measure the activity of the Factor Xa generated. This assay uses Thrombin to improve the efficiency of activation of Factor FVIII. The assay uses a single calibration curve from 100% down to 1% FIX using a sample dilution of 1:40.
Results: The assay reagents show excellent reproducibility of calibration curves verified across multiple runs per day and multiple days which showed pooled CV of 3.2% across the calibration interval. Preliminary estimation of the assay sensitivity showed LOB and LOD of 0.1% and 0.3% respectively. Upon reconstitution, the assay reagents are stable for up to 24 hours under refrigerated storage. The reagents also show on-board stability of 8 hours, consistent with other commercial kits. Recovery studies were performed with sample Factor IX levels across the calibration curve (88%, 5%, 2% & 1%) and showed good recovery and precision (≤ 12% CV) across the measured levels.
Conclusions: With a single calibration curve, this chromogenic assay offers a simplified and cost-effective approach towards the measurement of Factor IX in Hemophilia B patients

HEMOSTASIS AND ORGAN DYSFUNCTION

PB0351 | Effect of Warfarin Addition in Diabetic Foot Ulcer Patients Receiving Cilostazol In Mohammad Hoesin Hospital, South Sumatera, Indonesia

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Background: Diabetic foot ulcer is one of the most frequent chronic complication of diabetes, and is one of the most common cause of

hospitalization in diabetic patients. The treatment urges an integrative and holistic approach, including treatment of hemostatic disturbances which is commonly occured in diabetic patients. Cilostazol with its antithrombotic and vasodilatory effects has been a standardized therapy for diabetic foot ulcer with peripheral arterial disease. But until now, there has been less interest and attention in treating secondary hemostatic disorders using warfarin in diabetic foot ulcer.

Aims: To analyze the differentiation of wound healing in diabetic patients who get cilostazol and warfarin to those who only get cilostazol therapy.

Methods: This is a double blind randomized clinical trial, between the group that receives cilostazol and warfarin and the group that receives cilostazol and placebo. Evaluation of the diabetic foot ulcer with Texas criteria were also done before and after 3 weeks of treatment.

Results: Of 32 samples, 17 patients were in warfarin group, and 15 patients were in control group. There are significant reduction of ulcer area in both groups. The median of ulcer area reduction in warfarin group is greater (2 (0-28.11)cm^2) than in placebo group (1.7 (−5-39.72)cm^2), but not statistically significant different (p=0.71).

Conclusions: The reduction of ulcer area in patient receiving warfarin and cilostazol is greater than in patients receiving cilostazol only, but not statistically significant different.

PB0352 | Haemostatic Profile of Nigerian Children with Nephrotic Syndrome (NS)

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Background: Hypercoagulability occurs in NS with episodes of thrombotic complications.

Aims: This study evaluated the haemostatic profile of Nigerian children with NS and the relationship between haemostatic variables and the disease states.

Methods: Forty-six children with NS (2-18 years) attending the Paediatric Nephrology Clinic of the University of Nigeria Teaching Hospital Enugu and an equal number of age and gender-matched healthy controls were studied. APTT and PT were assayed manually. Fibrinogen was assayed with a semi- automated Coagulometer (KHB202 Kinghawk, China), Antithrombin (AT) by ELISA (Mindray MR-96A Plate-reader, China) and platelet count using a Haematology Analyzer (Mindray BC5300, China).

Results: There were 28 males and 18 females. Twenty (43.5%) of the 46 children with NS were in remission, 19 (41.3%) were in relapse and 7 (15.2%) had new disease.

The median fibrinogen levels in NS and controls group were 2.9g/L with IQR 1.1 to 7.2g/L and 1.4 to 4.5g/L respectively. The median fibrinogen levels were 4.9, 4.1, 2.3 and 3.0g/L in new disease, relapse, remission and controls respectively Sixteen (35%) children with NS had hyperfibrinogenaemia. The median fibrinogen levels of children in remission differed significantly with those of children in relapse (p = 0.001).

The median AT levels were not significant, 142% and 131% in children with and without NS respectively, (p=0.134).

The median APTT was significantly different in NS and control group (*p value = 0.02*). The median PT levels also differed significantly (*p = 0.004) and* 90% of children with NS had INR within reference range. Thrombocytosis occurred in 15% of NS group. The median platelet count in those with new disease was significantly higher (*(p = 0.01*).

Conclusions: We observed haemostatic derangements in Nigerian children with NS especially involving Fibrinogen, APTT, PT, INR and Platelet count, warranting a high index of clinical suspicion and monitoring in children with NS. Antithrombin was unaffected.

PB0353 | Role of Serpine2 (PN-1) in Diabetic Nephropathy

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Background: Diabetic nephropathy (DN), the primary cause of end-stage renal disease, is a multifactorial disease in which inflammation and angiogenesis play a crucial role. SerpinE2, or protease nexin-1 (PN-1), is a protease inhibitor of the serpin family, expressed by vascular and inflammatory cells, that has a strong antiangiogenic potential.

Aims: To address the role of PN-1 in DN.

Methods: Diabetes was induced in PN-$1^{-/-}$ and WT mice by repeted injections of streptozotocin. Vascularization was studied on kidney sections by isolectin labelling. Arterial flow was measured by Doppler. MK expression was analyzed by immunohistochemistry on kidney cryosections and by RT-PCR on renal cDNA.

Results: PN-$1^{-/-}$ diabetic mice presented histological features of an aggravated nephropathy, compared to WT mice, with higher hypertrophy of glomeruli and greater mesangial expansion. Moreover, renal function was worsened in PN-$1^{-/-}$ diabetic mice compared to WT mice, with much higher urine albumin to creatinine ratio in the absence of PN-1. At the very early stage, diabetes is known to induce an increase of glomerular vascularization. We observed that this increased glomerular vascularization was higher in PN-$1^{-/-}$- than in WT- diabetic mice. However at later stages, kidney vascularization was conversely significantly reduced in PN-$1^{-/-}$ diabetic mice. Similarly, reduction of arterial flow in response to diabetes was less accentuated in PN-$1^{-/-}$ than in WT kidneys in early diabetes but reached the level observed in WT kidneys in late diabetes.

Midkine (MK) is a growth factor involved in DN that has been shown to be overexpressed in retina and muscle of PN-$1^{-/-}$ mice. Our result evidenced higher MK overexpression in PN-$1^{-/-}$ compared to WT kidneys after diabetes induction that could thus be partly involved in the worsening of DN development in PN-1 deficient mice.

Conclusions: In conclusion, our results indicate that PN-1 may play a protective role in the development of DN, possibly via MK downregulation.

PB0354 | Liver Dysfunction Biomarkers Correlate with a Prothrombotic and Not with a Prohemorrhagic Hemostatic Profile in Cirrhotic Patients

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Background: Cirrhotic patients were demonstrated to be at increased thrombotic risk in studies using Calibrated Automated Thrombogram (CAT), a non-standardised/non-fully automated thrombin generation (TG) assay. However, due to the high analytical variability, these studies exhibit discrepancies. Moreover, different liver cirrhosis (LC) severity biomarkers are used to assess the bleeding risk of LC-patients.
Aims: We aimed to perform a larger study using automated/standardised TG-assays in order to
i) obtain robust data on LC-severity and TG; and:
ii) investigate relationships between LC-severity biomarkers and TG, questioning their adequacy for bleeding risk-assessment.
Methods: We performed a prospective single-centre study (n=200). TG was measured without/with thrombomodulin (TM) using ST Genesia® (Stago, Asnières-sur-Seine, France). We analysed the relationship between TG-parameters without TM and TM-mediated inhibition, and MELD-score/PT/INR/aPTT/FV-activity/albumin/total bilirubin.
Results: Without TM, the endogenous thrombin potential (ETP) and the peak height were slightly decreased and the velocity index (VI) was increased in LC-patients versus healthy donors (p=0.046/0.031/< 0.0001). The VI increased from Child A to B-patients (p=0.004). With TM, the ETP and the peak height were increased in LC-patients versus healthy donors (p=0.0002/0.003) and in Child B versus A-patients (p=0.009/0.028). This resulted in a decreased TM-mediated inhibition in LC-patients versus healthy donors and in Child B versus A-patients (p< 0.0001).
The TM-mediated inhibition showed a direct relationship with PT/FV-activity/albumin and an inverse relationship with INR/aPTT/bilirubin/MELD-score (R^2=0.448/0.483/0.310/0.389/0.326/0.295/0.250, respectively). Relationships between VI without TM and LC-severity biomarkers were coherent with results of TM-mediated inhibition (R^2=0.141/0.315/0.124/0.068/0.110/0.119, respectively).
Conclusions: Using an automated/standardised TG-assay (without and with TM), we documented that LC-patients are prothrombotic. We also confirmed a decreased TM-mediated inhibition, which is due to an acquired protein C-resistance. We demonstrated an increasing prothrombotic profile with increasing biomarker-disturbances in LC-patients. Therefore, these parameters should not be used for estimating bleeding risk in LC-patients. Alternative biomarkers for bleeding risk-assessment in LC-patients need to be developed.

PB0355 | Utility of Prophylactic Fresh Frozen Plasma Transfusion for Correction of Coagulopathy Prior to Image-Guided Drainage for Infected Collections

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Background: Current guidelines recommend prophylactic fresh frozen plasma (FFP) transfusion in patients with coagulopathy (PT-INR>1.2) prior to image-guided drainage, however there is limited evidence to support this practice.
Aims: To analyse the efficacy of weight-based standard FFP dose for coagulopathy correction in patients with infected collections planned for image-guided drainage.
Methods: In this prospective case-control study, 117 patients planned for image-guided drainage with Prothrombin time-International Normalised Ratio (PT-INR)>1.2-2.5 taken as cases and received FFP transfusion at 10-15ml/kg. Final analyses excluded 3 cases, due to non-availability of post-transfusion PT-INR. 112 patients planned for procedure with normal PT-INR≤1.2 taken as controls.
Results: This study included patients with liver abscess (80.35%), renal abscess (5.24%), psoas abscess (1.75%), pancreatic collections (2.62%), empyema and biliary collections (10.04%). Baseline characteristics were not statistically different among cases and controls. The mean PT-INR post-transfusion was 1.45 (SD=0.1776). Mean duration (admission to procedure) was 37.15 hours in cases and 20.5 hours in controls (p=0.001). FFP transfusion resulted in PT-INR normalization (INR≤1.2) in only 7.7% cases (n=9) while 15.7% cases (n=18) had PT-INR worsening. No bleeding complications were noted in 105 patients who underwent procedure despite persistent deranged coagulogram after FFP. Only 1 patient had local hematoma post-procedure, despite pre-procedure PT-INR normalisation. Among the cases, 23.9% had febrile non-hemolytic transfusion reactions, 6.8%

had urticaria, 5.9% had Transfusion-associated circulatory overload (TACO)/Transfusion-related acute lung injury (TRALI). Control group had no mortalities while study group had 5 mortalities that were attributed to worsening septic shock (p=0.060).

Conclusions: The efficacy of prophylactic FFP transfusion for coagulopathy correction prior to image-guided drainage is abysmally low while a significant number of patients experience transfusion-related complications. Risk of local bleeding complications is not increased in patients undergoing procedure with PT-INR>1.2. The practice of prophylactic plasma transfusion leads to a significant delay in procedure and may contribute to increase in sepsis associated mortality in patients with infected collections.

PB0356 | Mechanisms of Blood Clotting Activation by Amniotic Fluid

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Background: Amniotic fluid (AF) embolism is a rare, life-threatening obstetric syndrome with a high mortality rate. It is almost always associated with disseminated intravascular coagulation, but the pro-coagulant mechanism of amniotic fluid is still not clear.

Aims: To investigate pro-coagulant mechanisms of blood clotting activation by amniotic fluid.

Methods: Fresh AF samples were collected by amniocentesis from 7 patients. Plasma-based fibrin generation assays with donor pooled plasma (n=12), and plasmas deficient in factors XII, IX, XI, VII, V, X were used to characterize the pro-coagulant activity of amniotic fluid. Differential centrifugation was used to assess the pro-coagulant activity of different AF fractions. Flow cytometry was used to determine microvesicle count and analyze CD41+, TF+ (CD142+), and Annexin5+ populations.

Results: In normal plasma, AF at 1% concentration shortened clot time from 50 to 6 minutes on the average. This effect was not prevented by either corn trypsin inhibitor, or active site-inhibited factor VIIa, or their combination. AF was able to efficiently clot plasmas with severe deficiencies of factor XII, IX, or XI. The clotting time was prolonged in factor V- and, to a lesser degree, in some batches of factor VII-deficient plasma. AF did not cause coagulation in factor X deficient plasma. Although fluid fraction and cell fraction of AF possessed some clotting capacity, the predominant part of the activity was associated with the microvesicle fraction. Flow cytometry analysis revealed that most microvescles were Annexin5+ and CD142+, with few CD41+ events.

Conclusions: Our findings suggests the mechanism wherein amniotic fluid activate coagulation cascade via a microvesicle fraction at the factor X/V and/or factor VII level.

PB0357 | Predictive Role of Natural Anticoagulants in Prognosis of Chronic Liver Disease in Pediatric Age Group

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Background: Protein S and Protein C are sensitive markers of liver disease. There is significant reduction of Antithrombin III in decompensated cirrhosis.

Aims: To compare the frequency of protien C, antithrombin III deficiency and raised D-dimer in cirrhotic patients.

Methods: 80 patients suspected to have cirrhosis were included in this study by non-probability convenience sampling.

Results: The mean level of Antithrombin III showed significant reduction in patients with decompensated cirrhosis (50.0±4.05%) when compared with patients with compensated liver disease (84.52 ±15.36%). At the same time the mean PC level exhibited a more significant reduction in the decompensated patient groups (40.00±4.7%). The mean D-dimer levels showed significantly higher levels only in patients with signs of decompensated cirrhosis (840±11.85μg/L) when compared with compensated disease (629±6.29).In case of protein C, 9 (47.4%) compensated Patients showed protein C less than 70% while 61 (100.0%) compensated patients were with protein C 70 to 130%.11 (52.4%) compensated patients were with d-dimer more than 639ng/l while in the category of decompensation 10 (47.6%) patient showed d-dimer more than 639ng/l.

Conclusions: We concluded that natural anticoagulants AT III and PC reflect hepatocellular impairment in compensated as well as decompensated patients of cirrhosis. This is also supported that in decompensation there is marked decrease in these anticoagulant proteins. Our study further shows that patients with high D-dimer levels require careful monitoring since this observation is associated with decompensated liver disease.

PB0358 | Simvastatin Prevents Liver and Kidney Microthrombosis After Exposure to Lipopolysaccharide by Preserving the Antithrombotic Properties of the Endothelium

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Background: Microvascular thrombosis has an important role on the development of organ failure in sepsis and endothelial dysfunction represents a key-pathogenic mechanism of this process. Simvastatin preserves the anti-inflammatory and the vasodilator properties of endothelial cells and deserves potential anti-thrombotic effects during endotoxemia.

Aims: This study investigates the haemostatic changes in a rat model of endotoxemia (after exposure to lipopolysaccharide, LPS) and explores the protective effects of simvastatin against liver and kidney microthrombosis.

Methods: Rats exposed to LPS (5mg/Kg one i.p. dose) were compared with saline in the presence or absence of pre-treatment with simvastatin (25 mg/kg/24 h, orally, 3 days before LPS/saline injection). Peripheral- and organ-haemostatic profile were analysed using, respectively, ROTEM on whole blood and Morphological studies were performed by light-microscopy analysis to show intravascular fibrin deposition.

Results: LPS induced fibrin deposition and liver and kidney vascular microthrombosis. ROTEM analysis displayed a haemorrhagic profile in the peripheral blood that correlated with the degree of intrahepatic (R=0.789, p=0.001) and glomerular fibrin deposition (R=0.792, p=0.019). Simvastatin was able to prevent LPS-induced fibrin deposition in association with maintenance of the physiologic TM expression on endothelial cells and completely reverted the peripheral haemorrhagic profile associated with endotoxemia.

Conclusions: Simvastatin may preserve the antithrombotic function of endothelial cells after LPS exposure. The results of our study suggest a pharmacologic strategy to prevent the coagulopathy and organ failure elicited by sepsis.

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PB0360 | ADAMTS-13/VWF as Biomarker for Portal Vein Thrombosis Development in Cirrhotic Patients: A Prospective Study

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Background: Portal Vein Thrombosis (PVT) is one of the most relevant complication of cirrhosis. PVT prevalence, as showed in PROLIVER study, is 17%. Previous studies by our research group found that, a reduced portal flow velocity seems to be the most important predictive variable for PVT development in patients with cirrhosis. An unbalance between the decreased ADAMTS-13 level, due to myofibrillar transformation of stellate cells, and the inflammatioin-driven heightened levels of von Willebrand factor (VWF) released from endothelium, may predispose to platelet/VWF-rich thrombi formation in micro- and macrocirculation.

Aims: Our prospective clinical trial (ClinicalTrials.gov Identifier:NCT03322696) is aimed at unraveling the linkage between ADAMTS-13/VWF changes and PVT development in cirrhosis.

Methods: Currently, 118 cirrhotic patients (viral etiology represent ~42%, any other etiology ~58%) were enrolled (2017-2019). The CHILD-PUGH score assessed the severity of liver function failure. In all patients, portal vein flow velocity was measured. with eco-Doppler sonography. ADAMTS-13 activity (A13:act) was measured by a fluorescence resonance energy transfer-based assay, while VWF-antigen and VWF:activity were evaluated by chemiluminescence assays. The variables that resulted statistically significant in univariate Spearman analysis ($p< 0.05$), were used in multivariable regression analysis to identify prognostic factors (two-sided $p< 0.05$).

Results: CHILD-PUGH score was positively correlated with VWF (VWF:act: rho=0.347, $p< 0.0001$; VWF:Ag: rho=0.342, $p< 0.0001$) and inversely correlated with A13:act and A13/VWF:act ratio (rho=−0.249, p=0.01, rho=−0.306 $p< 0.0001$, respectively). Multivariable regression analysis showed that A13:act (β=−0.278, p=0.001) and A13:VWF:act ratio (β=−0.219, p=0.006) reflected the severity of liver failure and were inversely associated with PVT development (2/118 cases).

Conclusions: The inverse correlation between the A13:act/VWF:act ratio and the CHILD PUGH score would suggest that the enzyme availability (A13:act) decreases as a function of disease progression, triggering an accumulation of UL-VWF multimers, which may predispose to PVT. Thus, the A13:act/VWF:act is significantly associated with liver failure and could be a predictive biomarker for PVT development.

PB0361 | APML: 2 Cases of Discordant Coagulation Results and ROTEM Testing

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Background: Acute promyelocytic leukaemia (APML) is associated with a distinct pattern of coagulopathy predominated by hyperfibrinolysis.[1] The leading cause of death in APML is from haemorrhagic complications.[1] Annexin A2 is a fibrinolytic activator that increases plasmin generation and hyperfibrinolysis[2] and is heavily expressed on APML and cerebral endothelial cells, which likely contributes to the high incidence of intracranial haemorrhage (ICH).[2] Blood product replacement is generally guided by conventional coagulation parameters. There is little published data on the ROTEM parameters for blood product replacement in APML, however one study of 50 APML patients found an EXTEM MCF ≤30mm was an independent poor prognostic variable[3] and discrepant results between ROTEM and conventional parameters were also evident in some cases.

Aims: Here we present two illustrative cases of APML in which there is significant discordance between conventional coagulation parameters and ROTEM results. In these cases clinical bleeding correlated with the ROTEM findings.

Methods: Case 1 was a man with subdural haemorrhage (SDH) and new diagnosis of APML. At presentation he was coagulopathic (see table 1). He commenced ATRA and received cryoprecipitate achieving fibrinogen 2.1g/L and PT of 18s at the time of neck line insertion. Despite this his line was complicated by large neck haematoma and concurrent ROTEM showed FIBTEM A10 of 5mm. Fibrinogen concentrate (4g) and further platelets were administered, with improvement in ROTEM parameters and resolution of bleeding. Case 2 had similar presentation with severe intraparenchymal haemorrhage requiring neurosurgical intervention and was diagnosed with APML. At presentation coagulation profile was normal, but ROTEM FIBTEM A10 was reduced at 5mm (see table 2).

Results:

Conclusions: The 2 cases illustrate a discordant ROTEM and coagulation result. Why a discrepancy occurs is not certain. There may be value in utilizing ROTEM assays to assess for coagulation defects in APML and guide replacement therapy and further studies are warranted.

PB0362 | Diagnosis and Treatment of Obstetrics Disseminated Intravascular Coagulation in Resource Limited Settings

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Background: Disseminated intravascular coagulation (DIC) is one of the commonest causes of abnormal bleeding during pregnancy and puerperium. It's successful management requires early recognition, specific but vigorous treatment, a challenging feat in resource limited settings (RLS).

TABLE 1

	At Presentation	At time of haematoma	Following fibrinogen & platelet replacement
FIBTEM [A10 (7-23mm), MCF (9-25mm)]	Not performed	5, 5	8, 9
EXTEM [A10 (42-65mm), MCF (50-72mm)]	Not performed	5, 5	42, 53
PT (12-16.5sec)	19.5	18.0	17.8
Fibrinogen (2.0-4.0g/L)	1.2	2.1	2.2
Platelet (150-400x10^9/L)	19	39	127

TABLE 2

	At presentation	Following 2 adult cryoprecipitate, FFP and platelet replacement
FIBTEM [A10 (7-23mm), MCF (9-25mm)]	5, 6	12, 13
EXTEM [A10 (42-65mm), MCF (50-72mm)]	7, 13	28, 39
PT (12-16.5sec)	16.9	16.8
Fibrinogen (2.0-4.0g/L)	2.1	2.7
Platelet (150-400x10^9/L)	10	53

Aims: To determine Obstetricians' approach in diagnosing and treating obstetrics DIC in a RLS.

Methods: A semi-structured pre-tested 4-sectioned questionnairre was used to collect demographic data of Nigerian Obstetricians and data on their practice in the diagnosis and treatment of obstetrics DIC.

Results: A total of 171 Obstetricians responded with a majority of them (67.2%) having 5-15 years of specialist practice. Within the year under review, most of the respondents saw < 10 cases of DIC each. Preeclampsia was the most frequent cause identified (70.2%) followed by postpartum haemorrahge (58.3%). Platelet count determination was the test mostly used (95.9%) to make a diagnosis of DIC whereas, antithrombin assay was the least (20.6%) requested investigation. While about two-third would monitor the evolution of DIC, a little less than half of the Obstetricians would not repeat laboratory testing more than every 2 days, reason mainly (61.8%) due to patient's financial constraint. Likewise, majority will not look out for non-overt DIC. Majority would treat for low haemoglobin concentration and platelet count and a platelet transfusion trigger of 20-50 $x10^9$/L was used by 73.2% of the respondents. Almost three-quarter of them preferred fresh whole blood as the first line of treatment of DIC.

There was a non-significant association between years of practice and use of DIC score and index of suspicion for non-overt DIC, However, there was a significant association between years of practice and serial monitoring of evolution of DIC (X^2 10.8 p value 0.02).

Conclusions: DIC remains a challenge in the obstetrics practice in RLS especially in investigations, monitoring and index of suspicion for non-overt DIC.

PB0363 | State of the Hemostatic System in Diffuse Congenital Arteriovenous Malformations of the Extremities

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Background: Bleeding is one of the reasons that complicate the surgical treatment of diffuse congenital vascular malformations (CVM). Increased introoperative blood loss depends not only on the degree of vascularization of the diffuse CVM, but also on changes in the blood coagulation system, which is often manifested by hypocoagulation due to chronic disseminated intravascular coagulation syndrome.

Aims: The study goal was to investigate the characteristics of plasma coagulative and fibrinolytic systems activation degree for patients with diffuse arteriovenous (AV) CVM of the extremities before and after surgery.

Methods: Hemostasis system in 23 patients with diffuse AV CVM of the extremities were studied by indicators of blood clotting time, activated partial thromboplastin time, prothrombin time, INR, plasma fibrinogen content, immunodiagnostic test (simultaneous assessment of D-dimer, soluble fibrin and protein C activity).

Results: In the preoperative period in patients with AV CVM revealed an imbalance in the hemostasis system is shown by significant activation of the coagulation link: in 9 (39%) patients significantly increased plasma fibrinogen content (3.7 to 7.2 g / l, at the rate of 2-3 g / l), in 10 (43.5%) - soluble fibrin (from 3.6 to 50 µg / ml, at 3 mg / ml), 7 (30.4%) - D-dimer (from 119.3 to 1608.3 ng / ml, at normal 70 ± 20 ng / ml); protein C activity in 95% of patients was normal (85-100%); changes in the postoperative period depended on the degree of surgery correction of arteriovenous shunting, the extent of the surgical trauma, the degree of blood loss and its compensation.

Conclusions: Diagnostic test defining the status of coagulative and fibrinolytic systems are necessary to be done for timely correction of hemostasiological disturbances and for prevention of fatal hemorragic complications in the perioperative period in patients with diffuse AV CVM.

MICROPARTICLES

PB0364 | Micropartical-Associated Thrombin Generation in Patients with Philadelphia-Negative Myeloproliferative Neoplasms

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Background: Essential Thrombocythemia (ET) and Polycythemia Vera (PV) are Philadelphia-negative myeloproliferative neoplasms (MPN). PV and ET have some common properties such as relatively normal maturation of cells and similarity of clinical symptoms. As known, MPN patients have a high risk of thrombotic complications. Activation and damage of blood cells in patients with ET and PV can lead to microparticles (MP) release. MP expresses tissue factor (TF) and procoagulant phospholipids on their surface that can enhance prothrombogenic potential of blood plasma.

Aims: Estimation of MP significance for thrombin generation in the group of patients with ET and PV.

Methods: The study included 64 MPN patients (25-76 years, median = 40) with ET and PV which were combined in one group because of the similarity of pathogenesis. Reference blood samples were obtained from 30 healthy people. Thrombin generation was measured in platelet-free plasma with Calibrated Automated Thrombinography

TABLE 1 Thrombogram parameters in the group of patients and control group (Me, 95% CI)

Parameters	«PRP-Reagent» Patients (N=64)	«PRP-Reagent» Controls (N=30)	«MP-Reagent» Patients (N=64)	«MP-Reagent» Controls (N=24)
ETP (nM·min)	570.9* (128.0-1470.0)	471.8 (265.0-610.0)	798.0* (123.0-2366.0)	566.5 (57.2-1024.3)
Peak (nM)	17.7 (3.9-93.0)	18.4 (8.8-28.6)	72.0** (7.1-107.0)	46.3 (20.2-93.2)

(CAT). Following reagents were used: «FluCa-kit», «PRP-reagent», containing rTF (1pM), «MP-reagent», containing negatively charged phospholipids (4μM), with CTI (40 μ/ml). Endogenous thrombin potential (ETP, nM*min) and peak thrombin (Peak, nM) were evaluated. Data were described by non-parametric methods using the median (Me), 95% confidence interval (CI) and Mann-Whitney U test (Statistica 12.0). P< 0.05 was considered significant.

Results:

Parameters of CAT are presented in table.* - p< 0.05, ** - p< 0.0001 for difference with controls: «PRP-reagent» and «MP-Reagent» allow to estimate thrombin generation which depends on procoagulant phospholipids and TF, respectively. Significant differences were found when were used both of that reagents but using «MP-Reagent» allowed to give more considerable differences.

Conclusions: MP enhance thrombin generation and increase prothrombogenic potential of patients with MPN due to TF and procoagulant phospholipids expressed on their surfaces. MP carrying TF have more significance for hypercoagulability.

PB0365 | Microparticles Contribute to Hypercoagulation, but Not to Coagulation in Healthy Controls

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Background: Circulating cell-derived microparticles (MP) are important players in thrombogenesis because they carry tissue factor (TF), the initiator of blood coagulation. They are formed from membrane blebs of blood cells that are released from the cell surface by proteolytic cleavage of the cytoskeleton. All MPs are procoagulant because they provide a negative charged membrane surface for the assembly of coagulation factors.

Aims: With this study we performed thrombin generation assays on hypercoagulable plasma samples received at our Special Haemostasis Laboratory of the National Health Laboratory Service of South Africa. We isolated microparticles from these plasma samples and did thrombin generation assays again on these plasma samples without microparticles. We then determined the contribution of microparticles to the thrombin generation of these hypercoagulable plasma samples.

Methods: Microparticles were isolated from 50 hypercoagulable plasma samples and 50 normal plasma samples (control group), using the Ceveron microparticle filtration unit (MFU 500, Technoclone, Austria). Thrombin generation assays (TGA) were performed using the Technoclone Ceveron Alpha instument. Thrombin generation were measured over 90 minutes in both normal plasma samples and hypercoagulable plasma samples (with and without the presence of MPs). We calculated the peak thrombin concentration, velocity index and aeria under the curve.

Results: MPs from all hypercoagulable plasma samples exhibited increased thrombin generation with all TGA parameters. Microparticles contributed 49 ± 16% to the peak thrombin, 63 ± 19% to the velocity-index and 24 ± 10% to the AUC. Interesting to note is that MPs did not contribute at all to thrombin generation in normal plasma samples.

Conclusions: Microparticle mediated TGA is a novel way to assess functional procoagulant activity of MPs. Enhanced MP-mediated TGA was demonstrated in hypercoagulable plasma samples. These findings support a major role of MP in thrombogenesis.

PB0366 | miRNAs 126 and 223 in Microparticles from Patients with Coronary Ischemic Disease

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Background: Circulating microparticles (MP) are membrane vesicles derived from platelets, erythrocytes, leukocytes, endothelial cells and other cell types. They convey proteins, DNA, and miRNA from the cell of origin to other organs or tissues.

The miRNAs are small, non-coding ribonucleic acids (RNAs) of about 21 to 23 nucleotides that bind specifically to messenger RNA (mRNA) and directly influence gene expression.

They play a role in in the pathophysiology of many diseases.

Aims: To identify miRNAS in MPs of patients with acute coronary syndromes, TIMI 0 or 3.

Methods: This cross-sectional study was approved (18-1043) by the Scientific and Ethical Committee of the National Institute of Cardiology. All patients (n=86) signed an informed consent, had an angiographic study and were classified according to TIMI scale.

TABLE 1 Platelet aggregation in percentage, values are expressed as Median (minimum - maximum) value

	TIMI 0	TIMI 3	p
Platelet Aggregation (%)	133 (51-193)	115 (35-268)	0.01*

U Mann- Whitney test. *p<0.05.

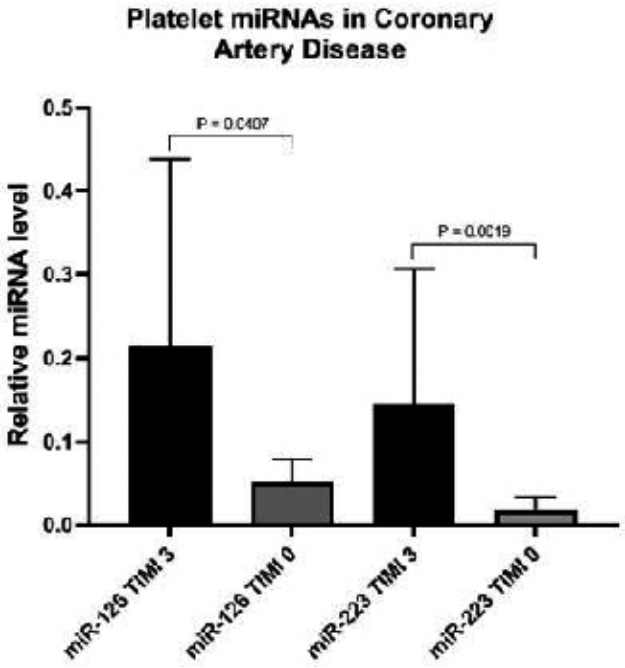

FIGURE 1 Expression profiles of miRNA from CAD patients. Bars represent average miRNA-126 and miRNA-223 expression (17 TIMI 3 and 15 TIMI 0 patients). Data rep

MPs were isolated from patient's plasma by centrifugation (20 000 g) and were used for miRNA identification (Direct-zol ™ RNA MiniPrep, Zymo-Research™, and TaqMan© miRNA Assay Thermo Fisher-Scientific) and platelet aggregation studies performed in a micro plate-based technique as follow. Platelets (250 000 platelets/μL) from healthy blood bank donors were first incubated with patients' MPs during 30 min at 37°C, and their aggregation in response to 10 mM ADP was detected in a microplate reader by measuring the absorbance at 595nm. The aggregation of platelets without MPs was considered as control 100% platelet aggregation.

Results: Platelet aggregation was increased 1.15-fold in TIMI 0 as compared to TIMI 3 patients, Table 1. The miRNAs 126 and 223 were decreased 20- and 7.5-fold in TIMI 0 (no spontaneous reperfusion) as compared to TIMI 3 (complete spontaneous reperfusion) patients after a cardiac ischemic attack, Figure 1.

Conclusions: miRNAs 126 and 223 are related to angiogenesis and platelet activity, both central mechanisms in ischemic disease.

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PB0367 | Influence of Specific Therapy on Microparticle-Associated Thrombin Generation in Patients with Primary Myelofibrosis

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Background: Primary Myelofibrosis (PMF) is philadelphia-negative myeloproliferative neoplasm with unique symptoms caused by bone marrow fibrosis. Thrombocytosis and leukocytosis are specific symptoms for PMF and appointment of specific therapy to PMF patients leads to reduce number of cells. Activation and damage of blood cells associate with microparticles (MP) release. Tissue factor (TF) and procoagulant phospholipids (PL) on MP surface can increase prothrombogenic potential of blood plasma.

Aims: To value influence of specific therapy on microparticle-associated thrombin generation in PMF patients.

Methods: The study included 46 PMF patients (38-78 years, median of age = 57.8). 24 patients (group 1) were given specific therapy (ruxolitinib and hydroxycarbamide) and 22 patients (group 2) were not. Thrombin generation was measured in platelet-free plasma with Calibrated Automated Thrombinography (CAT) according to Hemker et al. We used following reagents: «FluCa-kit», «PRP-reagent», containing rTF (1pM), «MP-reagent», containing negatively charged phospholipids (4μM) with CTI (40 μ/ml). Endogenous thrombin potential (ETP, nM*min) and peak thrombin (Peak, nM) were evaluated. STATISTICA 12.0 package was used in data analysis. The results are presented in the table as median with 95% confidence intervals, p< 0.05 was considered statistically significant (*).

Results: Significant differences were found when were used «PRP-reagent» allowing to estimate thrombin generation which depends on PL. ETP and Peak thrombin were lower in the group of patients on specific therapy. «MP-Reagent» allows to evaluate thrombin generation caused by TF. ETP and Peak had tendency to decrease in the group 1 compared the group 2 when «MP-Reagent» was used (p=0.26 and p=0.36, respectively).

TABLE 1 Thrombogram parameters in the groups of patients (Me, 95% CI)

Parameters	«PRP-Reagent» Group1	«PRP-Reagent» Group2	«MP-Reagent» Group1	«MP-Reagent» Group2
ETP (nM·min)	304.9* (102.7-1176.1)	557.0 (200.9-1306.9)	631.0 (305.0-1694.5)	968.2 (230.8-1881.5)
Peak (nM)	12.1* (2.9-66.92)	25.75 (8.1-59.54)	62.3 (19.0-300.2)	104.67 (17.6-336.39)

Conclusions: Specific therapy in patients with PMF leads to reduce of microparticle-associated thrombin generation. It can be associated with decline prothrombogenic potential of blood plasma and make significance for less development of thrombotic complications under specific therapy in patients with PMF.

PB0368 | Adhesive Properties of Plasma-Circulating and Platelet-Derived Microvesicles

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Background: Microvesicles produced by platelets upon activation or circulating in plasma are known to have high procoagulant activity, but their adhesive properties remain poorly understood. High concentrations of circulating microvesicles are reported to be a thrombosis marker in patients with cardiovascular diseases; still, direct experimental evaluation of the contribution of microvesicles to thrombus growth is lacking.

Aims: Study of the adhesive properties of circulating and platelet-derived microvesicles and their contribution to thrombus growth.

Methods: Flow cytometry was used for detection of PAC-1 and fibrinogen binding with microvesicles. Confocal microscopy was used for evaluation of microvesical adhesion to fibrinogen and visualization of thrombus formation in parallel-plate flow chambers. The experiments with flow chambers were produced on heparinized blood at shear rate 1000 s^{-1}, while immobilized type I collagen was used as an activator. The isolated microvesicles were stained with annexin V-Alexa Fluor 647, added to blood and the blood was perfused through flow chamber.

Results: No specific binding of PAC-1 antibody to the active form of integrin $\alpha_{IIb}\beta_3$ to the circulating and platelet-derived microvesicles was detected, and binding of fibrinogen was very low. The ability of microvesicles to adhere to immobilized fibrinogen was comparable with the non-specific ability of microvesicles to adhere to BSA and was lower than their specific ability to adhere to Monafram (F(ab)2 fragments of anti-integrin $\alpha_{IIb}\beta_3$ monoclonal antibody CRC64). We did not observe the signficant difference in the thrombus growth between two studied groups of thrombi as with or without circulating or platelet-derived microvesicles. The possibility of binding of microvesicles to a growing thrombus under flow conditions was studied, and we did not observe the circulating and platelet-derived microvesicles in the growing thrombi.

Conclusions: Our results suggest that the circulating and platelet-derived microvesicles are not involved in adhesion to a growing thrombus and not affect the thrombus growth.

PB0369 | Granulocyte Microvesicles with a High Plasmin Generation Capacity Promote Clot Lysis and Improve Outcome in Septic Shock

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Background: Microvesicles (MVs) have previously been shown to exert profibrinolytic activity, which is increased in patients with septic shock (SS) with a favorable outcome.

Aims: We therefore hypothesized that the plasmin generation capacity (PGC) could confer to MVs a protective effect supported by their capacity to lyse a thrombus, and we investigated the mechanisms involved.

Methods: MVs were characterized using a MV-PGC kinetic assay, ELISA, fibrin zymography and flow cytometry. Their lytic effect was investigated using *in vitro* lysis tests 1/ a fluorescent clot lysis test and 2/ a lysis front retraction assay by fluorescent microscopy. To test whether the fibrinolytic potential of MVs is protective *in vivo*, a murin model of SS was used.

Finally, to investigate whether factors differentially expressed in SS patients alter the MV-PGC, granulocytes from healthy subjects were incubated with SS patient plasma displaying low or high PGC levels which was screened by multiplex array.

Results: We found that granulocyte MVs (Gran-MVs) from SS patients display a heterogeneous PGC profile driven by the uPA/uPAR system. *In vitro*, these MVs lyse a thrombus according to their MV-PGC levels in a uPA/uPAR-dependent manner. Fibrinolytic activators conveyed by MVs contribute to approximately 30% of the plasma plasminogenolytic activity of SS patients. *In vivo* compared to the injection of low PGC Gran-MVs the injection of high PGC Gran-MVs significantly improved mouse survival and reduced the number of thrombi in vital organs. Using a multiplex array on SS plasma, we found that neutrophil elastase correlates with the effect of high-PGC-activity plasma and modulates the Gran-MV plasmin generation activity by cleaving uPA-PAI-1 complexes.

Conclusions: In conclusion, we show for the first time that high PGC level displayed by Gran-MVs reduce thrombus formation and improve survival conferring to Gran-MVs a protective role in a murine model of sepsis.

PB0370 | Plasma Platelets Microparticles as a Biomarker of Thrombotic Event in Thrombotic Thrombocytopenic Purpura Patients

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Background: Last decade, cell-derived microparticles plasma level has been used as a potential biomarker in several diseases. these particles play an important role in the patient's clinical condition and were related to various events such as thrombosis and inflammation.
Aims: Present study assessed the platelet microparticles (PMPs) role as a biomarker of thrombotic event in Thrombotic Thrombocytopenic Purpura (TTP) patients.
Methods: This is a cross-sectional study was conducted in TTP patients refer to all hospitals at Shiraz, Iran, from Jun 2015-Jun 2019. This study was approved by medical ethics committee of Shiraz University of Medical Sciences. We evaluate TTP patients at before plasma exchange (acute phase), after plasma exchange and remission phase. Microparticles were prepared by centrifugation and the size determining of PMPs was done via 'Dynamic Light Scattering (DLS)' Zetasizer. Platelet microparticles count, perform by Flowcytometery and for PMPs activity assay we used ZYMUPHEN MP-Activity kit. Data were analyzed by SPSS software v.21 and P-values less than 0.05 was considered statistically significant.
Results: Evaluation of PMPs level in TTP patients at 3 clinical phases revealed that PMPs count in TTP patients at before plasma exchange was higher than after plasma exchange and remission phase (P-value< 0.001) and also higher than normal healthy individuals (chart1), furthermore PMPs activity assay showed that PMPs activity in TTP patients was higher than normal individuals (table 1).
Conclusions: It seem that elevated PMPs in TTP patients could be related to patient's condition and thrombotic events, then PMPs level in TTP patients could be apply as a prognostic biomarker of patient's thrombosis. Nevertheless, more studies are needed to confirm these results.

PB0371 | Effects of Chemotherapy on Extracellular Vesicles and Coagulation Activation in Advanced Colorectal Cancer Patients

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Background: Cancer causes a hypercoagulable state and thus a high risk for thrombosis. Extracellular vesicles (EV) are elevated in colorectal cancer (CRC) patients and affect coagulation activation by exposing tissue factor (TF) and phospholipids.
Aims: We aimed to evaluate the long-term effects of chemotherapy on number and origin of EV and coagulation activation.
Methods: We included patients with advanced CRC receiving 5-fluorouracilbased chemotherapy. The number of EV was assessed by flow cytometry in fresh platelet poor plasma obtained immediately before chemotherapy. EV were defined by size (forward scatter, < 1 μm) and annexin V binding and labeled using antibodies to

TABLE 1 Number of extracellular vesicles (EV), levels of D-dimer, peak thrombin, and CEA in colorectal cancer patients before and during chemotherapy

	Before 1st ChTx	Before 2nd ChTx	p-value 1st vs 2nd	Before 3rd ChTx	p-value 1st vs 3rd	Before 4th ChTx	p-value 1st vs 4th	Before 5th ChTx	p-value 1st vs 5th
n	51	50		48		35		29	
EV (x 10^3 mL^{-1})	372 (277; 617)	348 (220; 520)	0.12	270 (184; 486)	0.04	318 (207; 435)	0.16	378 (204; 583)	0.48
PLT^+EV (x 10^3 mL^{-1})	175 (126; 351)	172 (100; 248)	0.02	125 (74; 208)	0.002	143 (80; 210)	0.03	106 (77; 254)	0.04
Proportion (%) of EV	47	49		46		45		28	
TF^+EV (x 10^3 mL^{-1})	14 (8; 35)	15 (9; 31)	0.7	11 (7; 26)	0.06	12 (9; 26)	0.05	13 (8; 23)	0.2
Proportion (%) of EV	4	4		4		4		3	
D-Dimer (μg mL^{-1})	1.07 (0.54; 2.73)	0.96 (0.56; 2.65)	0.9	1.03 (0.52; 1.98)	0.6	0.86 (0.49; 2.20)	0.1	0.77 (0.59; 1.49)	0.3
Peak thrombin (nM)	152.8 (73.7; 208.1)	180.5 (19.1; 239.6)	0.023	181.1 (135.3; 217.1)	0.011	172.2 (139.2; 214.35)	0.21	177.7 (118.3; 263.7)	0.16
CEA (μg L^{-1})	12.5 (2.9; 95.1)	11.1 (3.9; 70.6)	0.09	8.3 (2.4; 52.1)	0.11	9.3 (3.3; 24.7)	0.09	5.9 (3.1; 20.1)	0.09

categorize EV origin (anti-CD41a: platelet EV [PLT$^+$EV]; anti-CD142: TF EV [TF$^+$EV]). Additionally, D-Dimer, peak thrombin and CEA was determined. The paired *t*-test was used to compare baseline levels with levels obtained before chemotherapy. Data are given in absolute numbers (median [quartiles]) if not stated otherwise.

Results: 51 patients (mean age 63 years, 76% men) were enrolled. Table 1 shows the number of EV, EV sub-species, levels of D-dimer, peak thrombin and CEA at baseline and before 2nd, 3rd, 4th, and 5thchemotherapy, respectively. EV significantly decreased from 372 (277; 617)x10^3 mL^{-1}at baseline to 270 (184; 486)x10^3 mL^{-1}before the 3rdcycle. PLT$^+$EV significantly decreased from 175 (126; 351)x10^3 mL^{-1} at baseline to 125 (74; 208)x10^3 mL^{-1}before the 3rdcycle. The proportion of PLT$^+$EV was about 50% throughout chemotherapy. Number and proportion of TF$^+$EV were small at all time points. D-dimer levels were 1.07 (0.54; 2.73) µg mL^{-1} at baseline and remained stable. Peak thrombin was higher at each timepoint when compared to baseline levels. CEA decreased throughout chemotherapy.

Conclusions: In patients with advanced CRC, chemotherapy attenuates EV generation and affects coagulation activation as indicated by an increase of peak thrombin levels.

PB0372 | Plasma Levels of Platelet-derived Microvesicles Are Associated with Future Risk of Venous Thromboembolism

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Background: Microvesicles (MVs) are small double-membrane encapsulated particles shed from activated cells. High expression levels of negatively charged phospholipids, and in particular phosphatidylserine (PS), contribute to the procoagulant properties of microvesicles. Several case-control studies have shown elevated plasma levels of platelets-derived MVs (PDMVs) in patients with venous thromboembolism (VTE) compared to control subjects. As PDMVs is an acute phase reactant, it is not known whether PDMVs are a risk factor or merely a consequence of the acute VTE event.

Aims: To investigate the association between plasma levels of PDMVs and future risk of incident VTE.

Methods: A nested case-control study including 264 VTE cases and 601 randomly selected age- and sex-matched controls was derived from a population-based cohort (the Tromsø study). Plasma samples were collected at baseline and MVs were isolated by ultracentrifugation, stained for expression of PS and CD41a/isotype-control, and further analyzed by flow cytometry. Odds ratio (OR) with 95% confidence interval (CI) were calculated for VTE risk across quartiles of the proportion of PDMPs in control subjects.

Results: The plasma concentration of PS-positive MVs increased with increasing quartiles of PDMVs, as well as risk of incident VTE. Participants with PDMVs in the highest quartile had an OR of 1.61 (95% CI 1.07-2.44) for VTE compared to those in the lowest quartile. The OR was unchanged after adjustment for age, sex, body mass index, and CRP. The effect was most apparent for unprovoked deep vein thrombosis (DVT) (OR 2.35, 95% CI 0.92-6.78). A substantially higher risk for VTE was observed in samples taken shortly before the VTE event.

Conclusions: High levels of PDMPs were associated with future risk of incident VTE, suggesting that high plasma levels of PDMVs are not only a consequence of an acute thrombotic event, but also a risk marker of future VTE.

PB0375 | Expression of Circulating Microparticles Procoagulant Activity in Cirrhotic Patients

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Background: Cirrhosis is frequently associated to thromboembolic events particularly in portal vein. Moreover microparticles are considered as prothrombotic state biomarkers in many clinical diseases. Several studies have evaluated the expression of prothrombotic microparticles in cirrhotic patients but there is not enough data regarding their prothrombotic potential.

Aims: To evaluate the procoagulant activity expressed by circulating microparticles in cirrhotic patients and to correlate this activity with clinical and biological status.

Methods: A comparative case-control study was conducted in 32 cirrhotic patients and 14 controls. Laboratory tests included levels of both FVIII and PC and thrombin generation assay. Thrombin generation test (TGT) in platelet poor plasma was performed using 3 reagents from STAGO: PPP reagent low (R1), PRP reagent (R2) and MP reagent (R3). TGT parameters analyzed were: lag time, time to peak, Peak, endogenous thrombin potential (ETP) and velocity index. The ratio FVIII/PC was calculated to evaluate the hemostatic balance. TGT results were expressed originally as ratios: R2/R1 and R3/R1 to assess respectively the contribution of endogenous phospholipids and tissue factor.

Results: The mean age was 65.3 years old [35 and 90] and the sex ratio was 1. Patients were classified using CHILD-PUGH score as following: CHILD A (15 patients), CHILD B (10 patients) and CHILD C (7 patients). FVIII/PC ratio was significantly higher in cirrhotic patients than in controls (4.6 vs 1.14; p< 0.001). The ratio ETP (R3/R1) was significantly increased in controls compared to patients (1 vs 0.95; p=0.038). Whereas, ETP (R2/R1) was not significantly different in the two groups (1.14 vs 1.02; p=0.262). The other TGT parameters were also not significantly different in both groups. The ratio FVIII/PC was not significantly correlated to ETP ratios.

Conclusions: Although hemostatic balance evaluated with the ratio FVIII/PC reflected hypercoagulable state, micropaticle procoagulant activity assessed by TGT was not increased in cirrhotic patients.

PB0376 | Characterisation of Plasma Microvesicles in Type 2 Diabetes mellitus Patients with and without Microalbuminuria

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Background: Microvesicles (MVs) are membrane vesicles released into the bloodstream by vascular and blood cells upon injury. They initiate cell-to-cell communication in response to physiological or pathological signalling like cell activation, stimulation, inflammation and apoptosis. Abundant evidence suggests alteration in MVs in cardiovascular diseases. Less is known about the value of plasma MV analysis as diagnostic and prognostic tool in diabetes patients.

Aims: To characterise number, size and cellular origin of plasma MVs in diabetes patients with or without renal complications, as characterised by microalbuminuria (Albumin/Creatinine ratio ACR>2.5mg/mmol in males and ACR>3.5mg/mmol in females) and normoalbuminuria.

Methods: 20 patients (55-70 years) diagnosed with type 2 diabetes mellitus and 10 healthy controls were recruited from the Beat-DKD and VIBE studies (CI: Dr Kim Gooding, REC Ref: 17/SW/0180). The MV fraction was isolated by using the ultracentrifugation protocol as described. MVs were measured for their concentration and size using Nanoparticle Tracking analysis software: Nanosight (NS-300). Immunoblotting was used to characterise MV origin (platelet-specific marker= CD41; endothelial-specific marker=CD144).

Results: Type 2 diabetes patients with microalbuminuria showed significantly higher MV concentration (1.1x109 ± 0.6x109 ml^{-1} vs 0.2x109 ± 0.1x109 ml^{-1}, mean ±SEM, p=0.0031) and mean diameter (202 ± 38 nm vs 141 ± 57 nm, mean ±SEM, p=0.04) when compared with healthy controls (Figure 1). However, type II diabetes patients with normoalbuminuria did not show any significant difference for MV concentration and size when compared with controls (0.4x109 ± 0.2x109 ml^{-1} and 181 ± 25 respectively, p >0.05 compared to healthy controls).

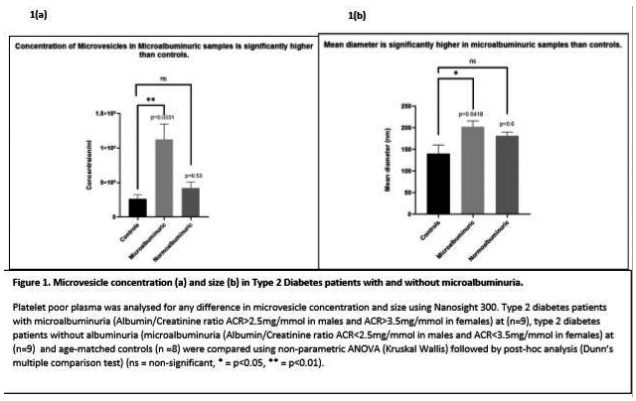

Figure 1. Microvesicle concentration (a) and size (b) in Type 2 Diabetes patients with and without microalbuminuria.

Platelet poor plasma was analysed for any difference in microvesicle concentration and size using Nanosight 300. Type 2 diabetes patients with microalbuminuria (Albumin/Creatinine ratio ACR>2.5mg/mmol in males and ACR>3.5mg/mmol in females) at (n=9), type 2 diabetes patients without albuminuria (microalbuminuria (Albumin/Creatinine ratio ACR<2.5mg/mmol in males and ACR<3.5mg/mmol in females) at (n=9) and age-matched controls (n =8) were compared using non-parametric ANOVA (Kruskal Wallis) followed by post-hoc analysis (Dunn's multiple comparison test) (ns = non-significant, * = p<0.05, ** = p<0.01).

FIGURE 1 Microvesicle concentration and size in Type II diabetes patients with and without microalbuminuria and healthy age-matched controls

Conclusions: Patients with type 2 diabetes and microalbuminuria displayed significantly higher plasma MV counts and size compared to healthy controls. Our future work will focus on the potential association between changes in plasma MV parameters and cardiovascular health in diabetic patients.

PB0377 | Procoagulant Microparticles Improve Hemostasis in Mice with Haemophilia A

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Background: Circulating microparticles (MPs) express phosphatidylserine, which provides a binding site for coagulation factors and cofactors. We have previously found that MPs improve thrombin generation and fibrin formation in hemophilia A (HA) plasma models *in vitro*.

Aims: To test the procoagulant effect of MPs in HA mice *in vivo*.

Methods: MPs isolated from pooled normal human plasma, either alone (3.8×10^4 MPs/g mice) or with recombinant FVIII (rFVIII, 5 or 25 IU/kg), were administered into HA mice intravenously. Following administration, a saphenous vein bleeding model was performed, and the bleeding parameters were recorded. After that, mice blood was collected, and tested using the whole blood thrombin generation and the overall hemostatic potential assays. The fibrin clot structure was analysed using scanning electron microscopy.

Results: The average bleeding time of HA mice were significantly shortened in mice treated with MPs compared to saline, while the number of hemostatic events tended to be higher. The bleeding phenotype was improved as expected after administration of rFVIII, however, the addition of MPs did not induce any further improvement. The thrombin generation, the overall coagulation potential and fibrin clot density were not improved after administration of MPs either alone or together with rFVIII.

Conclusions: It seems that administration of MPs improve the bleeding phenotype in HA mice, but does not enhance the procoagulant effect of rFVIII, suggesting that their mechanism of action may be different *in vivo*. Additionally, MPs cannot completely compensate for the FVIII deficiency. Mice treated with MPs did not show any improvement of the *ex-vivo* global hemostasis, suggesting that MPs might be either removed from the circulation *in vivo* or consumed due to incorporation during the formation of hemostatic plugs.

PROTEIN C PATHWAY

PB0379 | The Diagnostic and Prognostic Role of Protein C and Padui Scale for Prediction of Lung Vessel Thrombosis in Patients with Community-Acquired Pneumonia

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Background: There are limited data about lung vessel thrombosis in patients with community-acquired pneumonia (CAP). Moreover, there are no enough data about prognostic factors of this event.
Aims: To evaluate the diagnostic value of serum levels of protein C (PC) and Padui score as predictors of lung vessel thrombosis in patients with CAP.
Methods: This was a prospective observational study in 75 adults (80% men) with moderate (n=41) to severe (n=34) CAP. determination of biomarkers PC were performed at admission before starting of antibacterial treatment. Calculation scores by Padui scale. Statistic included ROC-analysis.
Results: We allocated 12 patients with lung vessel thrombosis (in 7 patients - on chest CT and in 5 patients - on autopsy).

TABLE 1 The result of ROC-analysis

Variable	AUC	95% CI	P
Padui scale	0.717	0.553 to 0.848	0.014
PC	0.836	0.685 to 0.934	0.001

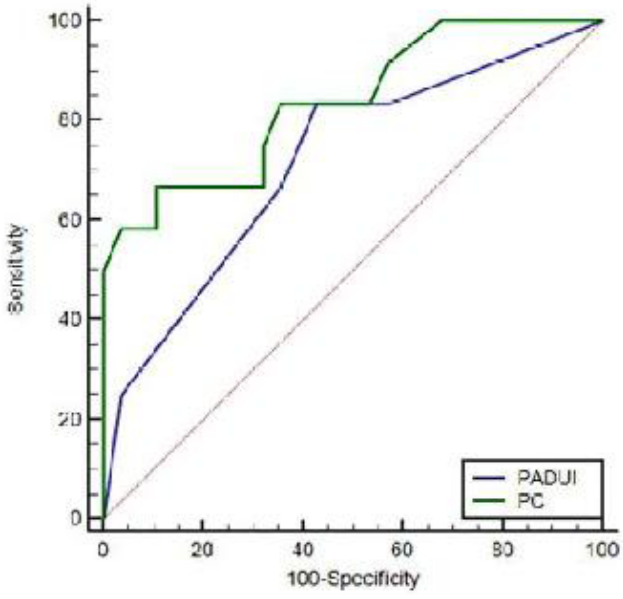

FIGURE 1 ROC analysis

Conclusions: Serum level of PC is independent and the more specific (89.3%) and sensitive (66.7%) biomarker for estimation of lung thrombosis in hospitalized patients with CAP. As for Padui scale specific is 83.3% and sensitive - 57.1%. Therefore, serum PC and Padui scale could be used for prediction of lung vessel thrombosis in patients with community-acquired pneumonia.

PB0380 | The Effect of Protein C on Various Links of Hemostasis

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Background: Protein C is a major coagulation inhibitor. Its active form binds and deactivates coagulation factors, thereby regulating the formation of a blood clot, slowing it down. In the bloodstream, it is inactive, activated on the surface of platelets and endothelial cells under the influence of thrombomodulin and thrombin.
Aims: Evaluation of the role of protein C in various links of hemostasis in the pathogenesis of thrombophilia in chronic leukemia and in hypocoagulation states.
Methods: The study included 120 patients. Of these, 30 patients with multiple myeloma (MM) and 30 with aplastic anemia (AA), 30 patients with chronic myelogenous leukemia (CML) and 30 with idiopathic thrombocytopenic purpura (ITP). The control group consisted of 20 healthy donors of comparable age without pathology of the hemostatic system.
Results: Diagnosis of disorders in the hemostatic system is extremely important, as it will allow you to prescribe antithrombotic therapy on time or to carry out the correction of an ongoing anticoagulation treatment. Thus, the most informative method of laboratory screening diagnosis of a prethrombotic condition has been established: a generally evaluating test of the protein C system.
The table below shows that the pro-inflammatory proteins we are studying clearly indicate the presence of multidirectional disorders. Marked changes in the hemostatic system in the study of chronic leukemia were characterized by hypercoagulation, thrombinemia, vascular-platelet disorders, which were exacerbated by a decrease in the activity of protein C.
Conclusions: In the studied groups with chronic leukemia, there is a decrease in the activity of protein C, but in the groups with

TABLE 1 Protein C indices in patients with hemostasis pathology

	Protein C (70-140%)	P
multiple myeloma (n=30)	68±6.6	≤ 0.5
CML (n=30)	66±8.3	≤ 0.5
AA (n=30)	160±8.1	≤ 0.05
ITP (n=30)	157±1.1	≤ 0.05
control group (n=20)	108±4.6	

hypocoagulation this indicator was equal to normal, which means that the bleeding syndrome in these patients is not associated with the activity in the blood of Protein C, but is associated with other disorders in the coagulation unit hemostasis.

PB0381 | Activated Protein-C up Regulates Microparticles Release from Ovarian Cancer Cell Line

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Background: The role of microparticles (MPs) in ovarian cancer and their involvement in thrombosis is poorly understood.

Aims: To identify and characterize *in vitro* the MPs generated by a human ovarian cell line and investigate the impact of activated protein C (APC) on their generation as well as their procoagulant activity.

Methods: The ovarian adenocarcinoma cell line (NIH-OVCAR-3) is cultured in three conditions [without stimulation, after stimulation either with protein C (PC), or with activated protein C]. Then, the microparticles generated in the supernatant, were isolated by ultracentrifugation. They were analyzed for their shape and property by several methods such as flow cytometry, electron microscopy, cryofracture analysis, DNA and RNA extraction and proteomic analysis. The level of TF on MP was evaluated by TF-induced shortening of $Ca2^{+}$ plasma coagulation time.

Results: Our results demonstrated that 1) OVCAR-3 cells produced heterogeneous MP size 91.92%, measured less than 100nm. 2) As tested by flow cytometry, the MPs contained TF, b2 microglobulin, and annexin V. 3) MPs were generated in greater amount when cells were incubated for 18 H with APC. 4) proteomic analysis show that the MPs contain several proteins involved in cancer progression. 5) by nucleotide extraction, they contained DNA fragments, 6) cytometry analysis showed that MPs have at their surface tissue factor, which induces a shortening of Ca^{2+} -induced plasma coagulation time. When MPs are derived from stimulated cells by APC, their level of TF is increased by 20%. Curiously, in contrast with the OVCAR-3 cells, the endothelial protein C receptor was not detected in MPs using specific antibodies in flow cytometry.

Conclusions: The MPs expressed procoagulant TF activity that is increased by APC.

PB0382 | A Single-Domain Antibody that Exerts an Anticoagulant Effect Dependent on Protein S

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Background: Protein S (ProS) is a natural anticoagulant acting as a cofactor for activated protein C (APC) and tissue-factor pathway inhibitor (TFPI). An immune library of single-domain antibodies (sdAb) generated from a llama immunized with recombinant human ProS (rhProS) was screened by phage display, allowing us to identify a strong ProS binder (PS003) after two rounds of biopanning on immobilized rhProS.

Aims: The sdAb PS003 was further characterized *in vitro* and *in vivo*.

Methods: Monovalent (PS003) and bivalent (PS003biv) forms of PS003 were produced in T7 SHuffle *E. coli* and purified. APC-cofactor activity of ProS was investigated in a commercial plasma clotting assay. The cofactor activity of ProS on TFPI-mediated FXa inhibition was evaluated in a purified system. The *in vivo* effects of sdAbs were evaluated in a $FeCl_3$-induced thrombosis mouse model on mesenteric vessels.

Results: In ELISA, PS003 was found to bind to rhProS and plasma-derived human ProS and not to other vitamin K-dependent proteins, including Gas6. Very unexpectedly, PS003 potentiated the APC-cofactor activity of ProS in a plasma clotting assay on ProS-deficient plasma supplemented with ProS, while having minor effects in the absence of ProS. To increase the potency of PS003, we generated a bivalent form of PS003 (PS003biv) that bound to immobilized rhProS with a 2-fold higher affinity than PS003 and again stimulated the APC-cofactor activity of rhProS. Neither PS003 nor PS003biv had any effect in the TFPI-cofactor activity of ProS.

PS003biv bound to recombinant murine ProS in ELISA and prolonged the occlusion times in mesenteric vessels, especially venules, in a murine thrombosis model, with thrombi that were very unstable and highly prone to embolization.

Conclusions: These results suggested that PS003biv, and possibly PS003, might exert antithrombotic effects *in vivo* through ProS-dependent anticoagulant mechanisms that need to be clarified.

PB0383 | Role of Endothelial Protein C Receptor Gene Polymorphisms (4678G>C and 6936A>G) in Young Indian DVT Patients

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Background: Endothelial Protein C Receptor (EPCR) augments PC activation via binding with thrombin-thrombomodulin complex. EPCR polymorphisms have been reported to influence the risk of DVT whereas their association with Indian DVT patient is not yet studied.

Aims: To identify the prevalence of EPCR gene polymorphisms (4678G>C and 6936A>G) and their role in young Indian DVT patients.

Methods: 75 DVT patients (M:36, F:39), median age 35 years range (20-45 years) and 75 age and sex matched healthy controls were studied. EPCR gene polymorphisms were detected by allele specific PCR.

Results: DVT was seen in lower limb in 65 (86%) patients and upper limb in 10 (14%) patients.

The variant allele C for 4678G>C was significantly higher in controls than patients (Patients: C=6%, Control: C=12.6%, p=0.047) but frequency of variant genotypes (GC, CC) were not significantly increased. (Patients:GC=20%,CC=2.6%,Controls:GC=9.3%, CC=1.3%. p=0.143, $\chi 2$=3.89). The allele frequency of variant allele G for 6936A>G polymorphism was significantly higher in patients than controls (Patient: G=16%, Control: G=6%, p=0.011, $\chi 2$=6.50). Genotypic frequency of AG and GG genotype was also significantly increased (Patients:AG=24%, GG=4%, Controls:AG=10.6%, GG=1.3%, p=0.040, $\chi 2$=6.04). Subjects carrying AG and GG genotypes had an increased risk of DVT (AG: OR: 2.55, CI: (1.11-6.62), GG: OR: 3.26, (CI: 0.680-32.46).

Conclusions: Protective role of 4678G>C polymorphism was identified which is similar to the results obtained from west, where as 6936G>A polymorphism has a causative role in the development of DVT. Therefore, EPCR 693A>G polymorphism detection may help in identifying young Indians having high risk of DVT.

PB0384 | Correlation between Protein C and CCL18 Levels in Multiple Sclerosis

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Background: Several studies suggest the crosstalk of coagulation with inflammation and immunity in the pathogenesis of multiple sclerosis (MS). Concentration of hemostasis inhibitors, which are known to possess anti-inflammatory properties, could influence this network and be associated with neurodegenerative processes.

Aims: To estimate plasma levels, and level correlations of hemostasis inhibitors, inflammatory/immunomodulatory- and adhesion molecules in relation to MS disease.

Methods: 138 MS patients and 42 healthy individuals (HI) were assessed with 3T MRI. Plasma levels of coagulation inhibitors (PC, TM, EPCR, PS, TFPI, HCII, ADAMTS13, PAI1), of chemokines (CCL5, CCL18), and soluble adhesion molecules (sNCAM, sICAM1, sVCAM1, sVAP1), assayed by Multiplex and ELISA, were analyzed by partial correlation. Association of protein levels with MRI outcomes was performed by regression analysis.

Results: In both MS patients and HI, levels of PC-CCL18 (r=0.31, p< 0.001; r=0.46, p=0.004), PC-PAI1 (r=0.27, p=0.002; r=0.41, p=0.009), PC-TFPI (r=0.21, p=0.014; r=0.46, p=0.003), and PAI1-CCL5 (r=0.55, p< 0.001; r=0.56, p< 0.001) were correlated.

Only in HI, levels of PC-PS (r=0.43, p=0.005), PS-PAI1 (r=0.41, p=0.008), PS-VAP1 (r=0.41, p=0.009) and PS-sVCAM1 (r=0.45, p=0.004) were correlated.

Only in MS patients, levels of TM were positively correlated with adhesion molecules, particularly with sICAM1 (r=0.31, p< 0.001), suggesting release from membranes caused by endothelial "injury"/alteration.

Increased PC levels were associated with decreased volumes of brain parenchyma (p=0.026), total gray matter (GMV)(p=0.003), cortex (CV)(p=0.008), deep gray matter (DGMV)(p=0.009), thalamus (p=0.007). Increased CCL18 levels were also associated with decreased volumes of CV (p=0.01), DGMV (p=0.006), thalamus (p=0.007). Regression model including both PC and CCL18 showed

that PC explains greater variance of GM-related volumes (i.e DGMV, PC p=0.001, CCL18 p=0.052).

Conclusions: Several correlation differences suggest disease-associated dysregulation of PC pathway components in MS patients. Taking into account that CCL18 is involved in immune cell chemotaxis, the correlation of PC with CCL18, both associated with neurodegeneration, suggests a novel hemostasis-immune network, warranting further investigation.

PB0386 | Inter-Laboratory Variability of the Standardized ETP-Based APC Resistance Assay

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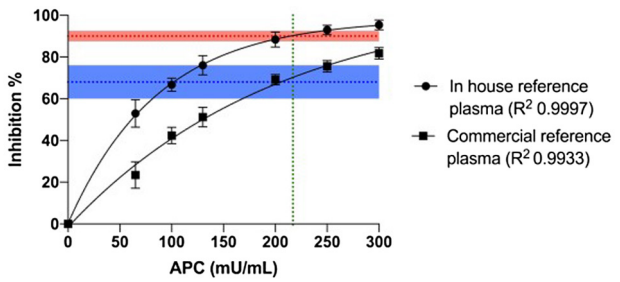
Background: Risk minimization measures that need to be implemented to minimize the thrombotic risk associated with the use of combined oral contraceptives (COC) is controversial. Despite the regulatory bodies recommend the assessment of the ETP-based activated protein C (APC) resistance during the development of steroid contraceptives; results are not comparable between studies due to lack of validation and standardization. To overcome this issue, Qualiblood recently validated and standardized this assay but wide implementation of this validated assay needs further investigations.

Aims: To assess the inter-laboratory variability of the validated ETP-based APC resistance assay.

Methods: Analyses were performed on three different Calibrated Automated Thrombogram (CAT) with Thrombinoscope software (version 5.0 for $CAT_{1,2}$; version 2.0 for CAT_3) by three different operators to assess the inter-laboratory variability. The method targets a 90% of inhibition of the ETP in healthy donors. Therefore, dose-response curves (n=3) were performed to define the amount of APC depending on the equipment used and the operator (n=3). A homemade and a commercial reference plasma were used. Quality controls (n=3) and a subset of real-life samples (n=11) were tested on each equipment. All measurements were performed in duplicate.



Curve with dots represents the mean dose-response curve (n=6) with in-house reference plasma; curve with square represents the mean dose-response curve (n=6) with commercial reference plasma. The dotted red line represents the reference range of in-house reference plasma (90±2.5%) and the blue dotted line represents the reference range of commercial reference plasma (68±8%). The green dotted line represents the defined concentration of APC (217 mU/mL) to fit the reference ranges.

FIGURE 1 Total variability between CAT1 and CAT2 (n=2 operators; n=3 dose-response curves/operator)

[APC]	In-house reference plasma			Commercial reference plasma		
	Inhibition %		p-value	Inhibition %		p-value
	CAT 1	CAT 2		CAT1	CAT2	
65 mU/mL	*26,69**	52,05	>0.9999	14,65	27,48	>0.9999
	*28,71**	56,13		17,18	31,35	
	44,24	59,57		25,53	24,7	
100 mU/mL	64,77	70,39	>0.9999	38,94	40,71	>0.9999
	62,63	64,34		37,51	47,3	
	69,72	68,21		44,32	45,7	
130 mU/mL	73,9	80,1	>0.9999	46,84	52,14	>0.9999
	68,15	76,11		44,37	56,42	
	80,23	77,84		52,77	54,82	
200 mU/mL	90,27	89,45	>0.9999	65,05	67,54	>0.9999
	87,92	81,62		68,86	71,88	
	92,09	88,72		71,27	69,99	
250 mU/mL	95,25	93,42	>0.9999	74,02	74,4	>0.9999
	91,58	89,48		72,16	78,83	
	95,47	92,47		79,06	74,66	
300 mU/mL	97,19	95,87	>0.9999	78,86	79,58	>0.9999
	96,13	*82,89**		83,06	85,8	
	96,55	95,08		83,69	79,92	
Defined concentration of APC (95%CI): 217 mU/mL [197 mU/mL-249 mU/mL]						

No statistically significant differences were observed between the two CATs (Kruskal-Wallis – Dunn's multiple comparison test; p-value >0.9999). Inhibition % in *blue** are outliers.

FIGURE 2 Inhibition percentage at 6 levels concentration of APC with in-house reference plasma and commercial reference plasma on CAT1 and CAT2

Results: Dose-response curves did not show any significant differences between CAT_1 and CAT_2 (Dunn's multiple comparison test; p-value>0.9999) (Figures 1-2), whatever the dose-response curves were performed with the in-house or the reference plasma. When comparing samples between CAT_1 and CAT_2 at the defined concentration of APC, the mean deviation was 7.83%. As the software version of the CAT_3 was older, some modifications must be performed to ensure the transferability of the methodology. This is due to important background noise which limits the performance of this older version of the CAT_3 at low ETP-values.

Conclusions: These results demonstrate the transferability of the validated ETP-based APC resistance test between CATs using the same software version.

PB0387 | Paradoxical Platelet Inhibitory Effect of Protein C Deficiency in Shear-dependent Thrombus and Fibrin Formation

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Background: In the protein C pathway, thrombin binds to thrombomodulin resulting in the activation of protein C (PC). Activated PC together with its cofactor protein S then inactivates coagulation factors Va and VIIIa. Patients with a PC deficiency have an increased risk of thrombosis.

Aims: Evaluation of platelet and coagulation responses of PC deficient patients in a whole blood flow perfusion assay.

Methods: Patients and corresponding control subjects were obtained from the screening program for familial thrombophilia at the University Hospital of Padua. Hematological parameters and von Willebrand factor (VWF) antigen levels were determined, as well as thrombin generation. Using GPVI-dependent microfluidics, recalcified whole blood was perfused at 1000 s^{-1} over microspots of collagen with(out) tissue factor (TF). Multicolor fluorescence microscopy was used to assess kinetics of platelet adhesion and activation, followed by fibrin formation.

Results: Platelet counts and VWF levels were all in the normal ranges. Whole blood perfusion revealed a remarkable decrease in platelet activation, aggregation, thrombus formation and contraction in 10 out of 14 PC deficient patients, in comparison to controls. In contrast, shear-dependent platelet adhesion was unaffected, suggesting an adhesion-independent platelet defect. In the presence of TF, platelet-dependent fibrin formation was reduced as well in the same 10 patients. In vitro inhibition of the protein C pathway did not lower the platelet parameters in control blood samples. Thrombin generation profiles in TF-triggered patients′ platelet-poor plasmas were normal.

Conclusions: Our findings collectively point to *in situ* platelet desensitization in a substantial fraction of patients with inherited protein C deficiency. The result is a partial impairment of thrombus and secondary fibrin formation in whole-blood flow. Such desensitization might be compensatory for the prothrombotic phenotype of the patients.

PB0388 | Clinical Presentation and Management of Homozygous Protein C Deficiency: Cukurova Experience

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Background: Protein C is a vitamin K-dependent coagulation protein that has a significant role in the regulation of thrombin. Homozygous protein C deficiency (HPDC) is a rare condition with a predicted prevalence of approximately 1:500,000 live births. The classical neonatal manifestation of homozygous protein C deficiency (HPCD) is a severe form of thrombosis of the large vessels, purpura fulminans and disseminated intravascular coagulation.

Aims: In this study, we aimed to report the clinical presentation, age at onset, genotype, and treatment modalities of 11 patients diagnosed with HPCD at Cukurova University, Adana, southern part of Turkey.

Methods: We retrospectively reviewed medical records of 11 patients with HPCD.

Results: Eleven patients with HPCD were included. six males and five females comprised the study population (n = 11). In all cases, purpura fulminans (PF) was typically seen in the neonatal periode with median day of 3 days (1-23 days). Fresh frozen plasma were given at a dose of 10-20 mL/kg every 6-12 h. Protein C concentrates were given in two patients. Anticoagulation therapy were initiated with administration of protein C replacement therapy (protein C concentrate or fresh frozen plasma) as long term secondary prophylaxis. Initial anticoagulation consists of either unfractionated heparin or low molecular weight heparin. As maintenance therapy, warfarine therapy was given. Blindness was seen in nine of eleven patients. The genetic study was carried out after obtaining an informed consent in seven patients. Four different PROC gene mutations were detected at these seven patients(c.1100T>C, c.658T>C, c.488C>A, c1361delC).

Conclusions: HPCD predisposes to reduce thrombin generation and a hypercoagulable. Laboratory and clinical findings, management of HPDC were limited due to its low prevalence. Increased evidence-based guidelines for the diagnosis and management of this patient population is required. In this study, we reported the cases and have encountered in management of patients with HPDC.

REGULATION OF COAGULATION

PB0389 | Thr90Ser Mutation in Antithrombin Causes Recurrent Thrombosis in a Heterozygous Carrier

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Background: Antithrombin (AT) is a principal serine protease inhibitor (serpin) in plasma that regulates the activities of the serine proteases of both intrinsic and extrinsic pathways of the blood coagulation cascade. We identified an AT-deficient patient with a heterozygous Thr90Ser (T90S) mutation in SERPINC1 who experience recurrent venous thrombosis.

Aims: The objective of this study was to determine the molecular basis of the clotting defect in the patient with T90S mutation.

Methods: We expressed AT-T90S in mammalian cells and characterized its properties in established coagulation assay systems.

Results: The inhibitory activity of the AT-T90S toward thrombin and factor Xa (FXa) were impaired in both the absence and presence of heparin. Kinetic analysis revealed that the stoichiometry of inhibition of both thrombin and factor Xa by AT-T90S was elevated 3-5-fold in both the absence and presence of heparin, suggesting that the reactivity of coagulation proteases with the mutant AT has been increased in the substrate pathway of the reaction. In a tissue factor-mediated thrombin generation assay, the anticoagulant activity of AT-T90S was significantly impaired with or without heparin as analyzed in the AT-deficient plasma. Furthermore, spectral analysis of heparin-mediated changes in the intrinsic tryptophan fluorescence of AT revealed that the affinity of heparin and pentasaccharide for AT-T90S has been impaired 4-5-fold.

Conclusions: AT-T90S mutation exhibits lower reactivity with coagulation proteases in the inhibitory pathway, but higher reactivity in the substrate pathway, thus responsible for the recurrent thrombosis in the patient.

PB0391 | The Effects of Acids on Whole Blood Coagulation is Depended on the Type of Acid and Concentration as Assessed by Thromboelastography (TEG)

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Background: Trauma is associated with coagulopathy explained on the basis of acidosis concurrently observed, due to findings of coagulation inhibition by dilute acids in *in vitro* studies. However, these studies have not taken into account the concentration and type of acids that have not been systematically investigated.

Aims: To compare the effects of HCL, lactic acid and sulphuric acids different acids at concentration ranges 0.1 M to 1.0 M on whole blood coagulation using Thromboelastography *in vitro*.

Methods: After ethical approval, blood was obtained from healthy human volunteers in citrate tubes and mixed in a ratio of 1:5 with HCL, sulfuric and lactic acids at concentration at 0.2 M. Thereafter, 320 µL of whole blood was added to 20 µL of calcium chloride in thromboelastography cups. Next coagulation effects were tested at concentration ranges of 0.1 M to 1.0 M. Tests were allowed to run for 60 minutes.

Results: In 0.2 M lactic acid TEG parameters were closer to control undiluted samples suggesting that it preserves coagulation (fig 1). However, with increase in acid concentration there was progressive inhibition of blood clotting in all the three acids. TEG flat trace in 0.3-0.4 M HCL, 0.3-0.6 M in lactic acid and only 0.2 M in Sulphuric acid. Paradoxically, coagulation started increasing in 0.3 M sulfuric acid, 0.5 M HCL, and 1.0 m in lactic acid. In 1.0 M HCL and sulphuric acid were hypercoagulable (Table 1). Surprisingly, significant lysis was observed in 0.2 M lactic acid, and sulphuric acid 0.3-1.0 M with concurrent diamond fibrinolytic pattern.

Conclusions: It appears that whole blood coagulation outcome is dependent on type of acid and particular concentration. Increase in acid concentration progressively inhibit coagulation most marked around 0.2-0.4 M. The study illustrates that acidic anions modulate coagulation independent of proton suggesting interaction with coagulation factors.

PB0392 | Neutrophil Extracellular Trap in Patients with Peritoneal Carcinomatosis of Several Origins

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Background: Neutrophil extracellular traps (NETs) are extracellular neutrophil DNA fibers that bind chromatin proteins (Histones H3 and 4) and granule proteins (myeloperoxidase and elastase) formed

TABLE 1 Mean Thromboelastography parameters in different acids at different concentrations

TEG parameters	Acid	Neat (control) N=7	0.1 M (N=7)	0.2 M (N=7)	0.3 M (N=7)	0.4 M (N=7)	0.5 M (N=7)	0.6 M (N=7)	1.0 M (N=2)
R (mm)	HCL Lactic acid H2SO4	14.36 12.70 14.69	14.01 11.90 61.2	37.64 31.10 ∞	∞ ∞ 4.029	∞ ∞ 2.729	24.67 ∞ 1.771	15.91 ∞ 1.243	2.829 22.95 0.9571
K (mm)	HCL Lactic acid H2SO4	6.786 4.700 6.314	5.057 4.100 8.557	16.16 8.500 ∞	∞ ∞ 2.643	∞ ∞ 1.171	10.97 ∞ 1.1	14.43 ∞ 1.343	4.85 25.00 1.5
alpha (degrees)	HCL Lactic acid H2SO4	33.91 38.70 31.47	36.59 41.30 1.557	10.34 11.00 0	0 0.0 57.91 0	0 0.0 73.87	4.843 0.0 74.76	16.71 0.0 76.54	41.95 6.700 77.1
Ma (mm)	HCL Lactic acid H2SO4	44.86 48.00 42.84	45.31 50.80 8.343	31.89 26.30 0	0 0.0 81.76	0 0.0 81.17	16.79 0.0 86.46	55.26 0.0 85.17	73 42.35 83.3
Lysis at 30 minutes (%)	HCL Lactic acid H2SO4	1.243 1.371 1.4	0.5286 0.2857 0.5143	0 13.90 0	0 0.0 50.34	0 0.0 9.329	1.429 0.0 52.86	0.4143 0.0 41.3	0 0.0 88.35

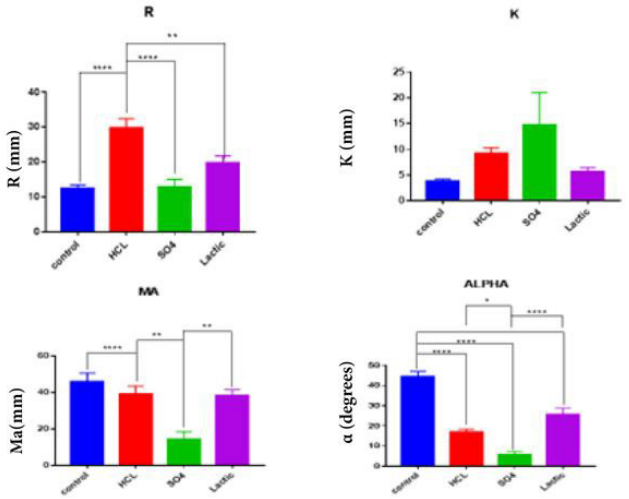

Figure 1Mean TEG parameters in whole blood mixed with 0.2 M of different acids.

FIGURE 1 Mean TEG parameters in whole blood mixed with 0.2 M of different acids

during neutrophil activation. It was established in the literature that tumors activate neutrophils to induce NETosis promoting metastatic progression and activation of coagulation. Extensive NET formation was evidenced in peritoneal colon cancer metastases in mice and patients and DNase administration in animal models markedly reduced the number of metastases in animals. In addition, NET is responsible of chemokine-dependent colon cancer cell migration and adhesion *in vitro*.

Aims: we were interested in evaluating before surgery different constituents of NETs in plasma of patients with peritoneal cancer of various origins.

Methods: Adult patients with peritoneal carcinomatosis were studied just before surgery. They were classified according to the origin of peritoneal carcinomatosis. All patients provided written or oral consent for participation in NET levels in accordance with local ethics committee requirements. dsDNA was measured using Quant-iT Picogreen dsDNA assay kit, (Thermo Fisher Scientific), Histones (H3 and H4) were evaluated by ELISA Kits (Clinisciences) and myeloperoxidase (MPO) was evaluated by ELISA kit (Biotechne).

NET in plasma was identified by confocal microscopy after staining by Picogreen.

Results: The different parameters of NETs were evaluated in the plasma of 35 patients with peritoneal carcinomatosis of several origins (peritoneal or ovarian n=10, mesothelioma n=3; pseudomyxoma n=6, colon n=12, oesophagus n=1 and stomach n=1). In almost all cases, H4, H3 and DNA levels were increased whereas that of MPO was increased in only 67% of cases. DNA filaments were found in plasma from cancer patients.

Conclusions: Many constituents of NETs being increased in the plasma of these patients whatever the origin of peritoneal carcimatosis, it should be interesting to analyze the evolution of the disease in these patients.

PB0393 | A Hereditary Bleeding Disorder Caused by a Novel Homozygous Mutation of Thrombomodulin Gene

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Background: A 19-year-old Vietnamese female presented with left ovarian hemorrhage. On admission, her blood investigation revealed thrombocytopenia and decreased fibrinogen with markedly elevated FDP and D-dimer levels, which suggested she was on disseminated intravascular coagulation (DIC). Despite hemostasis surgery or administration of nafamostat mesilate and tranexamic acid, her blood abnormalities were not improved and she repeated critical hemorrhagic events. We found that recombinant thrombomodulin (rTM) had a remarkable effect on her bleeding disorder, although her plasma levels of soluble TM fragments were always high even before rTM administration.

Aims: To understand abnormalities of her hemostasis and to solve the reason why rTM works.

Methods: A congenital or hereditary disease was suspected because she had also experienced several hemorrhagic events in her childhood. We screened mutation of the thrombomodulin (THBD) gene in her family members, because administration of rTM was effective on coagulation disorders.

Results: We found an unreported THBD mutation, c.793T>A (p.Cys265Ser), homozygously in the patient and heterozygously in her parents. Cys265 is one of the cysteine residues forming three disulfide bonds in the EGF-like domain 1 of thrombomodulin. Transient expression experiments using COS-1 cells demonstrated the significantly reduced expression of TM-Cys265Ser on the plasma membrane as compared with wild-type TM. The TM-Cys265Ser variant was degraded by endoplasmic reticulum associated-degradation (ERAD), probably because of its misfolding in the EGF-like domain 1. The reduced expression of TM on the endothelial cell surface may cause the pathogenesis of DIC-like symptoms of the patient.

Conclusions: We have identified a novel TM mutation c.793T>A (p.Cys265Ser). The patient who has the homozygous mutation may present severe bleeding events, and rTM administration seems to work well for this disorder.

PB0394 | Acute Deep Vein Thrombosis Is Associated with Increased Platelet Thrombin Generation and Increased Procoagulant Oxidised Phosphatidylethanolamine

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Background: Approximately one third of patients with acute deep vein thrombosis (DVT) have no risk factor identified and are labelled idiopathic. Previously we have shown that enzymatically oxidised phospholipids, 12-hydroxyeicosatetraenoic acid esterified to phosphatidylethanolamine (12- HETE-PE) or phosphatidylcholine (12-HETE-PC) generated rapidly by activated platelets, promote thrombin generation (TG) in vitro.

Aims: We investigated whether the thrombotic phenotype could be explained by the ability of platelets or microparticles (MP) to support thrombin generation (TG), mediated by phospholipids (PL) in the platelet membrane.

Methods: Washed platelets from healthy controls (HC) (n=27) or DVT patients (n=23) were isolated and activated with thrombin. TG assays on platelets resuspended in standardised platelet poor plasma were activated using liposomes containing tissue factor and phospholipid (0.5μM). 12-HETE-PL and externalised phosphatidylserine (PS) and phosphatidylethanolamine (PE) were measured by liquid chromatography mass spectrometry (LC-MS/MS).

Results: Peak TG on washed platelets was increased, median (IQR) 122 (97-151) for DVT vs 86 (68-121) nM, for HC p=0.008. Platelet-derived MPs supported similar TG in DVT and HC (Figure 1). There was no difference in externalised PS and PE in DVT patients compared to HC. Levels of three 12-HETE-PE species were significantly increased in the thrombotic cohort (P≤0.05) and a fourth was borderline elevated (P=0.06) (Figure 2). Levels of 12-HETE-PC were similar for DVT patients and HC indicating that this observation was specific for PE species.

Conclusions: In patients with acute DVT, an increased ability of platelets to support TG may in part explain the thrombotic phenotype. The increase in TG was associated with increased ability of activated platelets to generate procoagulant 12-HETE-PE, but not with 12-HETE-PC or externalised PS/PE. This suggests that the PL composition of the platelet membrane may be implicated in promoting thrombosis.

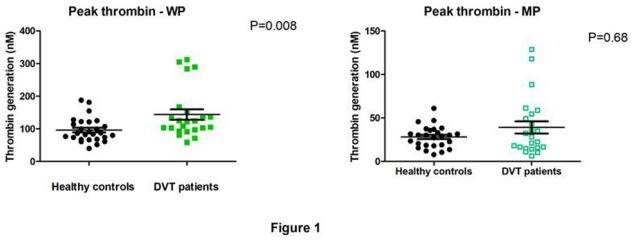

FIGURE 1 Peak thrombin generation in platelet and microparticles of healthy controls and DVT patients

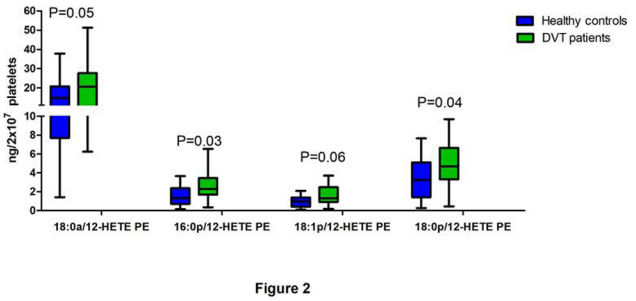

FIGURE 2 Generation of 12-HETE-PE species by activated platelets in healthy controls and DVT patients

Further studies are required to investigate whether increased amounts of TG and HETE-PE are causally associated with thromboses.

PB0396 | Heparin Affects the Induction of Regulatory T Cells Independent of Anticoagulant Activity and Suppresses the Allogeneic Immune Responses

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Background: Heparin, a widely used anticoagulant, which enhances the antithrombin (AT) activity. However, heparin also suppresses immune and inflammatory responses in various rodent models and in clinical trials, respectively. The mechanism by which heparin suppresses immune responses is unclear.

Aims: This research was aimed at clarifying the role of regulatory T cells (Tregs) in the immunosuppressive effects of heparin during the allogeneic immune responses.

Methods: The effect of heparin on Tregs in the allogeneic immune responses was analysed using an acute graft-versus-host disease (aGVHD) mouse model and mixed lymphocyte reactions (MLRs). *In vitro* culture systems were utilized to study the effects of heparin on Tregs.

Results: Heparin administration reduced mortality rates and increased the proportion of Tregs in the early post-transplantation period of aGVHD mice. In both murine and human MLRs, heparin increased Tregs and inhibited responder T cell proliferation. Heparin promoted functional $CD4^{+}CD25^{+}Foxp3^{+}$ Treg generation from naïve $CD4^{+}$ T cells, increased interleukin (IL)-2 production, and enhanced the activation of pre-existing Tregs with IL-2. Heparin-induced Treg increases were not associated with anticoagulant activity through AT but required negatively charged sulfation of heparin. Importantly, N-acetyl heparin, a chemically modified heparin without anticoagulant activity, induced Tregs and decreased mortality in aGVHD mice.

Conclusions: Our results indicate that heparin contributes to Treg-mediated immunosuppression through IL-2 production, suggesting that heparin derivatives may be useful for immunopathological control by efficient Treg induction.

PB0397 | Red Blood Cells Support Factor X Activation via Intrinsic Tenase

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Background: All major reactions of the coagulation cascade are membrane-dependent. There are different sources of negatively charged membranes such as activated platelets, fibroblasts, smooth muscle cells, endothelial cells and their microparticles. Was shown that RBC also can effect plasma coagulation by different ways but it's not considered which way is more important.

Aims: In this work was investigated the ability of RBCs to support generation of fXa by the intrinsic fX-activating complex and to bind the components of this complex.

Methods: RBCs were isolated from whole blood by washing. Written informed consent was given by all participants, and the study was approved by the institutional ethical committee. Flow cytometry were used to investigate interaction of FITC-labeled factors with the membranes of RBCs. The quantity of generated FXa was determined by chromogenic assay with specific substrate S-2765. For flow cytometry and chromogenic assay were used native RBCs and RBCs after osmotic shock.

Results: FX activation via instrinct tenase was studied in presence of either RBCs, or platelets. In both cases, the rate of the reaction was proportional to the concentration of added cells. The effect of RBCs in concentration 2x106 1/ul near 40% of normal haematocrite was similar to physiological concentration of activated platelets. Was shown PS-positive RBCs predominantly interacted with coagulation factors and increasing activation fX via intrinsic tenase.

Conclusions: We have shown that reaction of activation fX via intrinsic tenase is efficiently accelerated by RBCs.

This study was supported by the Russian Foundation for Basic Research grant 19-04-00615.

PB0398 | Inhibition of Protein Disulfide Isomerase (PDI) with PACMA-31 Amplifies LPS-Induced Expression of Tissue Factor (TF) in Monocytes

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Background: PDI mediates protein folding in the endoplasmic reticulum and contributes to vascular thrombosis through various mechanisms. The PDI antagonist quercetin-3-rutinoside, which reversibly binds to the b′ domain, has demonstrated antithrombotic activity, but the specific role of PDI in monocyte TF expression under inflammatory conditions is unclear.

Aims: To analyze the effect of PDI inhibition by PACMA-31, which tightly binds to the catalytic motifs of the a and a′ domains (IC_{50} 10 μM), on LPS-induced monocyte TF production.

Methods: Citrate-anticoagulated whole blood or isolated peripheral blood mononuclear cells (PBMCs) were incubated with lipopolysaccharide (LPS) in the presence of PACMA-31 or DMSO vehicle. Single-stage clotting and chromogenic Xa generation assays were used to assess TF-specific procoagulant activity (PCA) of cells and released microvesicles (MVs). TF antigen was quantified by ELISA and flow cytometry. Relative changes in TF mRNA were captured by qPCR.

Results: PACMA-31 alone did not increase monocyte TF in unstimulated whole blood. Co-incubation of whole blood with LPS and PACMA-31 (25 μM), however, significantly enhanced monocyte TF antigen, PCA and release of TF-bearing MVs when compared to LPS alone. PACMA-31 also significantly increased LPS-induced TF mRNA, but had no effect on phosphatidylserine membrane exposure. While PACMA-31 at 25 μM was cytotoxic in cultured PBMCs, resulting in complete inhibition of LPS-induced TF production, titration of the PACMA-31 concentration to 1 μM recapitulated the stimulating effect on TF antigen, PCA and mRNA.

Conclusions: While cytotoxic PACMA-31 concentrations abolish LPS-induced TF production in the PBMC model, lower concentrations amplify monocyte TF expression and release in response to LPS, an effect at least partially regulated on the mRNA level. Different concentration thresholds thus determine the cellular consequences of monocyte PDI inhibition by PACMA-31 under inflammatory conditions, with distribution of the PDI antagonist to other cells potentially explaining improved tolerability in the whole-blood model.

PB0399 | Correlation between ADAMTS13 and VAP1 Levels in Multiple Sclerosis with Cerebral Microbleeds

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Background: The progressive failure of blood-brain barrier (BBB) integrity, which may have the pathological feature of cerebral microbleeds (CMBs), leads to focal extravascular leakage of blood components and subsequently induces several inflammatory responses. CMBs have been associated with worsening of physical and cognitive disability in multiple sclerosis (MS). Adhesion molecules, which participate in BBB disruption and in inflammatory responses, could be involved in the formation of CMBs. The anti-inflammatory properties of hemostasis inhibitors offer a rational link for their study in the presence of CMBs.

Aims: To explore plasma levels, and correlations of hemostasis inhibitors and adhesion molecules in relation to CMBs in MS.

Methods: Plasma levels of coagulation inhibitors (PC, TM, EPCR, PS, TFPI, HCII, ADAMTS13, PAI1) and soluble adhesion molecules (sNCAM, sICAM1, sVCAM1, sVAP1), were evaluated by Multiplex and ELISA in 125 MS patients and 42 healthy individuals (HI) who underwent 3T MRI exams. CMBs were assessed on susceptibility-weighted minimum intensity projection images and susceptibility maps. Spearman's correlation and Mann-Whitney test were used, as appropriate.

Results: Patients with CMBs (n=12) showed, in addition to significantly lower ADAMTS13 levels (p=0.025), significantly higher sNCAM levels (p=0.017) and a trend for higher levels of VAP1 compared to MS patients without CMBs (n=113). Only in MS with CMBs, ADAMSTS13-VAP1 levels were correlated (r=0.67, p=0.020). In MS with CMBs and in HI, levels of PAI1-sVCAM1 were correlated (r=0.73, p=0.009 and r=0.33, p=0.031).

Conclusions: With the limitation of the low number of patients with CMBs, our findings suggest an intriguing molecular relationship between adhesion molecules and hemostasis inhibitors in development of CMBs in MS. The reactive oxygen species produced by VAP-1, an inflammatory adhesion molecule endowed with enzymatic properties, could interfere with ADAMTS13/von Willebrand factor function, a hypothesis that deserves further investigation.

PB0402 | Praxbind for Reversal of Dabigatran: Experience in a Tertiary Hospital

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Background: Praxbind is an antibody fragment that can effectively and safely reverses the anticoagulant effect of Dabigatran in bleeding patients as well as in patients facing urgent surgery. The 5 g dose used was planned to reverse the 99 percentile of dabigatran levels, however lower doses were also found to neutralize dabigatran effectively.

Aims: To evaluate the use of Praxbind according and the dose administered in bleeding and surgical candidate patients at our center.

Methods: A retrospective cohort of consecutive patients treated with Praxbind between 01/ 2016 to 12/2019 for major bleeding or before urgent invasive procedures. Praxbind dose ((5 gm - HD, 2.5 gm - LD) and the decision for repeated administration was at the discretion of the treating physician. In several patients dabigatran blood levels was take before after (30 min.) administration. Response to treatment was graded as adequate, fair or non-responding.

Results: Twenty six patients were included (13 males, median age 82 years). Dabigatran indication was atrial fibrillation in all patients. In most patients (19/26) Praxbind was administered at LD mainly to treat bleeding (11/19) while the 5g dose was used in 7 patients (3/7 bleeding). A single patient received repeated 5 g doses (15g). Median pre Praxbind blood levels were 104 ng/ml compared to 423 ng/ml (p=0.069) in LD vs. HD treated patients. Post administration median levels was 1 ng/ml in patients receiving LD compared to 0 ng/ml in a single HD patient with available data. In 3 patients response to Praxbind was graded as non-responding, one of which received repeated doses. Eight patients died (mainly infection, one patient died from GI bleeding). In 31% patients Dabigatran treatment was resumed pre-discharged.

Conclusions: In our study cohort low dose Praxbind seemed to be effective in achieving adequate hemostasis un bleeding and surgical patients. This should be confirmed in further patient cohorts.

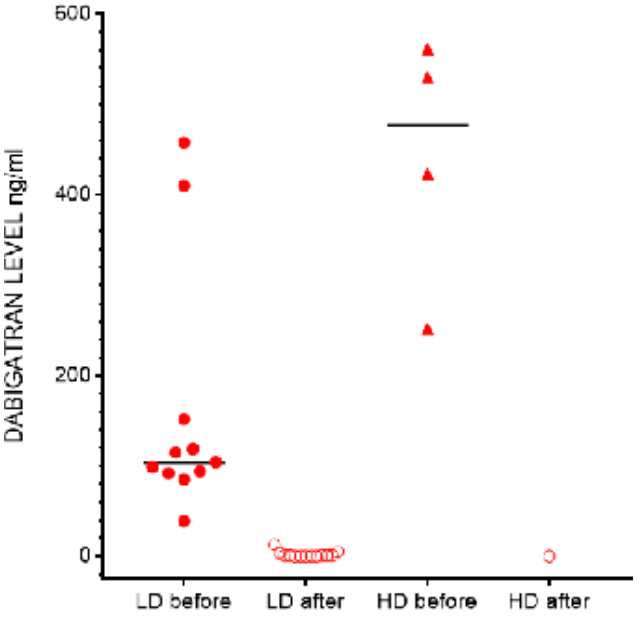

FIGURE 1 Dabigatran blood levels before and after Praxbind administration

PB0403 | Platelet Activation via GPVI Initiates FXII- and TF-independent Thrombin Generation

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Background: Collagen can trigger clot formation via activation of factor (F) XII and platelets to initiate coagulation and expose phosphatidylserine as a procoagulant surface, respectively. However, in the absence of FXII, collagen can still initiate coagulation.

Aims: To delineate the mechanism of coagulation initiation by platelet activation.

Methods: Thrombin generation (TG) triggered with collagen, the GPVI agonists collagen-related peptide (CRP) and convulxin, the α2β1 agonist GFOGER or tissue factor (TF) was measured in platelet-rich plasma (PRP) with or without FXII inhibitor (corn trypsin inhibitor, CTI) or active site-inhibited FVIIa (FVIIai) and in factor-deficient plasmas supplemented with washed platelets. In addition, FXa generation was measured in the presence of CRP or convulxin in a purified system consisting of FX, FVIII, washed platelets and a FXa fluorogenic substrate. Furthermore, in both assays, platelets were incubated with an inhibitory anti-human FIX antibody. Proteins from platelet lysates were analyzed by western blot and purified by immunoprecipitation.

Results: Both in the presence or abscence of CTI or FVIIai, collagen induced TG in PRP (Figure 1). Both GPVI agonists but not the α2β1 agonist could mimic the effect of collagen. In addition, collagen and CRP could trigger TG in FXII-, FXI-, or FVII-deficient plasma, but not FIX- or FVIII-deficient plasma, supplemented with washed platelets. Both GPVI agonists could significantly increase FXa generation in a purified system with FX, FVIII and washed platelets. Interestingly, both TG and FXa generation induced by GPVI agonists were dose-dependently inhibited by an anti-human FIX antibody (Figure 2). Furthermore, with western blot and immunoprecipitation, platelet lysates contained a protein recognized by anti-human FIX antibody, with a similar molecular weight as FIXa.

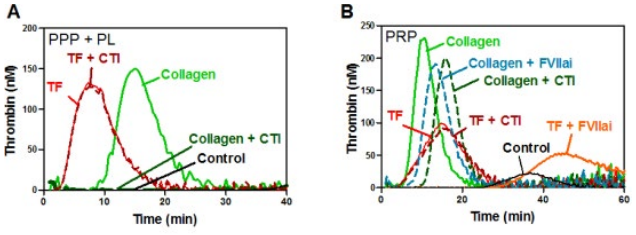

Figure1. Thrombin generation triggered with 1 pM TF, 25 µg/ml collagen or vehicle solvent (control) was measured in PPP with added phospholipids (PL) (panel A) and PRP (panel B). CTI and FVIIai were added to inhibit contact activation or TF pathway, respectively. Experiments were repeated with plasma of 3-5 donors and representative curves are shown.

FIGURE 1

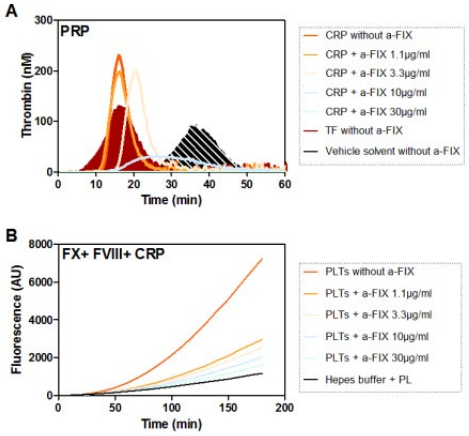

Figure 2. PRP preincubated with increasing concentrations of anti-human FIX antibody was triggered with 1 µg/ml CRP. As a control, thrombin generation (TG) was triggered with 1 pM TF or vehicle solvent. Shown are representative TG curves based on experiments done with 3 donors (panel A). Isolated platelets preincubated with increasing concentrations of anti-human FIX antibody or vehicle solvent, were activated with 1 µg/ml CRP and subsequently, a mixture of FX, FVIII, FXa fluorogenic substrate and $CaCl_2$ was added. Representative FXa generation curves based on experiments done with platelets of 3 donors are shown (panel B).

FIGURE 2

Conclusions: Platelet activation via GPVI (collagen, CPR and convulxin) could initiate TG via the release of a molecule similar to FIXa.

PB0404 | Exploring the Effectiveness of DNA and Glycosaminoglycans as Inhibitors of Histones

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Background: Neutrophil extracellular traps (NETs) have emerged as an interface between infection, innate immunity, and coagulation. These structures consist of DNA wrapped around a histone octamer containing two copies of histones H2A, H2B, H3, and H4. NETs aid in host defence by trapping and killing pathogens. However, histones are cytotoxic to endothelial cells and can impair protein C (PC) activation. Specifically, histones bind to PC and thrombomodulin (TM), thereby inhibiting activated PC (APC) generation. Once generated, APC can degrade histones.

Aims: (1) To measure the binding affinities between glycosaminoglycans (GAGs) with histones and (2) to explore the effectiveness of DNA and GAGs in neutralizing histone-mediated cytotoxicity and impairment of APC generation.

Methods: The binding affinities between GAGs and histones were determined by Bio-layer Interferometry. The GAGs used were unfractionated heparin (UFH;15kDa), enoxaparin (4.5kDa), Vasoflux (non-anticoagulant heparin;5.5kDa), and fondaparinux (1.7kDa). HEK293 cells were treated with histones in the presence or absence of DNA or GAGs, and cell viability was assessed. HEK293 cells expressing TM and EPCR, were treated with PC, thrombin, histones alone or in combination with DNA or GAGs. APC generation was quantified via an APC enzyme capture assay.

Results: UFH, enoxaparin, and Vasoflux bind histones H2A, H2B, H3, and H4 with high affinities (KD< $1x10^{-3}$nM to 85.51nM), whereas fondaparinux exhibited a low affinity (KD>8mM). DNA, UFH, enoxaparin, and Vasoflux dose-dependently neutralized

histone-mediated cytotoxicity. However, fondaparinux was not as effective as the other GAGs. Neither DNA nor GAGs neutralized the histone-mediated impairment of APC generation.

Conclusions: The ability of GAGs to neutralize histone-mediated cytotoxicity is dependent on the size of the GAG but is independent of the antithrombin-binding pentasaccharide. Our results also show that GAGs do not prevent histone-mediated impairment of APC generation. Future studies should investigate the use of a combination therapy of GAGs and recombinant APC to target the adverse effects of histones.

PB0406 | The Role of the Protein C Pathway in Hemostasis: The Importance of Physiological Context

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Background: Factor V Leiden (FVL) is the most common inherited risk factor for venous thromboembolism by significantly decreasing Factor Va inactivation by activated protein C (APC), thus demonstrating a critical role for APC as an endogenous antithrombotic. Protein C is activated by thrombin in complex with thrombomodulin on the surface of endothelial cells. However, recent studies show that during hemostasis in large vessels, thrombin activity is confined to the extraluminal compartment, raising questions about the role of APC-mediated thrombin inhibition in these settings.

Aims: Thus, we hypothesized that the contribution of the protein C pathway to hemostasis may depend on local conditions.

Methods: Homozygous FVL mice and a monoclonal mouse APC blocking antibody were used to examine the role of APC during hemostasis *in vivo* in both the micro- and macro-circulation. Anatomically correct computational models were developed to simulate thrombin generation in the presence and absence of the protein C system in small and large vessels.

Results: We found significantly greater platelet accumulation, activation, and fibrin formation following vascular injury in the microcirculation of FVL mice, and an even more dramatic pro-thrombotic phenotype utilizing the APC blocking antibody, suggesting enhanced thrombin generation compared to wild-type controls. Conversely, in a jugular vein puncture injury model, platelet accumulation, activation and fibrin deposition in FVL and APC blocking antibody infused mice were similar to controls. To reconcile these differences, we simulated thrombin generation following injuries in the micro- and macro-circulation. We found that in the microcirculation thrombin generation/activity can occur both intraluminally and extravascularly. Intraluminal thrombin generation results in APC generation. In contrast, simulations of macrovascular injuries showed that the combined action of flow extravasation and platelet deposition confines thrombin generation/activity to the extravascular space, resulting in negligible Protein C activation.

Conclusions: APC-mediated inhibition of thrombin generation depends on the vascular context and local hemostatic conditions.

PB0407 | Inhibition of Factor Xa by Specific Reversible Inhibitors Is Selective for Factor Xa Generated by Factor IXa

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Background: Coagulation activation in the thrombodynamics clot growth system follows two pathways:

(a) Tissue factor /factor VIIa to factor X and: (b) factor XIa/factor IX to factor X.

Both pathways have 2/3, 1/3 contribution to clot size and growth rate in normal plasma.

Aims: We tested inhibition by reversible factor Xa inhibitors (Xa-DOACs) on both pathways.

Methods: Normal and haemophilia plasma; spiking with purified factor VIII or emicizumab (emi). Rivaroxaban, apixaban, edoxaban, fondaparinux, heparin and emi from Pharmacy. Testing with and without 4 μM apixaban.

Results: Spiking factor VIII in Haemophilia A plasma increased stationary clot growth rate (Vst) about a factor of 1.5, reaching levels of normal plasma at 100% factor suppletion. Spiking of emi corrected clot growth similar to adding factor VIII with 80% correction at 20 μg/ml emi.

All Xa-DOACs had no effect on clot growth in haemophilia plasma. However, in normal and plasma corrected by spiking, all Xa-DOACs inhibited clot growth back to the level of the deficient plasma.

In contrast, fondaparinux and heparin inhibited also clot growth in haemophilia plasma, showing not the specificity of the Xa-DOACs.

In healthy control the tissue factor/ VIIa portion was the least variable. In 10 controls the portions were analysed using addition of 4 μM apixaban: the tissue factor /factor VIIa clot growth showed Vst of 21.3 (CV% 9) and the factor XIa/IX portion 14.0 with CV% 30.

Conclusions: Only the factor Xa generated by factor IXa can be inhibited by the Xa-DOACs, also when factor Xa is generated with emi as a cofactor. Addition of high apixaban enables detection of the tissue factor/ factor VIIa portion separately.

It shows that reversible Xa-inhibitors inhibit clot growth partially and specifically. It implies that in case of high factor VIII, IX or XI and with accidental high emi, Xa-DOACS are rational, specific inhibitors or antidotes.

PB0409 | System Wide Investigation into Coagulation Profile of Premature Myocardial Infarction Subjects

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Background: While plaque rupture is a pivotol causative event for myocardial infarction (MI) the amplitude of the haemostatic response may also be a key determining factor in whether the vessel becomes occluded. Elevated levels of some individual coagulation factors are seen in patients who have suffered an early MI but the reactions that occur in blood between these proteins are complex, involving multiple control mechanisms, meaning that it is difficult to predict the importance of changes on the extent and rate of thrombin and hence clot formation.
Aims: To investigate how small variations in multiple plasma coagulation proteins interact to control thrombin generation.
Methods: A mathematical representation of the coagulation cascade was used to predict system wide coagulation responses using profiles of coagulation factors measured in 162 donors who had suffered an MI< 50 years and 186 age/sex matched controls. Machine learning techniques were used to stratify donors and investigate patterns between data and model predictions.
Results: Differential response patterns were identified not only between healthy donors and premature MI patients, but also between males and females. The modelling highlights key points of control within the coagulation cascade and allows the identification of coagulation proteins that control the differential response seen between subsets of donors. In males the key proteins that contribute to the differences seen between case and controls are TF, FVIII, FIX and the inhibitor TFPI whereas in females FII, FVIII and FIX control the outcome.
Conclusions: We have identified key changes in procoagulant profiles that propagate through the coagulation cascade to produce shifts in thrombin generation. These changes are found to vary by gender and suggest that donor stratification, enhancing our understanding of therapeutic efficacy, may be possible.

PB0410 | A novel Interaction between Coagulation Factor XI and its Activated Form, FXIa, with the Complement Protein Properdin

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Background: A lack of understanding of the interactions between complement and coagulation means that existing mechanisms are often overlooked. Serious thrombo-inflammatory events can occur when these cascades become dysregulated. Components of the contact activation pathway of coagulation and the alternative pathway of complement both interact with polyanionic surfaces, thus we proposed that an interaction between the two pathways may exist.
Aims: To determine if a surface dependent interaction may occur between coagulation factor (F)XI and its activated form, FXIa, and the positive regulator of complement, properdin.
Methods: Surface plasmon resonance (SPR) was employed to determine binding characteristics between immobilised properdin, and injected FXI(a). Chromogenic assays were optimised to investigate several mechanisms: autoactivation of FXI in the presence of properdin, the effect of properdin on the catalytic activity of FXIa, and the inhibition profile of the serpin C1 esterase inhibitor (C1-INH) on FXIa with and without properdin, all in the in the presence and absence of high-molecular weight (500 kDa) dextran sulfate (DXS_{500kDa}). SDS-PAGE was used to analyse substrate specificity assays and mass spectrometry (MS) was utilised for band analysis.
Results: Properdin binds to FXI and FXIa with high affinity and properdin appears to inhibit the autoactivation of FXI by DXS_{500kDa}. DXS_{500kDa} inhibits FXIa through an uncompetitive mechanism and this study has shown that this is partially reversed by properdin. Incubation of FXIa, DXS_{500kDa} and properdin revealed unknown bands assessed by SDS-PAGE and were identified as properdin cleavage products by MS, suggesting FXIa was able to cleave properdin in the presence of DXS_{500kDa}, indicating a substrate specificity change of FXIa. Inhibition of FXIa by C1-INH in the presence of DXS_{500kDa} is reversed by properdin.
Conclusions: These findings suggest a novel regulatory mechanism of the alternative pathway of complement and the contact pathway of the coagulation cascade, influenced by the presence of a polyanionic surface.

PB0411 | Plasma Levels of DNase I Activity and Circulating-free DNA Are Associated to an Increased Risk of Venous Thromboembolism

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[1]*Medical Research Institute Hospital La Fe, Haemostasis, Thrombosis, Atherosclerosis and Vascular Biology Research Group, Valencia, Spain;* [2]*Medical Research Institute Hospital La Fe, Biostatistics Unit, Valencia, Spain;* [3]*La Fe University and Polytechnic Hospital, Haemostasis and Thrombosis Department, Valencia, Spain*

Background: Plasma circulating free DNA (cfDNA) comes from cellular death or *neutrophil extracellular traps* (NETs). Plasma cfDNA levels are increased in venous thromboembolism (VTE) patients and increase VTE risk.

Aims: Our aim was to compare plasma cfDNA levels and DNaseI activity (cfDNA inhibitor) in VTE patients and healthy controls.

Methods: One hundred and seven VTE patients and 53 healthy volunteers (controls) were recruited. Plasma samples were obtained between 6 and 24 month after the thrombotic event in VTE patients and at recruitment in controls. cfDNA was quantified with PicoGreen (Life Technologies) and DNAseI activity was measured with DNaseI assay Kit (Abcam) after optimization. Statistical analysis was performed with R (v3.5.0).

Results: cfDNA levels were slightly higher in VTE patients (1771.15 ± 223.81 ng/ml) than in controls (1724.69 ± 226.55 ng/ml) (P=0.15). DNase I activity was significantly increased in VTE patients (6.17 ± 1.61 μU/ml) vs controls (5.5 ± 1.25 μU/ml) (P< 0.001). In a bayesian regression logistic model an interaction between cfDNA levels and DNaseI activity was detected (OR=1.32, IC 95% [0.96, 1.97]).A synergistic effect was observed and simultaneous increasing of cfDNA levels and DNAseI activity, were associated with higher VTE risk. Patients and controls were distributed in tertiles according to DNaseI activity and VTE risk was calculated according cfDNA levels. VTE risk in subjects in the lowest tertile of DNaseI activity (4.42 μU/ml) ranged from 50 to 62%, in the medium tertile (5.95 μU/ml) ranged from 65 to 75%, and in the highest tertile (7.48 μU/ml) ranged from 76 to 95%.

Conclusions: Increased levels of cfDNA and DNaseI activity are associated with an increased risk of VTE with a synergistic effect. Thus, subjects with increased levels of both parameters show the highest risk of VTE.

ISCIII-FEDER (PI14/00079, PI14/00512, FI14/00269, CPII15/00002, PI17/00495), GVA (ACIF/2017/138) and SETH.

PB0412 | Study of a New Synthetic Thrombin Inhibitor IEM-2280

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Background: Modern views about hemostasis and thrombosis and the experience of using antithrombotic agents (anticoagulants, thrombolytic and antiplatelet agents) suggest that the development of synthetic low molecular weight direct thrombin inhibitors is promising because this enzyme is the key in the blood coagulation system, and its increased activity is capable to lead to thrombosis. IEM-2280 (N-[N′-tret-butyloxycarbonyl-4-amino-3-phenyl-butyryl]arginine hydrochloride) synthesized in the Department of Neuropharmacology named after S.V. Anichkov (Institute of Experimental Medicine) is one of these compounds.

Aims: The aim of this work was to investigate the ability of IEM-2280 to provide antithrombotic effect.

Methods: The enzymatic activity of purified thrombin was evaluated by cleavage of a chromogenic substrate H-D-phenylalanine-L-pipecolyl-L-arginine-4-nitroanilide dihydrochloride (S-2238) with the formation of p-nitroaniline and also by coagulation of fibrinogen. The kinetics of the reaction was studied by the photometric method at λ=405 nm. Formation of thrombin-induced platelets aggregates was analyzed by using turbidometric aggregometry by detecting changes in the light transmission of cell suspensions at λ=540 nm.

Results: Studies have shown that IEM-2280 in a concentration-dependent manner reduced the enzymatic activity of thrombin determined by the ability of the enzyme to cleave the chromogenic substrate. The concentration of half-maximal inhibition (IC_{50}) was 1.7±0.3 μM. Coagulation of fibrinogen completely blocked by 2 μM IEM-2280. It was found that IEM-2280 also effectively inhibited thrombin-induced (40 ng/ml) platelet aggregation, IC_{50} was 2.8±0.6 μM.

Conclusions: It was found that IEM-2280 is able to inhibit the enzymatic activity not only purified thrombin, but also thrombin-induced platelet aggregation. Since arginine is a precursor to NO, it is possible that the effect of IEM-2280 on platelets may be associated including with inhibition of platelet aggregation by NO. We can conclude that the IEM-2280 is promising compound to create on its basis of drugs with a pronounced antithrombotic action. This study supported by MD-1901.2020.4 grant.

PB0413 | Coagulation Factor Xa [(Recombinant) Andexxa®] for Reversal of Apixaban and Rivaroxaban in Patients Diagnosed with Intracranial Hemorrhage

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Background: In 2018, the FDA approval coagulation factor Xa [(recombinant) Andexanet alfa (AA)] for reversal of life-threatening or uncontrolled bleeding in patients receiving apixaban or rivaroxaban.

Aims: To describe our experience with AA for reversal of apixaban and rivaroxaban in patients diagnosed with ICH.

Methods: This retrospective, single-center, observational study included adult patients with ICH who received AA for reversal of apixaban or rivaroxaban between July 1, 2018 and September 1, 2019. See figure 1 for dosing guidelines. Patients were excluded if they received multiple doses of AA during the same encounter, or both four-factor prothrombin complex concentrate (4F-PCC) and AA. The primary endpoint of this study was the incidence of hemostatic treatment failure, indicated by progression of hematoma on neuroimaging within 24 hours of AA administration. Notable secondary endpoints included in-hospital mortality, incidence of thrombotic complications within 30 days, ICU and hospital length of stay (LOS), patient disposition, and change in National Institutes of Health Stroke Scale (NIHSS) and Modified Rankin Scale (mRS) at presentation and discharge.

Results: During the study period, 19 eligible patients receiving 20 doses (1 high-dose (800 mg bolus, followed by 8 mg/min) and 19 low-doses (400 mg bolus, followed by 4 mg/min)) of AA. 15 patients had repeat neuroimaging within 24 hours after AA was administered with 7/15 (46.7%) noted to have progression of hematoma. 2 patients (10.5%) developed thrombotic complications (deep vein thrombosis, ST-elevation myocardial infarction, and ischemic stroke), and one patient developed ventricular tachycardia with chest pain (Patient not included due to not receiving the full infusion of AA). Additional secondary endpoints are noted in Table 1.

Conclusions: In our patient population, rates of hemostatic treatment failure were higher compared to the ANEXXA-4 study. However, our definition of failure based on hematoma expansion differs from the ANNEXA-4 hemostatic efficacy scale.

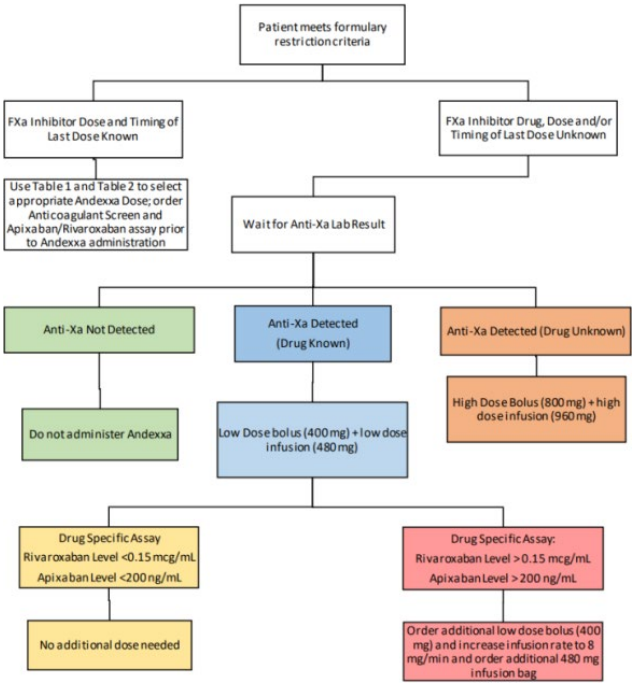

FIGURE 1 Andexanet alfa Dosing Guidelines

TABLE 1 Secondary Endpoints

	n=19
In-hospital mortality, n (%)	3 (15.8)
Discharge disposition, n (%) Hospice Skilled Nursing Facility Home	2 (10.5) 7 (36.8) 7 (36.8)†
ICU length of stay (days), median (IQR)	3.74 (2.30–7.13)
Hospital length of stay (days), median (IQR)	7.83 (4.37–15.89)
Confirmatory anticoagulation lab obtained (n=17) Time to confirmatory result (hours), median (IQR)	1.00 (0.62–1.42)
Resumption of DVT prophylaxis (n=13) Time to resumption of DVT prophylaxis (hours), median (IQR)	69.10 (47.95–69.90)
Resumption of systemic anticoagulation (n=8) Time to resumption of systemic anticoagulation (days), median (IQR)	26.5 (14–35)
Admission NIHSS, median (IQR), n=13 Admission mRS, median (IQR), n=11 Discharge NIHSS, median (IQR), n=7 Discharge mRS, median (IQR), n=7	10 (3–14) 1 (0–4) 6 (4–9.5) 4 (3.5–4)

TISSUE FACTOR PATHWAY

PB0414 | A Membrane-Adjacent Region in Tissue Factor Provides Substrate Selectivity

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Background: The membrane-adjacent tissue factor (TF) residues S160-S161-S162-S163 form a flexible, solvent-exposed loop that is poorly resolved in almost all crystal structures. Mutating some of these four serines to alanine decreases activation of factor X (FX) and factor IX (FIX). However, the exact role of this serine loop in facilitating substrate activation and selection by TF-FVIIa is still unknown.

Aims: Investigate if the TF serine loop facilitates selective activation of TF-FVIIa substrates.

Methods: We generated thirteen different TF serine loop mutants. In the first set of mutants, each serine was substituted to threonine. In the second set, the importance of loop length was tested by inserting one or more serines or threonines, or deleting one or more serines. Membrane-anchored TF was expressed in E. coli and affinity-purified. TF mutants were incorporated into liposomes and characterized using substrate activation assays as well as thrombin generation assays.

Results: Serine-to-threonine substitutions had little effect on rates of FX activation by TF-FVIIa. Surprisingly, however, increasing or decreasing the length of the serine loop by even one amino acid decreased rates of FX activation 20- to 200-fold, while having little to no effect on FIX activation. Thrombin generation assays with FVIII- and FIX-deficient plasma showed that the thrombin concentration was reduced two- to fourfold and time-to-peak was increased twofold for these TF serine loop mutants compared to wildtype TF.

Conclusions: The exact length of the serine loop on TF is essential for FX activation by TF-FVIIa, while altering its length has negligible effects on FIX activation. Thus, the precise length of this apparently unstructured TF loop plays a selective role in facilitating FX activation, which we believe is the most extreme example to date of substrate selectivity mediated by a portion of the TF structure.

PB0415 | Coagulation Factor IIIa (f3a) Knockdown in Zebrafish Leads to Defective Angiogenesis and Bleeding Phenotype

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Background: Mice lacking tissue factor (TF) are embryonically lethal (E10.5). The lethality has been attributed to defects in vascular development and circulatory failure, which suggests additional roles for TF in embryonic development beyond coagulation. Zebrafish has two TF paralogs (*f3a* and *f3b*) with unknown functions. Knockdown of *f3b* gene was shown to reduce coagulation and angiogenesis. Both *f3a* and *f3b* are necessary for normal hemostasis, albeit with differential contributions in the arterial vs. venous beds. The role of *f3a* in vascular development remains unknown.

Aims: To characterize the *in vivo* role of zebrafish *f3a* in vascular development.

Methods: Transgenic line of zebrafish with endothelial-specific reporter gene expression (*flk1:egfp-NLS/kdrl:mCherry-CAAX*) was used to image vascular development *in vivo*. The role of *f3a* during embryonic development was investigated by mRNA knockdown using Morpholino antisense oligonucleotides (MO). The *f3a* morphants were examined at 52 hours after fertilization (hpf) for defects in morphological appearance, bleeding, and vascular patterning.

Results: Phylogenetic and sequence homology analyses suggested that *f3a* exhibits greater similarity (amino acid and nucleotide sequence) to its human ortholog than *f3b*. *f3a* expression was seen in all developmental stages (64 cells stage to 72 hpf), indicating that *f3a* transcripts are maternally deposited, as well as zygotically expressed. MOs targeting the exon 3-intron 3 junction (*e3-i3*) dose-dependently reduced the expression of *f3a*, but not that of *f3b*, documenting target specificity. *F3a*MO-injected embryos exhibited a shortened body axis (11.45%), a short/crooked tail (27.5%) as well as bleeding in trunk (5.44%) and head (9.52%) regions. The caudal vein plexus (CVP), which forms through a process of sprouting immediately following the onset of circulation, showed a 3-fold decrease in the number of CVP loops and sprouting events, relative to controls.

Conclusions: This study reveals that zebrafish *f3a*, in addition to its function in coagulation, has an essential and non-redundant role in developmental angiogenesis.

PB0416 | Plasma Tissue Factor Pathway Inhibitor (TFPI) in Pediatric End Stage Liver Disease

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Background: Tissue factor pathway inhibitor (TFPI) is an intrinsic inhibitor of factor Xa. There is a paucity of data regarding the effect of TFPI in pediatric end stage liver disease (ESLD). TFPI is released from endothelium and metabolized by the liver and kidney, hence, plasma concentration of TFPI can potentially increase in liver failure.

Aims: Assessment of TFPI level in pediatric ESLD and potential role in bleeding risk.

Methods: In this pilot study, 13 pediatric ESLD patients included. Citrated plasma samples were analyzed for total TFPI via ELISA assay (Stago, USA). Corresponding prothrombin time (PT), activated partial thromboplastin time with hepzyme (HPTT), fibrinogen (FGN), platelet count (PLT) and ROTEM clotting time (CT) values were obtained from the electronic medical record. All data are expressed in mean±SD. Group means were compared by ANOVA (SPSS v25, IBM).

Results: Age 7.6±8 years, 77% female. Thirty-five samples were analyzed, 60% were collected at times corresponding with the bleeding episodes (Group I). Group I had higher TFPI (216±135 vs 108±33

ng/mL, p=0.006), but non-significant differences in PT (25±9 vs 31±11 sec, p=0.07), INR (2.3±1.0 vs 3.0±1.3, p=0.08), HPTT (49±19 vs 70±37 sec, p=0.05), FGN (165±53 vs 174±77 mg/dL, p= 0.70), EXTEM CT (75±12.5 vs 96±16 sec, p=0.05), INTEM CT(234±52 vs 300±57 sec, p=0.08), FIBTEM CT (13±5 vs 12±8 sec, p=0.85) and PLT (75±83 vs 68±56 ×10^3/µL, p=0.80) compared to the remaining 40% that did not correspond with the bleeding episodes (Group II). Overall mortality was 60%. Three patients with TPFI levels > 200 ng/mL (reference range 51.2–111.2 ng/ml) developed diffuse mucosal bleeding. One of them had additional heparinoid substance.

Conclusions: This is the first study to assess plasma TFPI level in pediatric ESLD. Plasma TFPI can be a clinically useful marker since it represents additional risk factor for bleeding that cannot be evaluated using routine testing.

PB0417 | Tissue Factor Pathway Inhibitor Is Increased in Plasma of Patients with a mild to Moderate Bleeding Tendency Independent of Known Genetic Variations in the factor V Encoding Gene

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Background: Increased levels of free tissue factor pathway inhibitor (TFPI) based on 2 mutations in the factor V(FV) gene (Transcript-NCBI ID:NM_000130.4) were reported as cause of bleeding tendencies (Vincent et al., 2013; Cunha et al., 2015). Recently, the intronic single-nucleotide variant (SNV) rs10800453 in the FV gene was related to elevated TFPI levels in a genome wide association study (Sun et al., 2018).

Aims: To investigate TFPI levels in a large cohort of adult patients with a mild-to-moderate bleeding tendency (MBD) and healthy controls and to identify possible underlying genetic alterations in the FV encoding gene.

Methods: Patients from the Vienna Bleeding Biobank study and 122 healthy controls were investigated after written informed consent. Free TFPI levels were measured using an ELISA (Stago, Asniéres France). Genetic data were generated by the ThromboGenomics assay (Downes et al., 2019) and using Affymetrix Axiom genotyping arrays. The genotypes for rs10800453 were imputed using the Sanger Imputation Service

Results: 620 patients (81.5% females) and 122 controls were investigated (Table 1). Median [IQR] free TFPI levels were higher in patients than in healthy controls (8.3 [5.7–11.8] vs. 7.8 [4.9–11.2] ng/mL; p=0.016). TFPI levels were higher in male patients (10.0 [7.8–13.2] vs. 7.7 [5.4–11.4] ng/mL; p=0.006) and associated with higher age (r_s=0.401). The statistically significant difference between TFPI levels in patients and healthy controls prevailed after adjusting for sex and age in multivariable linear regression analysis. We could not identify the reported mutations c. 2588 C>G or c.2440 A>G in our patients. The SNV rs10800453 did not affect TFPI levels in our patients (Figure 1).

Conclusions: Free TFPI levels were increased and might contribute to the bleeding tendency in our patients. We did not identify known genetic alterations for increased TFPI levels in our cohort. Further investigations are needed to understand the underlying mechanisms of increased TFPI levels in patients with MBDs.

TABLE 1 Patient characteristics including clinical data and parameters

	All Patients (n=620)	Healthy Controls (n=122)
Female, n (%)	505 (81.5%)	82 (67.2%)
	median [25–75 percentile]	median [25–75 percentile]
Age, years	40 [28–52.75]	38.5 [28.75–52]
Vicenza bleeding score	5 [4–8]	0 [0–0]
ISTH BAT bleeding score (available from 359 patients)	6 [4–9]	-
Platelets, x10^9/L	244.5 [209–283.75]	257 [221.8–292]
APTT, seconds	35.9 [33.7–38.9]	35.1 [33.2–36.4]
PT, %	95 [88–104]	100 [91–109]
TFPI, ng/mL (available from 607 patients)	8.3 [5.7–11.8]	7.8 [4.9–11.2]

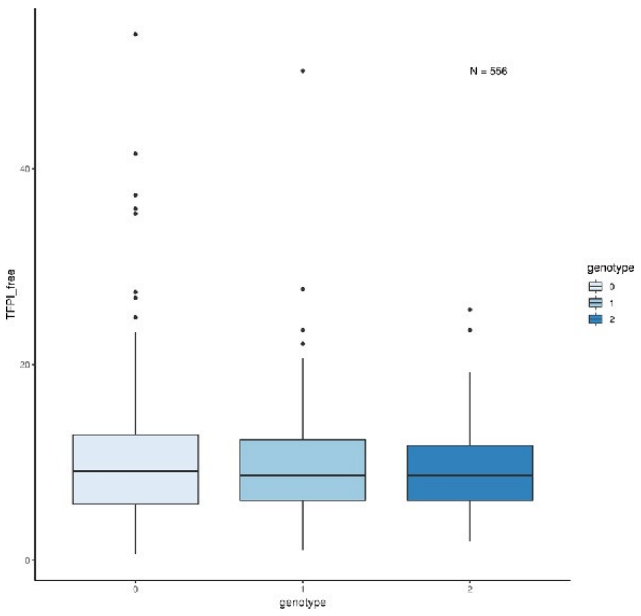

FIGURE 1 Genetic Analysis: Comparison of TFPI levels in patients with 0, 1 or 2 alleles of evaluated SNV rs10800453

PB0418 | Simulation of TF-dependent Factor X Activation on Phospholipid Vesicles: The Role and Importance of Individual Reaction Stages

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Background: Exposure of TF on the surface of cells or circulating microparticles to the blood is important for initiation of plasma coagulation. TF-dependent coagulation initiation is a multistage process, which includes the extrinsic tenase complex formation from transmembrane TF and circulating factor VIIa (FVIIa), interaction of the preferential extrinsic tenase substrate factor X (FX) with phospholipid membrane, diffusion of FX in the bloodstream or on the membrane surface, formation of ternary TF/FVIIa/FX complex and FX activation. The impact of individual reaction stages on the rate of FX activation is unclear.

Aims: Computational analysis of TF-dependent FX activation on the surface of phospholipid vesicles.

Methods: Three computational models of increasing complexity of the extrinsic tenase functioning were constructed: A, the homogeneous single-phase model, B, the homogeneous two-phase model and C, the heterogeneous model. The models were tuned to accurately describe experimental data from (Hathcock et al. 2005). The models were integrated in Matlab2012a and Comsol Multiphysics4.3a.

Results: Analysis of the models revealed that the rate-limiting stages for the FX activation depended on the TF surface densities. For high TF surface density (1000 molecules per μm^2) the reaction rate was determined by the rate of FX binding to the surface. For low TF surface density (30 molecules per μm^2) the reaction rate was limited by the rate of extrinsic tenase formation. In presence of vesicles without TF in the system, the heterogeneous model C described the experimental data better than the homogeneous models A and B.

Conclusions: Either the binding of FX to the vesicles or the tenase formation on the surface could be the rate-limiting stages of the tenase activity dependent on the TF surface density. In presence of vesicles without TF the rate of FX diffusion becomes of importance. The study was supported by the Russian Foundation for Basic Research grant 17-00-00138.

PB0424 | Anticoagulant Activity in Unclassified Bleeding Disorders

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Background: We have routinely used thrombin generation to investigate patients with unclassified bleeding disorder (UBD).

Aims: To investigate haemostatic abnormalities in patients with UBD that had abnormal thrombin generation on at least one occasion.

Methods: Investigation of 13 known UBD patients with thrombin generation and detailed haemostatsic testing was undertaken including TFPI assays but also thrombomodulin and fibrinogen-γ.

Results: 12 females and 1 male were included. No patient had a platelet function disorder or coagulation factor deficiency that explained the bleeding phenotype, though 2 patients had factor deficiencies; a factor X of 0.41IU/ml and a factor XI of 0.51IU/ml. ThromboGenomics revealed variants for these factors but no other abnormalities. Patients were included who previously had either prolonged lag-time or decreased endogenous thrombin potential (ETP) via high dose tissue factor (5pM) or low dose tissue factor (1.5pM) with corn trypsin inhibitor (CTI).. Tissue factor pathway inhibitor (TFPI) activity was significantly increased ($p < 0.001$; increased in 8 patients) compared to controls and abnormalities in soluble thrombomodulin (2 patients), fibrinogen-γ (1 patient), PAI-1 and tPA (4 patients for each) were seen. Total and free TFPI levels were not increased. Mixing studies of patient plasma with 50:50 normal

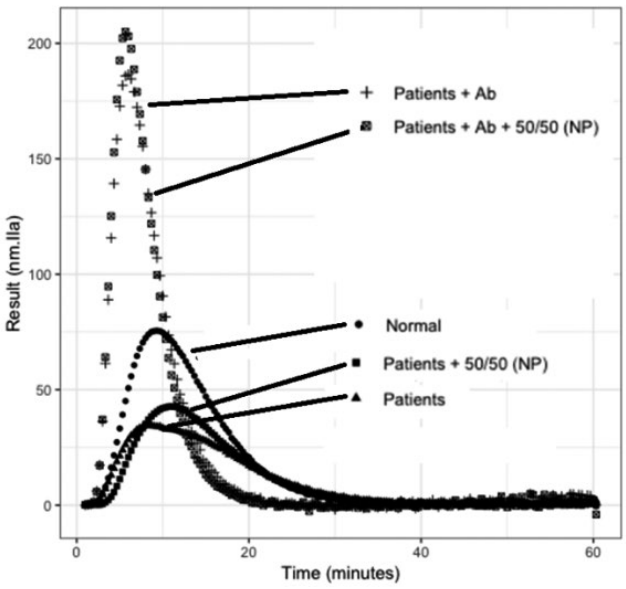

FIGURE 1 Thrombin generation profile of study patients when mixed with normal plasma and anti-TFPI antibody

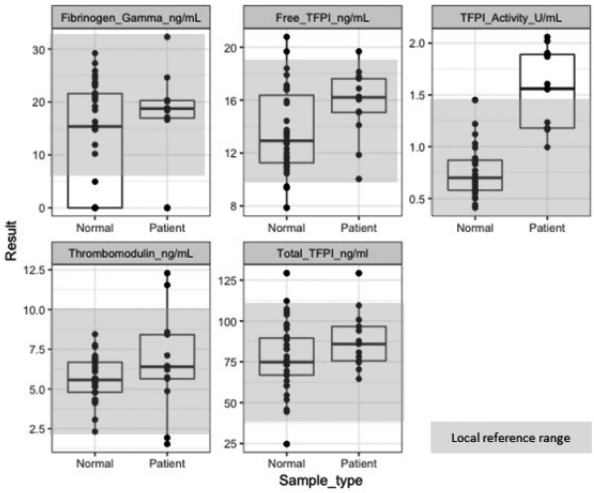

Figure 2: Relative measures of potential candidates for the thrombin generation results observed between normal volunteers and the patient cohort. TFPI – Tissue Factor Pathway Inhibitor. Shaded - locally derived reference range.

FIGURE 2 Anticoagulant levels observed between normal volunteers and the patient cohort. TFPI - Tissue Factor Pathway Inhibitor. Shaded - reference range

plasma for thrombin generation via low dose tissue factor failed to correct the ETP consistent with on-going inhibition. Addition of an anti-TFPI antibody corrected thrombin generation to normal levels. *TFPI* sequencing was unremarkable.

Conclusions: TFPI activity may be increased in a sub-set of UBD patients. Further research studies are warranted in UBD patients for coagulation inhibitor abnormalities.

DIAGNOSTICS AND OMICS

BIOMARKERS OF THROMBOSIS AND HEMOSTASIS

PB0425 | Gene-Gene Interactions of Folate Cyclein as a Potential Factor of the Risk of Thrombosses in Patients with Breast Cancer

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Background: Establishing the relationship between polymorphic genes and phenotypic manifestations of thrombophilia in breast cancer is an essential condition for establishing key pathogenesis links that affect certain physiological functions, through which the risk of thrombosis is formed.

Aims: To study the association of complex carriage of folate metabolism polymorphism with the level of homocysteine in the blood serum of patients with breast cancer.

Methods: The study included 122 patients with breast cancer aged 56 ± 10.0 years. The diagnosis is histologically confirmed. The control group consisted of 142 conditionally healthy women of the Transbaikal Territory at the age of 40.2 ± 9.5 years without a history of cancer at the time of blood sampling. The determination of serum homocysteine level was carried out by high performance liquid chromatography according to A.A. Zhloba and E.L. Blashko (2004). Genotyping for the detection of polymorphism MTHFR (C677T), MTHFR (A1298C), MTR (A2756G), MTRR (A66G) was carried out by polymerase chain reaction in real time. Statistical analysis was performed using Ms Excel 10.0, Statistica 6.0., MDR v.3.0.2. The significance of differences was determined by the Mann-Whitney test and the median and percentiles (25th and 75th) were calculated.

Results: All possible combinations of polymorphic variants of folate metabolism genes were analyzed in patients with sporadic breast cancer and in the comparison group. Combinations MTR2756AG x MTRR66AG and MTHFR677CT x MTHFR1298AA x MTR2756AG x MTRR66AG were found to be associated with a higher concentration of homocysteine in serum compared to carriers of combinations of alternative genotypes and in the control ($p < 0.05$).

Conclusions: The additive effect of polymorphism of folate metabolism genes, leading to hyperhomocysteinemia, has an independent effect in the formation of the "pathogenetic profile" of thrombophilia due to synergistic interaction, which contributes to the phenotypic realization of thrombosis, a serious complication in breast cancer patients.

PB0427 | The Diagnostic Significance of Platelet-Derived Microparticles Quantification by Flow Cytometry in Egyptian Patients with Ischemic Heart Disease

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Background: Ischemic heart disease (IHD) is a major health catastrophe in developing countries. Acute coronary syndrome (ACS), the major IHD component where platelet-derived microparticles (PMPs) have a primitive role, is diagnosed by facilities of different specificities,

PMPs are platelet- vesiculated particles of < 1.0 µm, expressing surface CD61. The activation marker (glycoprotein GP IIb/IIIa receptor (CD41)) starts the activated platelets' cross-linking, and hence primary platelet aggregation.

Aims: to assess the value of PMPs by flow cytometry (FCM) for diagnosing Egyptian ACS patients, compared to troponin I (Tnt I).

Methods: The study was conducted on 130 Egyptian individuals: 100 patients with IHD (68ACS and 32 non- ACS), presented to Ain-Shams cardiology unit, and 30 controls. All had a written consent approved by the Ain Shams University ethical committee. They were all negative for any inflammation, or platelet activation, and had history taking, Tnt I, and the FCM- PMPs assay of both the number/micro Liter (n/µL), and the % activation in platelet- free plasma of EDTA- anticoagulated blood samples. CD61, CD41-double+ve PMPs

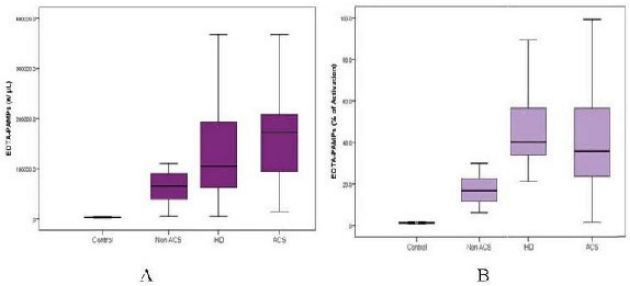

FIGURE 1 Diagrammatic representation of EDTA-PMPs n/μL A), and % of activation among all study population

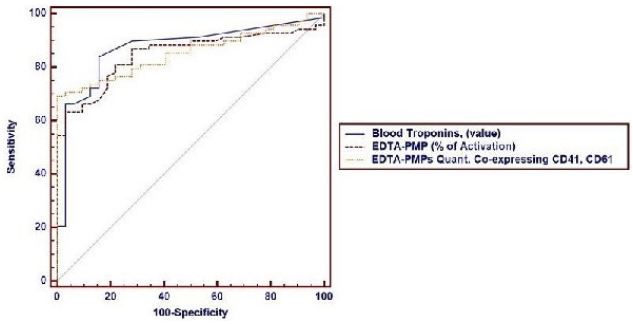

FIGURE 2 The performance of TnI, EDTA-PMPs both % of activation, and n/μL in discriminating ACS from non-ACS

were assayed, against standard beads. Mann-Whitney's test of significance and a ROC curve were applied.

Results: Both PMPs' % of activation and n/μL had a highly significant relation (p< 0.001) with the increased clinical severity, being highest in ACS patients, figure (1). The performance of EDTA-PMPs by FCM in detecting ACS of the % of activation with a cut-off > 42.1% had a 96.87% specificity, and the n/μL at a cut-off value of 110512 showed a 100% specificity, while using Tnt I, with a cut-off value of > 0.04 ng/ml had a 84.37% specificity, Figure (2).

Conclusions: The EDTA-PMPs- FCM assay in IHD is an inexpensive, non-invasive and specific independent predictor of ACS in Egyptian population, especially in cases with very early negative troponins.

PB0428 | High TAFI Level as a Predictor of VTE Recurrences during Long-Term Warfarin Therapy

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Background: Our previous study showed that low (<90%) thrombin activatable fibrinolysis inhibitor (TAFI) level was the independent predictor of the deep veins total clot lysis and bleedings in a target international normalized ratio (INR) in venous thromboembolism (VTE) pts during long-term warfarin (W) therapy. The influence of TAFI level on the risk of VTE recurrences is not yet clear, but it may be assumed that high TAFI level increased this risk.

Aims: To study the TAFI level effect on the risk of VTE recurrences during the long-term W therapy.

Methods: Ninety-eight VTE pts (70 men) aged 18–76 (mean 54 ± 14) years were included in the study. Pts received unfractionated or low-weight-molecular heparin for at least 5 days followed by long-term W therapy (target INR 2.0–3.0). TAFI level was measured once during the first month of W therapy. The follow-up period was 18 months. Endpoints were VTE recurrences.

Results: TAFI level ranged from 50 to 217% (median 106%, IQR 90 to 133%). TAFI level >106% was defined as high, TAFI level ≤106% was defined as low. Median of follow-up period was 18.00 (IQR 16.95 to 18.10) months. During 18 months of W therapy, the rate of VTE recurrences was 16.3%. Kaplan-Meier analysis showed that pts with high TAFI level had higher cumulative risk of VTE recurrences (chi-square = 5.05; p=0.025). Cox regression with age and sex adjustments confirmed that high TAFI level was associated with a 3.4-fold increase in the risk of VTE recurrences during 18 months of W therapy (HR 3.39; 95% CI 1.09 to 10.53; p=0.034) compared with low TAFI level pts.

Conclusions: During 18 months of W therapy, the rate of VTE recurrences was 16.3% or 10.9% per year. High (>106%) TAFI level had a prognostic value and was associated with the 3.4-fold increase in the risk of VTE recurrences.

PB0430 | Soluble Glycoprotein VI is an Independent Predictor of Major Bleeding in Patients with Suspected Heparin-Induced Thrombocytopenia

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Background: Patients with suspected heparin-induced thrombocytopenia (HIT) have a high incidence of major bleeding (Pishko, JTH 2019). Recent studies have implicated elevated soluble glycoprotein VI (sGPVI) levels as a risk factor for bleeding (Muthiah, JHLT 2016).

Aims: To determine if elevated sGPVI plasma levels are associated with major bleeding events in patients with suspected HIT.

Methods: We utilized a cohort of 310 hospitalized adult patients with suspected HIT, who had a citrated plasma sample collected at the time HIT was suspected. Plasma sGPVI levels were measured by ELISA. Patients transfused with platelets within 1 day of specimen collection were excluded because of the presence of high levels of

TABLE 1 Clinical features of patients with and without bleeding events

	Bleeding EventN=89	No Bleeding EventN=167	p-value
Age, Median (range)	65 (19–86)	67 (24–92)	0.373
Critical Care, n (%)	66 (74.2%)	68 (40.7%)	<0.0001
Surgery, n (%)*Cardiac BypassNon-Cardiac Bypass*	39 (43.8%)14 (15.7%)	47 (28.1%)29 (17.4%)	0.0110.739
Sepsis, n (%)	38 (42.7%)	38 (22.7%)	0.001
Platelet Count on day of blood collection x 10^9/L	54.5 (8–200)	79 (13–422)	0.0004
Serotonin Release Assay Positive, n (%)	10 (11.2%)	15 (9.0%)	0.863
Platelet Factor 4/Heparin ELISA*≤0.40.4–1.01.0–2.0≥2.0*	63 (70.8%)7 (7.9%)7 (7.9%)12 (13.5%)	117 (70.1%)16 (9.6%)17 (10.2%)16 (9.6%)	0.722

TABLE 2 Univariable and multivariable logistic regression model for bleeding

	Univariable Logistic Regression		Multivariable Logistic Regression	
	OR (95% CI)	p-value	OR (95% CI)	p-value
sGPVI*<43 ng/mL≥43 ng/mL*	1 (ref)4.59 (2.65–7.96)	-<0.001	1 (ref)3.07 (1.67–5.64)	-<0.001
Critical Illness*NoYes*	1 (ref)4.18 (2.37–7.36)	-<0.001	1 (ref)2.97 (1.58–5.56)	-0.001
Sepsis*NoYes*	1 (ref)2.52 (1.45–4.40)	-0.001	1 (ref)1.72 (0.91–3.26)	-0.092
Platelet Count x10^9/L>151100–15050–100<49	1 (ref)1.44 (0.44–4.67)1.26 (0.42–3.47)3.58 (1.20–10.66)	-0.5420.6800.222	1 (ref)0.98 (0.27–3.57)0.99 (0.30–3.22)1.97 (0.60–6.49)	-0.9790.9830.264

sGPVI in platelet concentrates. We reviewed the electronic health record for ISTH major bleeding events from time of sample collection to hospital discharge or death. We compared median sGPVI levels between patients with and without major bleeding events. We selected a dichotomous cut-off point for sGPVI that maximized the associated Youden's index. We then assessed the association of sGPVI with bleeding outcomes by multivariable logistic regression, adjusting for other clinical risk factors for bleeding.

Results: 54 patients were excluded due to recent platelet transfusion. 34.8% of patients had a major bleeding event. Table 1 displays clinical features by bleeding outcome. Median sGPVI levels were significantly elevated in patients with major bleeding events compared to those without major bleeding events (49.09 ng/mL vs. 31.93 ng/mL, p< 0.001). The odds of a major bleeding event were 3.07 times (95% CI 1.67–5.64) greater in patients with a sGPVI level ≥43 ng/mL after controlling for platelet count, sepsis, and critically ill status (Table 2).

Conclusions: In this cohort of patients with suspected HIT, sGPVI was associated with major bleeding. sGPVI may be a novel biomarker to predict bleeding risk in patients with suspected HIT.

PB0431 | Global Coagulation Assays in Patients with Chronic Kidney Disease

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Background: Chronic kidney disease (CKD) confers both increased bleeding and thrombotic risks in addition to traditional cardiovascular risk factors. Global coagulation assays (GCA) may advance our understanding of the individual's haemostatic profile beyond standard coagulation tests.

Aims: To evaluate GCA in CKD patients compared to healthy controls.

Methods: In this prospective observational study, CKD subjects were recruited into 2 groups: eGFR< 30ml/min/1.73m2 (pre-dialysis) (n=24) and dialysis patients (n=51 including 40 haemodialysis and 11 peritoneal dialysis). The results were compared to healthy controls (n=153). Blood samples were collected for baseline investigations and GCA including whole blood thromboelastography (TEG5000) and platelet-poor calibrated automated thrombogram (CAT) and overall haemostatic potential (OHP).

Results: Compared to controls (mean age 42 years, 64% female, eGFR 101), CKD subjects (mean age 66 years, 39% female, eGFR 12) had increased von Willebrand factor (vWF) antigen, factor VIII level and D-dimer (Table 1). CKD patients showed hypercoagulable

TABLE 1 Shows the comparisons between normal controls and patients with CKD

	Controls (n=153)	CKD (n=75)	p-value
Factor VIII (%)	115.7	194.5	<0.001
von Willebrand factor antigen (%)	114.5	206.0	<0.001
D-dimer (ng/mL FEU)	286	1145	<0.001
Maximum amplitude (mm)	60.2	69.0	<0.001
Endogenous thrombin potential (nM.min)	1335.1	1314.4	0.566
Peak thrombin (nM)	219.7	244.0	0.015
Velocity index (nM/min)	69.5	86.6	0.002
Overall fibrinolytic potential (%)	50.2	41.0	<0.001
Tissue factor pathway inhibitor (ng/mL)	21.5	51.1	<0.001

parameters compared to controls - increased maximal amplitude (MA) (p< 0.001) on thromboelastography and thrombin generation (peak thrombin p=0.015, velocity index p=0.002) with impaired overall fibrinolytic potential (OFP) (p< 0.001). Elevated urea did not predict hypocoagulability. Comparison of pre-dialysis and dialysis subpopulations did not identify significant differences for TEG and OHP parameters but dialysis patients, interestingly, demonstrated reduced thrombin generation (peak thrombin 232 vs 269 nM; p=0.046, velocity index 78 vs 103 nM/min; p=0.030) despite higher TFPI levels (59.2 vs 35.6 ng/mL; p=0.032). Peritoneal dialysis patients showed higher peak thrombin (279.9 vs 218.6 nM; p=0.007) and velocity index (104.6 vs 71.3 nM/min; p=0.014) with comparable MA and OFP compared to haemodialysis patients.

Conclusions: CKD appears to confer a hypercoagulable state with impaired fibrinolytic potential. Interestingly, dialysis patients demonstrated reduced thrombin generation compared to pre-dialysis patients but comparable to controls despite higher TFPI levels. Further longitudinal studies evaluating GCA and clinical outcomes in renal patients are required.

PB0433 | Evaluation of Global Coagulation Assays in Pregnancy

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Background: Women are at higher risk of venous thromboembolism (VTE) in pregnancy and postpartum. Current routine coagulation tests cannot discern the physiological hypercoagulability of pregnancy. Global coagulation assays (GCA) may be more representative of the coagulation process.

TABLE 1 Shows global coagulation assay parameters in pregnant women compared to normal controls

	Variable	Controls (n=47)	Pregnant women (n=60)	p-value
TEG	Maximum amplitude (mm)	60.6	71.5	<0.001
	Lysis 30 (%)	0.6	0.0	<0.001
CAT	Endogenous thrombin potential (nM.min)	1399.3	1895.2	<0.001
	Thrombin peak (nM)	240.0	320.9	<0.001
	Velocity index (nM/min)	84.0	110.7	0.001
OHP	Overall haemostatic potential (U)	25.4	33.9	<0.001
	Overall coagulation potential (U)	54.8	79.0	<0.001
	Overall fibrinolysis potential (%)	53.9	56.1	0.022

Aims: To evaluate the ability of GCA to detect the hypercoagulability of pregnancy and differentiate coagulability depending on VTE risk profile.

Methods: Women undergoing term elective Caesarean section were recruited. Pre-operative blood sample were collected for baseline blood tests and experimental testing with thromboelastography (TEG), thrombin generation using calibrated automated thrombography (CAT) and fibrin generation using the overall haemostatic potential assay (OHP). Citrated whole blood was used for TEG while platelet-poor plasma for CAT and OHP was obtained from double-centrifuged citrated whole blood. Data from 47 healthy non-pregnant women aged 18–45 years were used as controls.

Results: Sixty women with term singleton pregnancies were included. 41.7% (n=25) were obese (≥30kg/m^2) at booking and 88.3% (n=53) were multiparous. All GCA parameters were significantly more hypercoagulable in pregnant women compared to normal controls (Table 1), particularly with increased maximum amplitude (clot strength) (71.5 vs 60.6 mm, p< 0.001), endogenous thrombin potential (1895.22 vs 1399.33 nM.min, p< 0.001) and fibrin generation (79.01 vs 55.87 units, p< 0.001). Pregnant women with booking BMI ≥30kg/m^2 had significantly higher maximum amplitude compared to pregnant women of normal BMI (18.5–25kg/m^2) (73.2 vs 66.1 mm, p< 0.001). Statistical significance was maintained after controlling for age, parity, smoking status and diabetes. GCA parameters did not differ when comparing women of high and intermediate VTE risk with women of low risk.

Conclusions: GCA are able to detect the hypercoagulability of pregnancy and may potentially correlate with obesity in the pregnant population. GCA hold promise as adjuncts to risk factor-based criteria for VTE thromboprophylaxis during pregnancy and the puerperium - larger prospective studies are required.

PB0434 | Laboratory Accuracy Assessment of a Novel INR Point-of-Care Test Compared with Laboratory Reference Methods Used Across Four Independent Clinical Sites

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Background: The LumiraDx Platform is a novel, point of care *in vitro* diagnostic system that can be used for INR Testing by Healthcare Professionals. It has been shown that comparing results from the LumiraDx INR Test to those obtained with common laboratory reagents shows that the LumiraDx INR Test correlates well with IL ACL Elite Pro (ACL) with HemosIL RecombiPlasTin 2G.

Aims: A sample comparison was carried out to demonstrate the performance of IL ACL Elite Pro (ACL) and Sysmex CS instruments (CS-2500 and CS-5100) with plasma as reference methods for the LumiraDx INR Test using a capillary blood sample.

Methods: 101 blood samples were collected from patients attending anticoagulation clinics and their INR values were established using the LumiraDx INR Test (capillary) and ACL Elite Pro (plasma). Frozen plasma samples from routine calibration testing were sent to four independent laboratories for INR testing using the Sysmex CS Instruments, with Siemens Dade® Innovin® Thromboplastin. Passing-Bablok regression was used in a method comparison of data obtained.

Results: Analysis confirmed a good agreement between the LumiraDx INR Test results (capillary blood sample) from plasma samples using both the ACL Elite Pro and also the Sysmex CS 2500/5100.

Furthermore, data from the Sysmex CS 2500/5100 was demonstrated to correlate across 4 independent hospital laboratories.

Conclusions: The LumiraDx INR Test, ACL Elite Pro and the Sysmex CS2500 and 5100 laboratory reference methods returned comparable results, and agree closely when compared across 4 independent sites.

Funding: This study was sponsored by LumiraDx UK Ltd. 09206123

PB0435 | Platelet Procoagulant Membrane Dynamics in Cardiac Surgery with Cardiopulmonary Bypass: Outcomes of a Pilot Study

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Background: Platelet dysfunction following cardio-pulmonary bypass (CPB) is a well-established contributor to perioperative haemorrhage and blood product transfusion, yet the underlying mechanism is unclear.

Aims: 1) Leverage the systematic analysis of platelet Procoagulant Membrane Dynamics (PMD), to gain new insights into platelet procoagulation and the haemostatic response following open-heart surgery with CPB. 2) Explore the potential clinical correlation between PMD elements and post-operative blood loss.

Methods: Impedance aggregometry and 4D confocal imaging. PMD analysis for 12 patients undergoing coronary artery bypass grafting was at 6 time-points, namely: baseline (T_0), following induction of general anaesthesia (T_{GA}), after 60-minutes of CPB (T_{CPB}), following administration of protamine (T_{PROT}), and at 6 and 24-hours after chest closure (T_6 and T_{24}).

Results: Platelets adhesion to fibrillar collagen diminished significantly at T_{CPB} compared to T_0 and persisted to T_{24} ($p< 0.01$), despite a minimal change in platelet count. Similarly, platelet ATP secretion in response to 3 µg/ml and p-selectin membrane expressions were reduced from T_{PROT} through T_{24}. In some patients, an increase in platelet oxidative stress was associated with diminished microvesiculation and exposed phosphatidylserine on the platelet membrane after CPB and up to T_{24} ($p< 0.05$). Platelet PMD analysis indicated that post-operative haemostatic plugs were smaller at T_{PROT}, T_6, and T_{24} ($p< 0.01$). These marked PMD changes were not reflected in whole blood aggregometry, which indicated no significant impairment of coagulation up to T_{24}. Preliminary fitted value for blood loss 24 hours after surgery vs PMD residuals plot (−18.54 mL to 11.579 mL; adjusted R2 = 0.9978) demonstrated good fit. Pilot LASSO regression analysis identified PMD parameters with non-zero regression coefficients, distinguishing them as promising variables for inclusion in our final model construction.

TABLE 1 ACL Elite Pro vs Sysmex (average results from 4 sites)

Laboratory	Slope	95% CI Upper	95% CI Lower	Intercept	95% CI Upper	95% CI Lower	n
Average over 4 sites	0.9470	0.9927	0.9019	0.1025	0.2070	−0.007072	101

TABLE 2 LumiraDx vs Sysmex (average results from 4 sites)

	Slope	95% CI Upper	95% CI Lower	Intercept	95% CI Upper	95% CI Lower	n
Average over 4 sites	1.001	1.060	0.9481	−0.06174	0.04839	−0.1860	223

Conclusions: PMD has the potential to provide novel mechanistic insights and reveal biomarkers of platelet/coagulation dysfunction, as well as correlate with bleeding following open-heart surgery requiring CPB.

PB0436 | Plasma Levels of von Willebrand Factor and Future Risk of Incident Venous Thromboembolism

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Background: Several case-control studies have shown elevated plasma von Willebrand factor (VWF) levels in patients with venous thromboembolism (VTE) compared to control subjects. As VWF is an acute phase reactant, it is not known whether VWF is actually a risk factor or merely a consequence of the acute VTE event. Limited information exists on the association between plasma VWF levels and future risk of VTE.
Aims: To investigate whether plasma VWF levels were associated with future risk of incident VTE.
Methods: A nested case-control study including 414 cases and 843 randomly selected age- and sex-matched controls was derived from a population-based cohort (the Tromsø study). Baseline information was collected by blood samples and physical examinations in 1994–95. Unconditional logistic regression was used to estimate odds ratios (OR) with 95% confidence intervals (CI) for VTE across quartiles of VWF determined from levels in the control group. Ethical approval and informed written consent were obtained.
Results: The risk of VTE increased linearly across quartiles of plasma levels of VWF (*P* for trend=0.001). Participants with VWF in the highest quartile had an OR of 1.82 (95% CI 1.28–2.60) for VTE compared to those in the lowest quartile in the age- and sex-adjusted model. The OR was marginally affected after adjustment for body mass index (1.69, 95% CI 1.18–2.42). The effect was most apparent for unprovoked VTE (OR 2.55, 95% CI 1.50–4.35) and a substantially higher risk for VTE was observed in samples taken shortly before the VTE event. The OR for unprovoked VTE was 4.41 (95% CI 1.62–12.0) when the follow-up time was restricted to 5 years.
Conclusions: We found a dose-dependent relationship between plasma VWF levels and future risk of incident VTE, and unprovoked events in particular. Our findings suggest that plasma VWF levels may represent a promising biomarker of future risk of incident VTE.

PB0437 | Circulating MicroRNAs and Platelet Extracellular Vesicles as Novel Biomarkers in Acute Ischaemic Stroke

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Background: Circulating microRNAs and extracellular vesicles (EVs) were found to be modulated in stroke.
Aims: We identified all miRNAs as well as determined EVs from platelets (PEVs), leukocytes (LEVs) and endothelial cells (EEVs) as biomarkers to diagnose stroke.
Methods: Blood collection at days 1 / 7 after the onset of stroke patients, and controls. Plasma EVs: Flow cytometry (Apogee A60); pletelets (CD61, CD62p; PEVs), leukocytes (CD45; LEVs) and endothelial cells (CD146; EEVs). Plasma RNA extraction: mirVANA Kit and quality assesment: Agilent 4200 TapeStation. MiRNA profiling: Affymetrix GeneChip 4.0.
Results: Patient with stroke had higher platelet reactivity comparison to controls (ADP p< 0.001, TRAP p< 0.001). Most down-regulated; miR-3135b (p< 0.001), associated with vascular calcifications/HF; miR-1275 (p=0.028), involved in atherosclerosis; miR-4467 (p=0.003), and miR-7170 (p< 0.001) modulated in neurodegenerative diseases. Most up-regulated; miR-18a (p=0.004), associated with stroke/FraminghamCohort; the platelet-enriched miR-22-5p (p=0.004), modulated in Huntington Disease; miR-199a (p=0.012), marker of brain microvascular injury/stroke severity, miR-106b (p=0.009), regulator of neural stem-cell proliferation/differentiation was found (figure1). PEV concentrations were elevated, both at day 1 / 7 (p=0.035, p=0.059, respectively). LEVs were comparable at day 1 (p=0.83) and decreased at day 7 (p=0.059), whereas EEVs decreased at day 1 (p=0.048) and normalized to control levels at day 7 (p=0.91) (figure2).
Conclusions: Several miRNAs were identified that are known to play a role in the pathophysiology of neurovascular diseases, paving the way to a new class of smart pathophysiology-based biomarkers in stroke. PEVs concentrations are elevated in patients with stroke

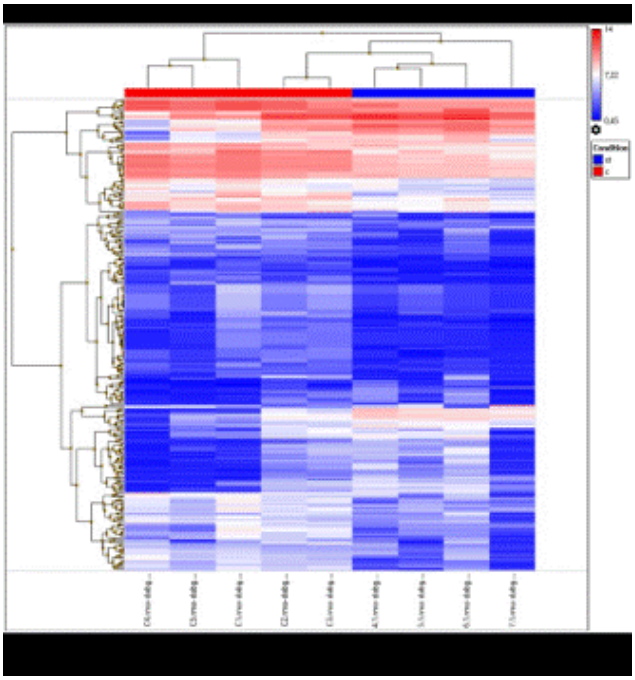

FIGURE 1 Heatmap showing results of miRNA profiling. Microarray results from the Affymetrix GeneChip miRNA 4.0 Array

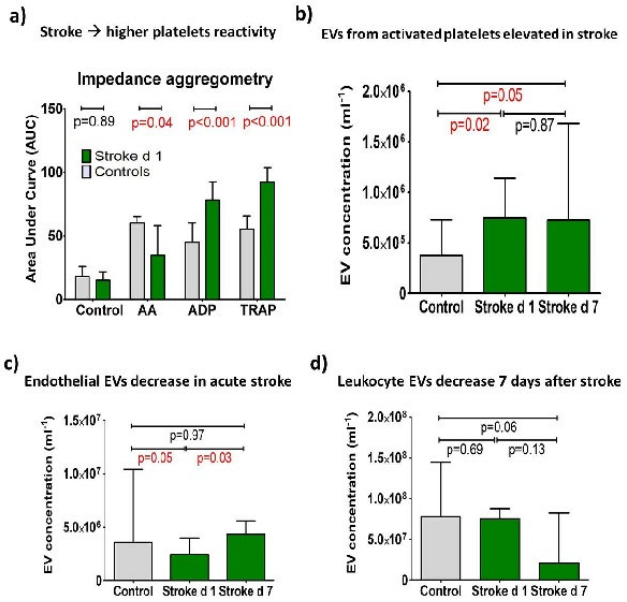

FIGURE 2 Impedance aggregometry and extracellular vesicles concentrations from activated platelets, endothelial cells and leukocytes

both at day 1 and day 7, compared to controls. LEVs and EEVs are fluctuate between day 1 and day 7 after stroke, likely reflecting activation of immune system and endothelium following brain damage.

Study was supported by Polish Science Center grant ''Preludium'' number 2017/25/N/NZ5/00545.

PB0438 | The Presence of Anti-Phosphatidylserine/Prothrombin Antibodies Enhance Differences in the Thrombin Generation Phenotype among Subjects with Lupus Anticoagulant Not Meeting Antiphospholipid Syndrome Criteria

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Hospital Universitario Fundacion Jimenez Diaz, Madrid, Spain

Background: The lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and anti-β_2-glycoprotein-I (β_2GPI) are the laboratory criteria of antiphospholipid syndrome (APS). In the last decade, anti-phosphatidylserine/prothrombin (PS/PT) antibodies have emerged as a possible additional marker for APS. An increase in thrombin generation (TG) is correlated with prothrombotic status.

Aims: To evaluate whether the presence of anti-PS/PT influences TG phenotype of LA-positive subjects.

Methods: We evaluated samples from 80 LA-positive patients in which both a complete APS laboratory profile [aCL/anti-β_2GPI/LA] and anti-PS/PT were assayed. Cases were prospectively selected at Hospital Universitario Fundación Jiménez Díaz in Madrid from May 2018 to June 2019. ACL, anti-β_2GPI and anti-PS/PT were determined by automated chemiluminescence using BIO-FLASH® technology platform (Instrumentation Laboratory, MA, USA). None of them met clinical criteria for APS since they had no historical evidence of thrombosis or gestational complications. Thrombophilia study (Factor V Leiden, Prothrombin G20210A, antithrombin, protein C and protein S) was negative in all of them. LA was determined according to ISTH guidelines, using both diluted Russell Viper Venom time (dRVVT) and Silica Clotting Time (SCT) integrated tests. Both LA tests were measured on ACL-TOP700 analyzer (Instrumentation Laboratory, MA, USA), and results were expressed on normalized *ratios* (our cut-off values: dRVVT>1.2 and SCT>1.3). Calibrated automated TG was measured in PPP using ST-Genesia® analyzer (Diagnostica Stago, Asnières sur Seine, France). TG-derived assessed parameters were: lag time, peak height, time-to-peak, endogenous thrombin potential (ETP), velocity index (Vel.index), and ETP%inh (percentage of ETP inhibition after adding thrombomodulin).

Results: Results are summarized in Figure 1.

Conclusions: The presence of anti-PS/PT increased both lag time and time-to-peak in single LA-positive subjects, and diminished the sensitivity to thrombomodulin (higher activated protein C resistance). A similar tendency was observed in double and triple positive subjects, but the sample size was probably insufficient to demonstrate significant differences. Further studies are needed to confirm these findings.

	Single positive			Double positive			Triple positive		
Anti-PS/PT	NEG n= 11	POS n= 37	*p*	NEG n= 2	POS n= 11	*p*	NEG n= 1	POS n= 18	*p*
Lag time (min)	2.77 ± 0.75	3.89 ± 0.96	0.001	2.45 ± 0.02	4.84 ± 1.74	0.088	2.14	5.20 ± 1.49	-
Peak height (nM)	242.34 ± 85.49	226.90 ± 61.09	NS	259.45 ± 67.38	218.14 ± 52.84	NS	260.6	234.04 ± 42.11	-
Time to peak (min)	5.39 ± 0.94	6.57 ± 1.35	0.009	4.93 ± 0.11	7.42 ± 1.91	0.103	4.35	7.83 ± 1.92	-
ETP (nM.min)	1336.5 ± 409.1	1241.14 ± 268.1	NS	1372.01 ± 345.07	1197.04 ± 267.9	NS	1279	1280.85 ± 178.9	-
Vel. index (nM/min)	127.94 ± 71.99	119.28 ± 54.69	NS	132.80 ± 28.14	110.70 ± 42.08	NS	151.7	121.53 ± 42.02	-
ETP %inh (%)	57.55 ± 16.95	38.83 ± 20.41	0.009	52.44 ± 4.43	33.91 ± 14.81	0.117	45.96	23.75 ± 12.22	--

FIGURE 1 Comparison of TG-derived parameters (mean ± SD) depending on the presence of anti-PS/PT and the APS profile. Mann-Whitney U test was used

PB0439 | Canonical Signaling Pathways Dysfunctions in the Development of Major Cardiovascular Complications, Circulating Micrornas as Novel Biomarkers and their Link to Inflammation in Diabetic Patients

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Background: Cardiovascular disease (CVD) is a major cause of mortality among people with type 2 diabetes (T2DM).

Aims: We performed gene expression profiling (GEP) from T2DM patients plasma that had developed a major cardiac events during 6 years follow up, compared to patients that had not. Prognostic ability was assesed of selected circulating miRNAs to predict CV complication.

Methods: Six patients per group were randomly selected, from 304 T2DM patients from AVOCADO study. RNA extraction: mirVANA PARIS Kit, quality of RNA check: fluorometric assay. GEP analysis: Clariom D pico chips, Affymetrix. PCR validation in 238 patients, Taqman universal. MiRNA related to platelet function/antiplatelet treatment were chosen among those with the most relevant modulation between the groups.

Results: Profiling results showed a significant modulation involved in: i) EIF2 ii) EIF4 and iii) mTOR signaling in the development of cardiovascular complications. In fact, a highly significant differential expression was found both at unpaired and paired analysis after matching samples for sex, age and cardiovascular risk profile. MiR-223, miR-126, Let-7e, miR-16, and miR-125a-3p were measured in 283 patients (47 patients with primary end point). We found that miR-126 (p< 0.000), Let-7e (p< 0.000), miR-16 (p=0.003), and miR-125a-3p (p=0.001) were able to predict future occurrence of the primary endpoint. MiR-16 and miR-125a-3p showed week positive correlation with hsCRP levels (r = 0.190, p =0.005 and r=0.166, p=0.013, respectively). Besides, miR-223 were positively correlated with HbA1c (r = 0.147, p =0.027).

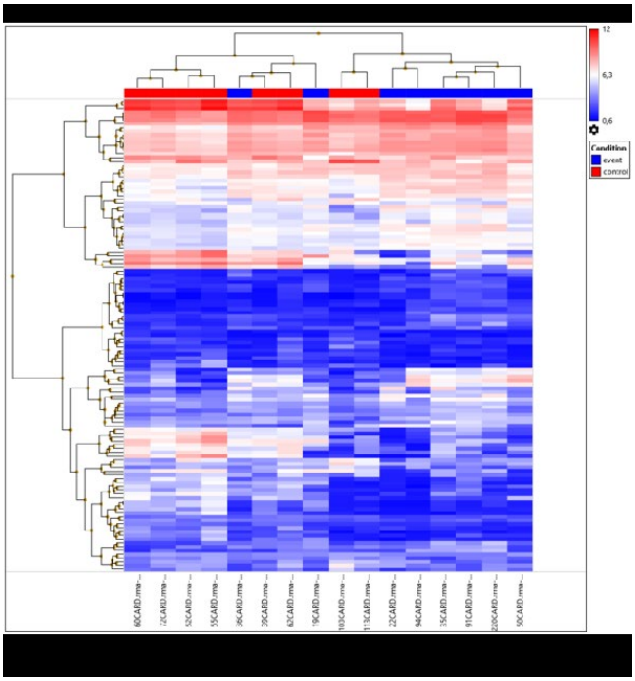

FIGURE 1 Heatmap, Microarray results from the Affymetrix GeneChip miRNA 4.0 Array

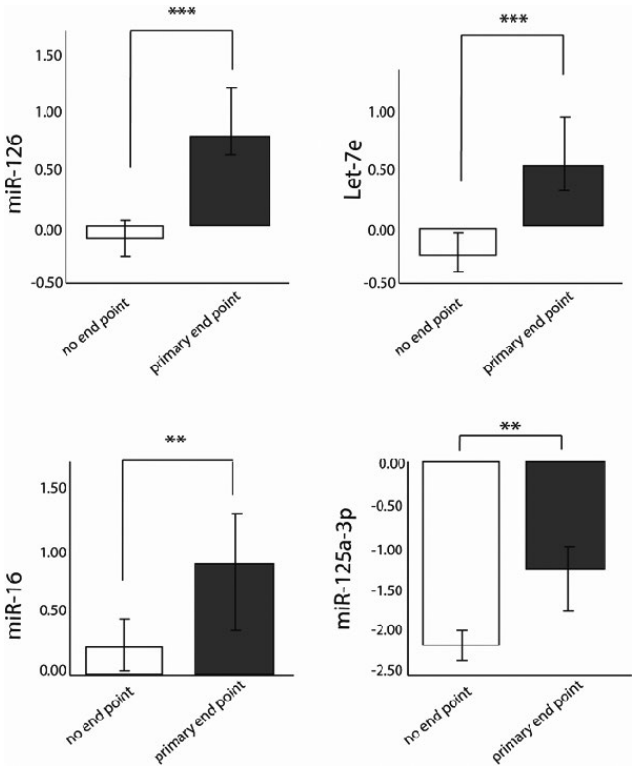

FIGURE 2 Comparison of miRNA levels based on primary end points. miR-126 (p<0.000), Let-7e (p<0.000), miR-16 (p=0.003), and miR-125a-3p (p=0.001)

Conclusions: Our results demonstrate a strong association between dysfunction of key canonical pathways, such as EIF2, EIF4 and mTOR signaling pathway and the risk of major cardiovascular complications in T2DM patients. Additionally, miR-126, Let-7e, miR-16, and miR-125a-3p might be useful for the clinical risk assessment during the follow up in high risk patients with chronic inflammatory conditions, such as diabetes, reflecting the residual risk to guide their clinical management. Supported by Polish Science Center "Preludium" 2017/25/N/NZ5/00545

PB0440 | The Importance of Altered Symmetric Dimethylarginine Levels with Platelet Hyperreactivity in Patients with Acute Coronary Syndrome Undergoing Percutaneous Coronary Intervention

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Background: Myocardial infarction (MI) is the leading cause of mortality and is a condition where platelet hyperreactivity is commonly present. Nitric oxide (NO) likely plays a pivotal role in the pathogenesis platelet hyperreactivity. Methylated arginines-asymmetric dimethylarginine (ADMA) and its structural isomer symmetric dimethylarginine (SDMA)-are endogenous by-products of proteolysis that inhibit NO production.

Aims: In particular, we sought to identify the impact of impaired nitric oxide (NO) signaling to platelet reactivity in patients with acute coronary syndrome (ACS).

Methods: We assessed plasma ADMA and SDMA levels and analyzed the data, biospecimens, and adenosine diphosphate (ADP)-induced platelet aggregation from prospective, observational, multicenter ATLANTIS-SWITCH sub-study to assess its value in 344 consecutive ACS patients undergoing percutaneous coronary intervention (PCI). The plasma concentrations of SDMA and ADMA were evaluated using a Waters Acquity Ultra Performance Liquid Chromatograph coupled with a Waters TQ-S Triple-Quadrupole Mass Spectrometer. The mass spectrometer operated in the multiple-reaction monitoring (MRM)-positive electrospray ionization (ESI) mode.

Results: Hyper-responsiveness of platelets to ADP was inverse correlated with plasma concentrations of symmetric dimethylarginine (SDMA, $r=-0.252$, $p < 0.001$). Multivariate analysis identified that SDMA is a predictor factor for platelet hyperreactivity [adjusted OR 0.704, 95% CI 0.517–0.960; P =0.027].

Conclusions: We conclude that platelet hyperreactivity, where present in the context of ACS, may be engendered by impaired availability of NO signaling.

TABLE 1 Multivariate regression analysis of factors which correlates with platelet hyperreactivity

variable	Coefficient	p value	OR	2.5% OR	97.5% OR
SDMA	−0.351	0.027	0.704	0.517	0.960
Smoking	−1.303	0.126	0.272	0.051	1.445
Sex	−11.265	0.950	0.000	0.000	0.000
Diabetes	−13.891	0.980	0.000	0.000	0.000
Intercept	2.497	0.239	12.140	0.190	774.110

Study was supported by Polish Science Center grant "Preludium" number 2017/25/N/NZ5/00545.

PB0441 | Standardization of a Flow Cytometry Platelet Sialylation Assay, and reference Values for Beta-Galactose Exposure in Healthy Subjects

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Background: A correct identification of the causes of thrombocytopenia is crucial for the appropriate management of the patients. The major mechanisms underlying a reduced platelet count are decreased production and/or increased destruction of platelets. It is now known that, in many cases, multiple mechanisms may contribute to the development of thrombocytopenia, and hyposialylation/desialylation characterized by an increase of β-galactose exposure accelerate platelet clearance.

Aims: A reference range of β-galactose exposure in the healthy population was never defined. Therefore, this step is a prerequisite before to identify thrombocytopenic diseases linked to sialylation variation, paving the way for a more widespread use of oseltamivir treatment in the future.

Methods: Our goal in this study was to develop a robust and standardized flow cytometry test in platelet-rich plasma by using RCA lectin allowing β-galactose exposure measurement in a clinical lab.

Results: Several pre-analytical and analytical conditions have been evaluated (RCA concentration, stability, platelet count and the storage conditions after blood sampling, positive and negative controls). We proposed that platelet β-galactose exposure has to be expressed with a ratio $R^{RCA/FSC}$ taking into account platelet size. We then used this test to study platelet β-galactose exposure variability in a healthy population (127 adults) and we defined normal ranges. The analytical performances of our assay are good with acceptable precision results (within-run and between-day CV less than 12%).

Conclusions: Having reliable reference values is crucial for obtaining an adequate diagnostic accuracy. Based on our results, further investigations in large group of patients with thrombocytopenia will determine the value of this new biomarker.

PB0442 | Upregulation of Inflammatory Biomarkers in Pulmonary Embolism as Studied by a Biochip- Array Profiling Approach

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Background: The pathophysiology of pulmonary embolism (PE) is complex including both the systemic and localized involvement. Hemostatic activation, inflammatory processes, and cellular dysfunction, along with hemodynamic aberrations contribute to the progression and outcome of PE. Inflammatory mediators such as the interleukins, oxidative stress molecules, and other cellular products contribute to the overall pathophysiology.
Aims: The purpose of this study is to profile biomarkers of inflammatory processes in the baseline blood samples collected from patients diagnosed with PE.
Methods: Citrated blood plasma samples from patients with clinically confirmed diagnosis (n=119) were collected under an IRB approved protocol. These samples were retrospectively analyzed for such biomarkers as IL2, IL4, IL6, IL8, IL10, vascular endothelial growth factor (VEGF), gamma interferon (IFNG), tumor necrosis factor a (TNFa), IL1a, IL1b, monocyte chemotactic protein (MCP1), and epidermal growth factor (EGF). The Randox Signature System (Crumlin, North Ireland, UK) was used for profiling these markers. The control samples represented a pool prepared from healthy normal individuals for the comparison of each of these biomarkers.
Results: In comparison to the normal controls, marked increase in IL6 (53 fold), IL8 (8 fold), IL10 (6 fold), VEGF (0.9 fold), TNFa (0.9 fold), IL1a (0.5 fold), IL1b (0.7 fold), MCP1 (0.5 fold), EGF (4 fold). In contrast, IL2 (14%), IL4 (3%), and IFNG (25%) showed a decrease in the circulating levels in PE patients in comparison to the controls. Marked variations in the circulating levels of such biomarkers as IL6, IL8, IL 10 and VEGF were evident by a large scatter in the data. No correlation between the individual mediators profiled in this study was noted.
Conclusions: These results demonstrate that the pathophysiology of PE is associated with the activation of inflammatory processes resulting in the upregulation of various biomarkers. IL6, IL8, and IL10 showed the most significant increase suggesting the role of inflammation in PE.

PB0443 | Preanalytical Considerations for Evaluation of NETosis Biomarkers

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Background: With the large implication of NET in physiopathological processes and intensive use of biomarkers of NET in clinical fields, preanalytic standardization is required.
Aims: 1) Evaluate 3 types of blood sampling (serum, plasma citrate and EDTA) on DNA quantitation, MPO-DNA complex and DNase activity.
2) Evaluate the stability of these parameters in citrated plasma at 2, 6 and 24h after blood collection.
Methods: - Cell-free DNA was quantified using Quant-iT™ PicoGreen® dsDNA Reagent and MPO-DNA complex according a modified approach of the Cell-Death Detection Elisa kit. For plasmatic DNase activity we used a fluorometric determination in microplates containing salmon-DNA as substrate and recombinant human DNaseI as calibration.
- Preparation and storage of serum, plasma citrate and EDTA samples was carried out in accordance with the French guideline procedures.
- For Aim 1 we used blood from 10 healthy controls and for aim 2, 9 healthy volunteers and 9 patients with myeloproliferative neoplasms.
Results: - Aim 1: Cell free-DNA levels were significantly higher in serum. MPO-DNA quantification was not significantly different whatever the type of blood sampling; nevertheless with EDTA, the values were more dispersed. In citrated plasma we noted a significant increase of DNase activity compared to sera or EDTA plasma. This could be due a better preservation of DNase activity in citrate, due to lower deprivation of divalent ions.
- Aim 2: No significant variation was observed for DNA levels and DNase activity at the different time points. For MPO-DNA, we observed significant differences between 2–6h in citrate.
Conclusions: As highlighted by Meddeb in 2019, standardizing preanalytical conditions is required to compare results for clinical research. Based on our results, citrate blood sampling seems to be more relevant for a simultaneous analysis of biomarkers of NETosis. To maintain analyte integrity, sampling should be carried out no more than two hours after blood collection.

PB0445 | Cell-free DNA Is Elevated in Chronic Myeloproliferative Neoplasms: Correlation with Thrombosis and Disease Progression

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Background: Thrombosis remains the main complication in myeloproliferative neoplasms (MPN). Although age, previous thrombosis and JAK2V617F are associated with thrombotic risk, additional predictive factors are lacking. Cell-free(cf) DNA is associated with thrombosis in diverse prothrombotic conditions.

Aims: To assess whether cfDNA is useful as a biomarker for thrombosis and clinical outcome in MPN.

Methods: cfDNA was measured by fluorometry using PicoGreen.

Results: Circulating cfDNA was elevated in MPN (n=107) vs. controls (P< 0.0001). Levels were higher in myelofibrosis (MF) (n=46) vs. essential thrombocythemia (ET) (n=31) and polycythemia vera (PV) (n=30) (P< 0.001). cfDNA was higher in ET/PV patients with thrombosis vs. those without (P< 0.05), whereas no difference was found for MF. To evaluate whether cfDNA could identify patients at risk, PV/ET patients with elevated (>mean+2SD of controls) cfDNA were compared to those with normal levels; the frequency of thrombosis tended to be higher in the former, 42% vs. 21% (P=NS). Levels did not differ according to driver mutation or cytoreductive treatment. cfDNA was elevated in 76% MF patients; those with high-risk (DIPSS-Plus) disease had higher values vs. low-risk categories (P< 0.0001). To assess the association of cfDNA and outcome, cfDNA was dicothomized into high (upper quartiles) and low values. MF patients with high cfDNA had increased blasts (P< 0.01), ASXL1 mutations (P< 0.05), a trend for anemia and thrombocytopenia and shorter survival (P< 0.05). Levels decreased during ruxolitinib (P< 0.05). Correlation was found between cfDNA and tumour burden parameters, such as circulating nucleosomes, leukocyte counts and LDH.

Conclusions: The finding of higher cfDNA in patients with thrombosis should encourage prospective studies to assess its value as a predictive thrombotic marker in PV and ET. The association between cfDNA and advanced disease in MF suggests its potential role as a prognostic factor, although larger studies should evaluate whether it is independent of other prognostic parameters.

PB0446 | Influence of Antiphospholipid Antibodies on Arterial Thrombosis in Myeloproliferative Neoplasia Patients: One Centre Experience

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Background: The major complications of *BCR-ABL* negative myeloproliferative neoplasms (polycythaemia vera, essential thrombocythaemia and primary myelofibrosis) are thrombosis, the pathogenesis of which is multifactorial. In myeloproliferative neoplasia patients, circulating platelets are already activated and they secret higher levels of surface P-selectin and tissue factor. Anti-β2 glycoprotein 1 antibodies (β2GP1) bind to the β2GP1 receptors on the platelet surface, activating them that is important for thrombus formation. Antiphospholipid antibodies activate endothelial cells, whose dysfunction also plays an important role in the pathogenesis of arterial thrombosis in *BCR-ABL* negative myeloproliferative neoplasia.

Aims: Our aim was to evaluate the antiphospholipid antibodies in relationship with the risk of arterial thrombosis in combination with other risk factors in patients with MPN's.

Methods: The study was conducted at the Lithuanian University of Health Sciences. It included 108 patients with *BCR-ABL* negative MPN. Data of previous arterial thrombosis and thrombosis risk factors were collected. There were two different screening and one confirmatory test for the lupus anticoagulant determination. The detection of antibodies against β2-glycoprotein-1 (β2GP1) was performed using a chemiluminescent method. The detection of antibodies against cardiolipin (aCL) was performed by ELISA using commercial Aeskulisa Cardiolipin-GM (AESKU.DIAGNOSTICS, Germany).

Results: Arterial thrombotic complications were present in 49 (45.4%) of those with MPN. These patients were diagnosed with transient ischaemic attack (TIA) or ischaemic stroke more often than other arterial complications. aPL were significant more frequently found in TIA and ischaemic stroke patients compared to MPN patients with other

arterial thrombotic complications or MPN patients without thrombosis, respectively (38.5%, 29.4% and 4.6%; p< 0.001).

Conclusions: Myeloproliferative neoplasm's patients with ischaemic stroke or TIA should be screened for antiphospholipid antibodies that increase the risk of thrombosis development in patients with *BCR-ABL* negative myeloproliferative neoplasms.

PB0447 | Evaluation of Plasma Level of Soluble CLEC2, a Novel Platelet Activation Marker, in Patients with Sepsis Induced Coagulopathy -A Pilot Study

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Background: C-type lectin-like receptor 2 (CLEC2) is a platelet-activated receptor expressed on the surface of platelet membranes and soluble CLEC2 (sCLEC2) is receiving attention as a predictive marker for evaluating thrombotic predisposition such as cerebral or myocardial infarction.

Aims: We examined the relationship between plasma sCLEC2 level and the degree of coagulation disorder, especially platelet activation in patients with sepsis-induced coagulopathy (SIC).

Methods: This is a single-center retrospective observational study from April 2015 to March 2018. 71 sepsis patients were enrolled and divided into sepsis with disseminated intravascular coagulation (DIC) and without DIC at the time of ICU admission, according to Japanese Acute Medical Association (JAAM) DIC criteria (JAAM DIC score ≥4). In addition, 37 healthy adult volunteer were enrolled as a control. We measured plasma sCLEC2 level by ELISA and compared with this level in each group.

Results: Since we suspected sCLEC2 levels were likely to be affected by platelet count, we attempted to calculate the sCLEC2/platelet count ratio (called the C2PAC index). The C2PAC index in healthy volunteer, sepsis without DIC and sepsis with DIC was 0.34 ± 0.15, 1.26 ± 0.69, 2.77 ± 2.44, respectively, and the C2PAC index of sepsis patients was significantly higher than that of healthy volunteers (p< 0.001). Moreover, the C2PAC index of sepsis patients with DIC was significantly higher compared with the sepsis patients without DIC (p< 0.001).

Furthermore, we divided the sepsis induced DIC patients into four groups (JAAM DIC 0–1, 2–3, 4–5 and ≧6) using the JAAM DIC scoring system. The C2PAC index of each group were 1.18±0.62, 1.38±0.86, 2.41±2.47, and 3.91±2.20, and as the DIC score increased, the value of the C2PAC index also increased significantly (p=0.002).

Conclusions: We suggested that the evaluation of C2PAC index might be a predictor of SIC progression in sepsis patients.

PB0448 | Quantification of Neutrophil Extracellular Trap by Flow Cytometry: Technical Development

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Background: Neutrophil extracellular Traps (NET) are involved in various clinical settings such as thrombosis. They have been recently used as thrombosis biomarkers.

Aims: We aimed to develop a new reliable and reproducible method to quantify NETs using flow cytometry (FCM), and compare it to a quantitative ELISA assay for MPO/DNA complexes.

Methods: We used whole blood from 32 healthy donors and MPA to activate NETosis. To validate our gating protocol, we used various markers of detection and quantification of NETs (Myeloperoxydase (MPO), Propidium iodide (PI) and citrullined histone H3 (H3c)) and gated on the neutrophil window (targeting NET bound to neutrophil) and on cell fragments window (circulating NET remnants).

We then applied this test to 21 patients with *non-CML* myeloproliferative *neoplasms* (MPN), who are known to have more NETs than healthy controls.

Results: The triparametric gate MPO/IP/H3c+ showed significantly more events on MPA- stimulated neutrophil versus unstimulated samples (median: 10% vs 0.48%, p< 0.05) and offers the best specificity of quantification. Using the *Bland-Altman* difference plot, we observed that this triparametric gate offered the best statistical consistency, when compared to ELISA. However we did not report a significant correlation between both techniques.

Lastly, using FCM, we detected more NETs in MPN patients samples compared with healthy controls, both in the triparametric gate on neutrophil NET window and NET remnants.

Conclusions: NETs detection by FCM, is a recent, specific and reliable quantification methods of NETosis that allows rapid quantification of NETosis from whole blood. In this study, we have for the first time quantified NETs remnants by FCM. FCM and ELISA lack of correlation may be linked to the heterogeneity and complexity of NETs structures, which can affect MPO/AND complexes dosing. Finally, cytometric NETs detection on whole blood is probably the most closely reflect of NETosis *in vivo*.

PB0449 | Evaluation of the Accuracy of INR Values Measured by a POCT System in a German Clinic with Patients Taking Phenprocoumon as VKA Therapy

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Background: The LumiraDx INR Test is a new point of care *in vitro* diagnostic system for the quantitative determination of Prothrombin Time expressed as International Normalised Ratio (INR) at the point of care (POC). Within Europe, there are country to country differences in preferential vitamin K antagonist (VKA) prescription. In Germany and surrounding countries, Phenprocoumon is the most commonly prescribed. Agreement between the LumiraDx INR test and the IL ACL Elite Pro has been demonstrated previously in patients taking Coumadin.

Aims: The aim of this study was to evaluate the INR results in a German clinical setting with patients taking Phenprocoumon using the LumiraDx INR, and comparing the results to the laboratory reference methods, the ACL Elite Pro and Sysmex CS5100.

Methods: This study was an observational, cross-sectional study with 102 subjects (24 female and 78 male) taking Phenprocoumon. Ages ranged from 36–88 years. Devices used were CE marked for INR measurement, conducted by healthcare professionals. In part 1 (25 subjects), samples of venous whole blood were analysed on the LumiraDx instrument and compared to venous plasma INR determined by both reference methods. In part 2 (77 subjects) a comparison of capillary whole blood INR (direct fingerstick application) determined by LumiraDx INR Test was made to venous plasma INR determined by the laboratory reference methods.

Results: The LumiraDx INR Results were plotted against the reference methods in part 1 and 2 and showed strong agreement across the range 1.3–5.2 INR.

Conclusions: Different VKA anticoagulation therapies, i.e. warfarin and phenprocoumon, do not influence the accuracy of the LumiraDx INR Test results. Overall, the LumiraDx INR Test can be used by healthcare professionals at the POC to provide reliable INR monitoring of patients who are receiving VKA therapy.

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PB0450 | Comprehensive Platelet Phenotyping Supports the Role of Platelets in the Pathogenesis of Acute Venous Thromboembolism - Results from Clinical Observational Studies

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Background: The pathogenesis of arterial and venous thrombosis is in large part interlaced, however to which extend platelet function relates to acute venous thromboembolism (VTE) independent of the underlying cardiovascular profile is presently poorly investigated.

Aims: To compare platelet function variables between VTE cases and controls and to identify the most relevant characteristics associated with VTE from a large array (N=58) of clinical risk factors and platelet variables.

Methods: Platelet count, mean platelet volume (MPV), platelet aggregation in whole blood and by the light transmission aggregometry (LTA) in platelet rich plasma, platelet-dependent thrombin generation (TG) and platelet surface activation markers by flow cytometry were measured under standardized conditions.

Results: VTE cases (N=155) presented with lower platelet count and MPV compared to controls (N=139). Whole blood aggregation showed shorter collagen/Epinephrine closure times in cases, particularly within acetylsalicylic acid (ASA) users. LTA results were distinct according to therapy; within ASA users, higher aggregation

TABLE 1 Summary of the Method Comparison Results of the LumiraDx INR Test

Sample	Reference Method	Slope	Slope CI	Intercept	Intercept CI	r
Plasma part 1	ACL Elite Pro	1.055	[0.937, 1.161]	−0.156	[−0.375, 0.184]	0.981
Plasma part 1	Sysmex CS5100	1.038	[0.917, 1.136]	−0.104	[−0.300, 0.225]	0.982
Plasma part 2	ACL Elite Pro	1.002	[0.945, 1.073]	−0.029	[−0.173, 0.108]	0.959
Plasma part 2	Sysmex CS5100	1.000	[0.923, 1.000]	−0.050	[−0.093, 0.142]	0.975
Capillary Blood Part 2	ACL Elite Pro	0.951	[0.874, 1.033]	−0.028	[−0.248, 0.154]	0.949
Capillary Blood Part 2	Sysmex CS5100	0.923	[0.846, 1.000]	0.015	[−0.200, 0.186]	0.950

response after adenosine diphosphate (ADP), epinephrine, collagen and arachidonic acid was observed in cases vs controls. Within non-ASA users and/or subjects on anticoagulants, cases presented with lower aggregation after ADP and collagen vs controls. Lower platelet-dependent TG, higher platelet CD63 surface expression on resting platelets and lower PAC-1 expression after collagen/ADP in vitro stimulation further characterized VTE cases vs controls, independent of therapy. Lasso regression analysis identified 34 variables associated with VTE. Platelet related-biomarkers comprised 76% of the variables selected and presented with the highest predictive strength.

Conclusions: Comprehensive phenotyping of platelet function identified a large proportion of low responders to ASA in VTE cases. Lower platelet-dependent TG and lower platelet reactivity after ex-vivo stimulation characterized the "platelet exhausted syndrome" in cases compared to controls. Finally, from a large array of covariates including clinical profile, platelet biomarkers comprised 76% of all selected variables differentiating VTE cases vs controls.

PB0452 | Neutrophils Extracellular Traps (NETs) Contribute to Thrombin Generation (TG) Potential in Patients with Essential Thrombocythemia (ET)

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Background: ET is a myeloproliferative neoplasm characterized by a high rate of thrombotic complications. Our group has previously demonstrated that, in ET patients, TG-potential is increased, and neutrophils circulate in an activated status. Recent studies suggest that NETs can promote thrombus formation by several mechanisms, including TG induction.

Aims: In ET patients, we aimed to measure circulating levels of NETs and evaluate the influence on these levels of somatic mutations [i.e. JAK2V617F, Calreticulin (CalR)], and concomitant therapies. Furthermore, we aimed to assess whether a correlation may exist between plasma NETs levels and TG-potential.

Methods: Sixty-four ET patients (M/F: 27/37; median-age: 60 years) were analyzed: 35 JAK2V617F-positive and 19 CalR-positive; 26 on hydroxyurea+aspirin (HU+ASA), 5 on HU, and 20 on ASA therapy. Citrullinated Histone-DNA complexes were measured by an ELISA: results are expressed as ODx1000=mOD. TG was evaluated by the calibrated-automated-thrombogram (CAT) assay.

Results: ET patients displayed significantly higher NETs values compared to healthy controls (1,106±678 *vs* 635±423 mOD, $p< 0.05$). NETs inversely correlated with age: in ET patients, this correlation remained significant ($p< 0.05$) after correction for gender and therapies. ET CalR-positive patients (1,261±819 mOD; $p< 0.05$) showed significantly higher NETs levels compared to other subjects (JAK2V617F= 1,004±537 mOD; no mutations= 1,114±784 OD). After multivariate analyses corrected for gender, age and therapy, CalR mutation was still independently associated with elevated NETs. Significantly higher TG-potential was found in ET *vs* controls. High NETs levels were significantly ($p< 0.05$) associated with shorter time-to-peak and higher peak, also after correction for age, gender and HU therapy.

Conclusions: ET patients are characterized by elevated NETs and higher TG-potential, particularly in CalR-mutated subjects. We also demonstrate that NETs may significantly contribute to the *ex-vivo* increase of TG-potential in ET. Further studies are needed to assess the correlation between NETs levels and thrombotic risk.

PB0453 | Extensive Characterization of the Hemostatic Changes during Cytoreductive Surgery (CRS) with Hyperthermic Intraperitoneal Chemotherapy (HIPEC) in Patients with Peritoneal Carcinomatosis

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Background: CRS in combination with HIPEC is a therapeutic option for treatment of peritoneal carcinomatosis. It is a long and complex procedure with significant blood and fluid loss and important pathophysiological alterations, which lead to an important raise of the hemorrhagic risk.

Aims: In a cohort of patients with ovarian or colorectal cancers and peritoneal carcinomatosis, we aimed to characterize extensively the impact of CRS and HIPEC on perioperative hemostasis by a panel of global and specific coagulation assays.

Methods: 34 patients (21F/13M; age: 56 years) were recruited after informed consent. Rotational thromboelastometry (ROTEM), PT, aPTT, D-Dimer, fibrinogen, FVII, and FVIII levels were measured before and after CRS, and after HIPEC. Clotting time (CT), clot formation time (CFT) and maximum clot firmness (MCF) by ROTEM were evaluated. Platelet count, hemoglobin and transfusion needs were registered.

Results: Before surgery, patients showed significant prolongation of PT and EXTEM-CT, reduced FVII levels, and increased FVIII and D-dimer levels compared to controls. After CRS, there was a significant decline in plasma fibrinogen, FVII, platelets and hemoglobin levels, which were associated with prolongation of PT and EXTEM-CFT and reduction in EXTEM- and FIBTEM-MCF ($p< 0.01$). No changes were observed in FVIII levels, while D-dimer further increased. HIPEC did

not add any additional hemostatic changes. Significant correlations were found between fibrinogen and FIBTEM-MCF (R^2=0.7), and between platelet count and EXTEM-MCF and EXTEM-CFT (R^2=0.6). During surgery, 50% of patients received packed red blood cells [median 2U (1–6)], whereas 9% received fresh frozen plasma [median 1U (1–4)].

Conclusions: Our data show that CRS-HIPEC is associated with a pro-hemorrhagic state, secondary to a consumptive coagulopathy, determining an increased bleeding risk. ROTEM is able to capture the dynamic and different hemostatic changes during the complex CRS-HIPEC procedure, making this point-of-care a useful complementary tool in this setting to inform clinicians on hemostatic intervention needs.

PB0454 | The Role of Phosphatidylserine-Exposing Microparticles and Glycophorin-Exposing Microparticles in Hemostasis System in Children with Hemolytic Anemia

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Background: Patients with hemolytic anemia (HA) are characterized by the increased risk of thrombosis.

Aims: To investigate the role of microparticles in hemostatic state in children with HA.

Methods: The hemostatic status of 4 children (boys, age: 4÷12yr) with hereditary spherocytosis (HS) and 6 children with β-thalassemia major (β-TM)(girl, 5 boys, age: 2÷15yr) was assessed using clotting times and D-dimer levels. The global hemostasis assay, thrombodynamics(TD), was used to evaluate the hypercoagulation state in patients and the procoagulant activity of microparticles. The concentration of microparticles was assessed by flow cytometry.

Results: TD parameters were increased in 6 children (2 with HS and 4 with β-TM) compared to normal range (59.2±1.9μm/min for clot growth rate, normal range 38–56μm/min): they had transfusion-dependent anemia. The other 4 patients have non-transfusion-dependent anemia and were in normal range by TD. APTT, TT and PR were normal in all patients. The patients with hypercoagulation had the highest concentration of phosphatidylserine-exposing microparticles ($^{PS+}$MP) and glycophorin-exposing ($^{CD235a+}$MP) microparticles identified by flow cytometry through the use of antibodies compared to patients in normal range by TD (1617±579^{PS+}MP/μl vs 557±199^{PS+}MP/μl;105±62$^{CD235a+}$MP/μl vs 40±15$^{CD235a+}$MP/μl). Interesingly, the patient with highest clot growth rate (62.2μm/min) and highest D-dimer (506ng/ml, range 0–243ng/ml) had the highest concentration of microparticles (1939^{PS+}MP/μl;205 $^{CD235a+}$MP/μl).

To compare the microparticles activity from plasma of patients and of 5 healthy donors we measured TD parameters dependence on microparticles concentration in the pool of microparticles depleted plasma of healthy donors. At similar concentrations the influence of microparticles from plasma of patients and of healthy donors on the clot growth did not differ significantly.

Conclusions: The global hemostasis test TD revealed hypercoagulability in patients with HA. More dramatic changes were observed in patients with the highest concentrations of phosphatidylserine-exposing and glycophorin-exposing microparticles. The microparticles impact to hypercoagulation was explained by increase in concentration while their activity did not differ from that of healthy donors.

PB0456 | Usefulness of D-dimers Levels Determination in the Prediction of Thromboembolic and Cardiovascular Events in Patients with Atrial Fibrillation during Anticoagulant Therapy

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Background: Atrial fibrillation (AF) is associated with hemostatic abnormalities even during oral anticoagulant therapy. D-Dimer levels reflect a prothrombogenic state and thus might serve as a marker of thromboembolic (TE) and cardiovascular events.

Aims: The aim of the present study was to evaluate whether elevated D-Dimer levels can predict subsequent thromboembolic and cardiovascular events in patients with atrial fibrillation during oral anticoagulant therapy.

Methods: This was a prospective, cross-sectional study. Patients with AF (41 patients, mean age 63.6 ± 9.38) were treated with vitamin K antagonist (Target Prothrombin level-International Normalized Ratio: 2.0 to 3.0). D-Dimer levels were measured to evaluate the relationship of this parameter with thromboembolic and cardiovascular events.

Results: The average level of D-Dimer was 356.82 ± 497 (20–3070) at entry and 215.88 ± 127.08 after one moth. This rate was high (> 500μg/l) in 05 patients (12.20%). During a follow-up period of four months, 02 patients developed thromboembolic event (Stroke), 01 patient had cardiovascular event (heart failure) and 03 patients had bleeding complications. Patients with high D-Dimer levels experienced higher thromboembolic and cardiovascular events. It was found that high d-Dimer levels were associated with TE ($\rho < 0.05$) and cardiovascular ($\rho < 0.05$) complications.

Conclusions: D-Dimer is a useful marker for thromboembolic and cardiovascular events in patients with atrial fibrillation during oral anticoagulant therapy.

PB0457 | Analysis of Biomarkers of Hemostatic Dysregulation and Inflammation in Lymphoma Patients

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Background: Several biomarkers are associated with the occurrence of venous thromboembolic events (VTE) in cancer patients but the role of inflammation and hemostasis dysregulation in pathophysiology of VTE in lymphoma patients is not clarified yet.

Aims: The aim of this study was to profile hemostatic and inflammatory biomarkers in patients with aggressive and indolent non Hodgkin and Hodgkin lymphoma and to validate potential connection with development of thrombotic events.

Methods: Plasma samples were collected from lymphoma patients (n=62) treated at Clinic for hematology, Lymphoma Center, Clinical Center Serbia. Samples were analyzed to profile biomarkers of coagulation activation (von Willebrand Factor (vWF), Factor XIIIa, Protein S, β_2Glycoprotein I (β_2GPI), D-dimer, Microparticles (MP), Urokinase-type Plasminogen Activator Antigen (uPA), Fibronectin) and inflammation (Human Tumor Necrosis Factor- α (TNF-α), C-Reactive Protein (CRP), Plasminogen Activator Inhibitor Type 1 (PAI-1)). The lymphoma patient population for each biomarker was compared to normal human pooled plasma.

Results: We analysed 41 patients (66.1%) with aggressive non-Hodgkin lymphoma (NHL), 13 (21%) with indolent NHL and 8 (12.9%) with Hodgkin lymphoma (HL). All three groups of lymphoma patients had significantly higher following markers of coagulation (vWF, d-dimer, Factor XIIIa fibronectin) and inflammation (PAI-1 and CRP) compared to healthy controls, except indolent NHL and Hodgkin lymphoma to PAI and vWf, respectively (Figure 1). In lymphoma population 10 patients had thrombosis. Patients with thrombosis had significantly higher Factor XIIIa in comparison to lymphoma patients without thrombosis (103.1 (range 94.6–122.1) *vs.* 91.3 (range 24.4–128.6) %; $p < 0.005$).

Conclusions: This study confirmed significant hemostatic dysregulation and inflammation in our group of lymphoma patients, which may reflect increased thrombogenic potential. Further studies are required to link clinical data and biomarker levels, with special emphasis on the involvement of FXIII in the development of thrombotic events in lymphoma patients.

PB0458 | Clot Contraction Drives Structural Redistribution of Platelets, Fibrin and Red Blood Cells through Active Cell Sorting

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Background: Blood clots or intravascular thrombi undergo volume shrinkage driven by activated platelets. Clot contraction results in the compression of red blood cells (RBCs) into polyhedral structures (polyhedrocytes) in the core of the clot, while most fibrin and platelets concentrate at the clot periphery.

Aims: Determine the physical mechanisms driving this segregation.

Methods: The redistribution of the components in contracting clots were quantified using histology and scanning electron microscopy. Coupling experimental data with an active mechanochemical model allowed us to analyze why this segregated distribution was more favorable than a homogenous distribution.

Results: Platelets exert tension on the fibrin network leading to pressure on and compression of the RBCs. RBC compression and densification of fibrin leads to stiffening of the overall clot. A stiffer clot yields more resistance to platelet contraction, upregulating mechanosensitive pathways in platelets that trigger the generation of larger contractile forces and decreased active potential. This results in a lower energy for the overall system coupled when compared to a hypothetical homogenous clot distribution, therefore revealing an active cell sorting mechanism drives the structural non-uniformity of contracting clots. The finding that contraction drives the structural segregation of clots due to minimization of active and passive energy provides a theoretical basis for this important biological phenomenon.

Conclusions: Structural remodeling of blood clots contributes to the course and outcomes of thrombosis as it changes the properties of intravital blood clots and thrombi, including their ability to block a vessel lumen as well as to withstand mechanical deformations, rupture, and enzymatic lysis.

PB0459 | Histidine-rich Glycoprotein Levels Contribute to Prolonged Clot Lysis Time in Acute Pulmonary Embolism

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Background: Histidine rich glycoprotein (HRG) displays anticoagulant and antifibrinolytic properties in animal models, but its effects in humans are unclear.

Aims: We investigated serum HRG levels and their associations with the disease stage and prothrombotic alterations in acute pulmonary embolism (PE) patients.

Methods: In 114 adult patients with acute symptomatic PE aged 57.1±14.5 years we measured HRG levels in association with clot permeability (K_s), clot lysis time (CLT), and endogenous thrombin potential (ETP).

Results: Patients with intermediate-high (n=25) compared to intermediate-low (n=73) or low PE mortality risk had increased HRG levels. Based on optimal cut-off value of 87 µg/ml (sensitivity, 92% and specificity, 50%, area under curve 0.711, 95% confidence interval 0.564–0.858, p=0.0048), which discriminated PE patients with intermediate-high PE risk compared to the remainder, increased HRG was found in 62 (54.5%) PE patients. Individuals with HRG values above the cut-off compared to the remainder did not differ with regard to demographic and clinical variables, except higher incidence of coronary heart disease in subjects with higher HRG levels. Increased HRG levels were associated with 11.8% higher ETP, 16% prolonged CLT, and 10.9% lower K_s (all p< 0.01). HRG was associated with N-terminal brain natriuretic propeptide levels (r=0.43, p< 0.0001), factor VIII activity (r=0.19, p=0.047), and ETP (r=0.36, p< 0.0001). Moreover, we found correlations of HRG with CLT (r=0.48, p< 0.0001) as well as with PAI-1 (r=0.43, p< 0.0001) and plasminogen (r=−0.21, p=0.027) levels. Thrombophilic patients did not differ from the remainder regarding HRG levels.

Conclusions: Our study is the first to show that HRG levels are associated with the disease stage and hypofibrinolysis in acute PE patients. This work was supported by the Polish National Science Centre (UMO-2015/B/NZ5/02215 to A.U.).

PB0460 | Platelet-Associated Tissue Factor Predicts 5-Years Mortality in Coronary Artery Disease Patients

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Background: Improvement of thrombotic risk stratification in coronary artery disease (CAD) patients is a major challenge. The currently used risk scores, designed to predict mortality and/or the incidence of adverse cardiovascular events, include clinical and laboratory parameters. None of them takes into consideration the increased platelet activation status, although it has been consistently reported in CAD.

Aims: To evaluate the capacity of platelet-associated activation markers, assessed by whole blood flow cytometry at hospital admission, to predict mortality in CAD patients.

Methods: Surface expression of P-selectin, activated GPIIbIIIa, Tissue Factor [TF] was evaluated in 624 CAD patients (73% stable, 27% NSTEMI) and associated with 5-years all-cause mortality by COX regression model. Euclidean distance method was used to calculate the best cutoff value of platelet-associated TF expression and adjusted (age, gender, diagnosis, antiplatelet therapy, kidney function) Kaplan-Meier survival analysis was performed. The capacity of TF to improve the mortality prediction on top of TIMI Risk Score for Secondary Prevention (TRS2P) was calculated by integrated discrimination improvement (IDI).

Results: All-cause mortality rate was 9.7%. Multivariate analysis indicated that, among the platelet activation markers analyzed, only TF was an independent predictor of all-cause mortality (HR=2.02; 95% CI 1.03, 3.98). In a ROC curve analysis, a cut-off value of 4% TF-positive platelets was calculated. Patients were classified into 2 groups according to TF-cutoff. Adjusted Kaplan-Meier survival analysis showed that patients with levels of TF-positive platelets >4% had worse prognosis (p< 0.0001) compared to those with < 4% TF-positive platelets. Moreover, when the predictive capacity of platelet-associated TF was analyzed on top of TRS2P, it increased the AUC from 0.719 to 0.732 with $IDI_{(TRS2P+TF\ vs\ TRS2P)}$=0.012 (p=0.018).

Conclusions: Our findings highlight platelet-TF as novel marker useful to implement risk stratification since, among the platelet-associated activation markers, it is an independent predictor of all-cause mortality in CAD patients.

PB0461 | Dyslipidaemia Associated with Increase of Thrombin Generation in Renal Transplant Recipients

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Background: Dyslipidaemia is common adverse outcomes in Brazilian Renal Transplant Recipients (RTR) which are correlated with an increase in cardiovascular risk, mainly associated with other comorbidities, such as atherosclerosis and thrombotic diseases. Thrombin holds a significant role in haemostasis. Its assessment may be a potential tool for cardiovascular risk stratification.

Aims: This study aims to explore the relation between thrombin generation and dyslipidaemia in RTR.

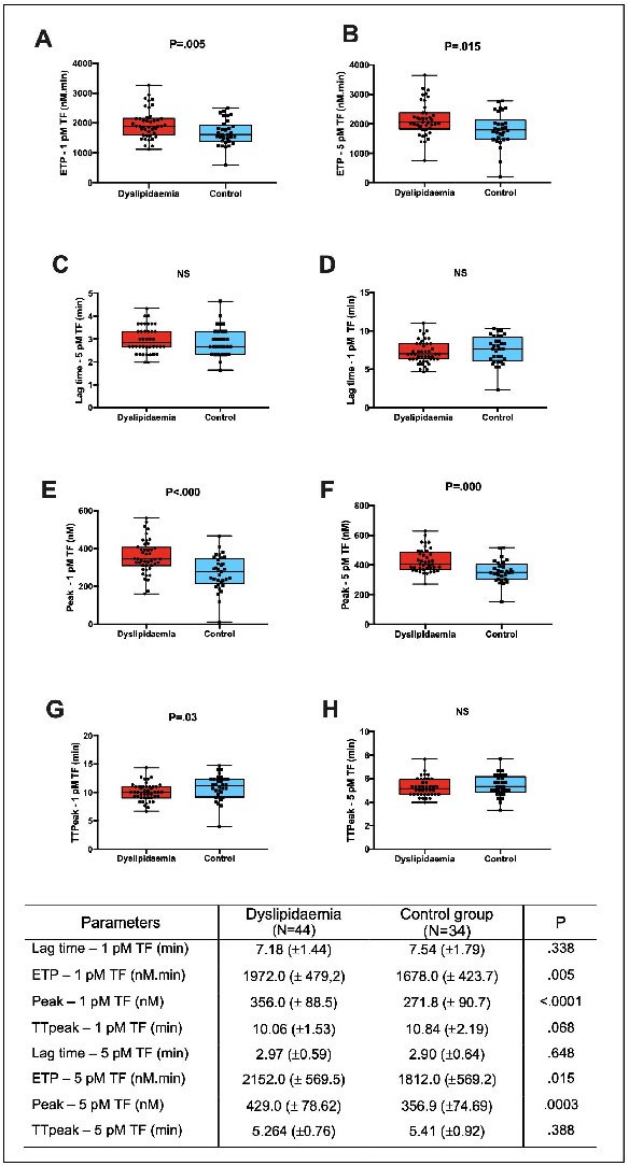
Methods: In this cross-sectional study, 78 RTR was included, 44 dyslipidaemic RTR and 34 normal lipidic profile RTR (control group). Informed consent was obtained and this study was approved by the local Ethics Committee. Lipidic profile values and other parameters were obtained from medical records. Thrombin Generation test (TGT) was performed by the Calibrated Automated Thrombogram (CAT) method at 1 and 5 pM concentration of tissue factors (TF) trigger in platelet-poor plasma. Lag time, endogenous thrombin potential (ETP), peak and time to peak (TTpeak) were acquired from the thrombin generation curve. Data were presented as mean and standard deviation (Unpaired t-test). Additionally, Spearman′s correlation test was performed. GraphPad Prism and SPSS software were used. P< .050 was significant.

Results: Dyslipidaemic RTR showed higher levels of ETP and peak at 1 pM and 5 pM TF concentrations and decreased TTpeak levels in 1 pM TF concentrations compared than the control group (Figure 1). Lag time and TTpeak were positively correlated with HDL cholesterol (P< .05 for both in 5 pM TF). Triglycerides were positively

TABLE 1 Spearman's correlation assay in renal transplant recipients

Parameters	r	*P
ETP 1 pM TF vs Cholesterol total	.247	.035
ETP 1 pM TF vs Triglycerides	.284	.015
ETP 1 pM TF vs non-HDL cholesterol	.252	.038
PEAK 1 pM TF vs Triglycerides	.362	.002
ETP 5 pM TF vs Triglycerides	.322	.007
PEAK 5 pM TF vs Triglycerides	.469	.000
Lag time 5 pM TF vs HDL cholesterol	.259	.034
TTpeak 5 pM TF vs HDL cholesterol	.280	.023

correlated with ETP and Peak in both TF concentrations (Values of P< .00 for all) (Table 1).



Parameters	Dyslipidaemia (N=44)	Control group (N=34)	P
Lag time – 1 pM TF (min)	7.18 (+1.44)	7.54 (+1.79)	.338
ETP – 1 pM TF (nM.min)	1972.0 (± 479,2)	1678.0 (± 423.7)	.005
Peak – 1 pM TF (nM)	356.0 (+ 88.5)	271.8 (+ 90.7)	<.0001
TTpeak – 1 pM TF (min)	10.06 (+1.53)	10.84 (+2.19)	.068
Lag time – 5 pM TF (min)	2.97 (±0.59)	2.90 (±0.64)	.648
ETP – 5 pM TF (nM.min)	2152.0 (± 569.5)	1812.0 (±569.2)	.015
Peak – 5 pM TF (nM)	429.0 (± 78.62)	356.9 (±74.69)	.0003
TTpeak – 5 pM TF (min)	5.264 (±0.76)	5.41 (±0.92)	.388

FIGURE 1 Thrombin generation test in Brazilian Renal Transplant Recipients allocated in groups of dyslipidaemia and control

Conclusions: The presence of dyslipidaemia was associated with a procoagulant profile between RTR, which may favour thrombotic diseases and, consequently, allograft rejection and death. The TGT may be a promising tool for the management of renal transplanted recipients, which deserves further investigations.

Support: Capes and CNPq.

PB0462 | Active Thrombin and its Value: New Approach to Diagnosis of Thrombosis?

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Background: The diagnosis of thrombosis is heavily dependent on D-dimers determination. Evaluation of D-dimers is one of the most used diagnostic tools for assessment of thrombogenic state, but their all-embracing meaning is hindering their diagnostic value. The determination of active thrombin might be an additional independent important value in the D-dimers assay.

Aims: Aim of this study was to determinate the presence of active thrombin bound to D-dimers in various pathological condition.

Methods: Patients samples were obtained in accordance with The Ethic committees of participating institutions (IHBT, Prague and MUH, Prague). Qualifying criterions were elevated D-dimers and consent to the study. From 98 patients, medical history and treatment protocols were acquired from 83 of them.

Results: The presence of active thrombin bound to D-dimers was shown to be linked with thrombotic diagnosis - more than 60% cases of thrombogenic states had active thrombin bound to D-dimers. More than 33% of other pathological conditions had active thrombin bound to D-dimers - out of them 70% was linked with complications or death.

Conclusions: The presence of active thrombin bound to circulating D-dimers has been proposed to help to differentiate between thrombogenic state and other pathological conditions linked with elevated D-dimers. Detection of active thrombin bound to D-dimers might be used as another qualitative step in assessment of the patient's condition and prognosis.

Acknowledgement: Authors would like to highlight the contribution of M Maly, J Suttnar, O Kucerka, J Louzil, P Salaj, M Zemanova, E Ceznerova, K Svobodova and I Hrachovinova to the acquisition of samples.

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PB0463 | FM and D-di in Preeclampsia and in vitro Fertilization

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Background: Coagulation activation is observed during pregnancy and its complications. Laboratory markers may rate the degree of activation and predict complications or help to determine prevention tactics. Fibrin monomer (FM) seems to be promising though less investigated than D-dimer (D-di) in pregnancy.

Aims: To compare the level of FM and D-di in women with normal pregnancy (NP), preeclampsia (PE) and in the process of in vitro fertilization (IVF).

Methods: 180 females were included: during IVF at the stage after the cycle of ovulation induction (n=78), with mild or severe PE (n=47) and uncomplicated NP (n=55) as the control. FM and D-di levels were measured by immunoturbidimetry (STA-Liatest FM and STA-Liatest D-Di, Stago, France). The results are presented as median, 2.5th–97.5th percentile, Mann-Whitney comparison p-value or rank correlation coefficient (r; p).

Results: The median FM level in NP did not exceed the reference interval for population and was 5 μg/ml (5.0–31.24). The median levels FM-IVF and FM-PE were the same but with larger spread of values in PE group - 5 μg/ml (5.0–16.71) and 5.0 μg/ml (5.0–97.09), respectively. There were significant differences in IVF (p=0.0457) and PE (p=0.0054) in comparison to NP. FM was also higher in PE vs IVF group (p< 0.0001). D-di was 0.50 μg/ml (0.27–1.82), 0.47 μg/ml (0.27–2.44) and 1.77 μg/ml (0.52–8.25) in NP, IVF and PE, respectively. Significant D-di differences were observed between control and PE (p< 0.0001), PE and IVF (p< 0.0001). We also observed FM and D-di correlation in PE (r=0.469; p=0.0013).

Conclusions: The results reflect a significant coagulation activity scatter in the studied groups, as well as in NP. However, women with PE show increased cross-linked fibrin formation (D-di) and coagulation activity in progress (FM), reflecting a higher VTE and complications risk. Further research is required to enable prediction of adverse outcomes based on the markers studied.

PB0464 | Identification of a Circulating Lipid Profile Associated with Venous Thromboembolism

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Background: Cell membrane plays an essential role in the different reactions that occur during coagulation, highlighting the presence of phospholipids, lipids and glycolipids, with pro- and anticoagulant actions.

Aims: To identify, through an advanced lipidomics analysis, the main lipid groups associated with venous thromboembolic disease (VTE).

Methods: Plasma samples of 30 VTE patients and paired 30 healthy volunteers were selected. We analysed the lipids present in the plasma samples by LC-MS/MS. The annotation of the lipid groups was carried out using the specific package of *R*: Lipid MS. Results were analysed by logistic regression analysis. We also analysed the correlation between lipid groups and coagulation parameters.

Results: A total of 379 lipids were identified in our plasma samples. These lipids were grouped according to the lipid class to which they belonged, identifying a total of 18 classes of circulating lipids, of which 11 had a statistical difference between both groups. We obtained decreased levels of CEs and increased levels of carnitines, ceramides, DGs, FAs, LPGs, MGs, PCs, PEs, PGs and SMs in patients compared to controls (P < 0.05). In addition, we found that FAs and SMs were associated with thrombotic recurrence (P < 0.05). Next, we performed a predictive model with the lipid classes with the highest discriminatory capacity between both study groups (LPGs, FAs, SMs and ceramides), and obtained an AUC of 0.940 (95% CI: 0.87–1). Finally, we observed an association between some of the lipid classes and free circulating DNA in plasma.

Conclusions: We have identified 11 plasma lipid classes associated with VTE. These results suggest the existence of new molecules and routes of action involved in the coagulation system. It would be necessary to carry out further studies to identify the specific lipids responsible for the effect on thrombotic risk.

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PB0465 | Circulating MicroRNA Levels Indicate Platelet Activation in Endotoxemia Despite Platelet $P2Y_{12}$ Inhibition

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Background: There is emerging evidence for effects of platelet inhibition on innate immune activation. Levels of circulating microRNAs (miRNAs, miR) have been implicated as markers of platelet and immune cell activation. Here, we assess the effects of ticagrelor and clopidogrel on circulating miRNA levels during low dose endotoxemia.

Aims: To investigate whether miRNA levels can indicate platelet activation in endotoxemia despite platelet $P2Y_{12}$ inhibition.

Methods: 30 healthy volunteers were randomly assigned to receive oral ticagrelor (n=10), clopidogrel (n=10) or no drug (n=10) for one week, followed by an intravenous bolus of 2ng/kg endotoxin. Serum was collected at baseline, after one week of antiplatelet treatment and 6 and 24 hours after endotoxin administration. 92 serum-miRNAs were screened using a custom-made LNA-based qPCR assay, followed by validation of 16 candidate miRNAs by TaqMan-qPCR (Figure 1).

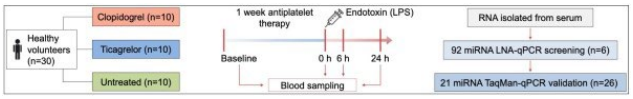

FIGURE 1

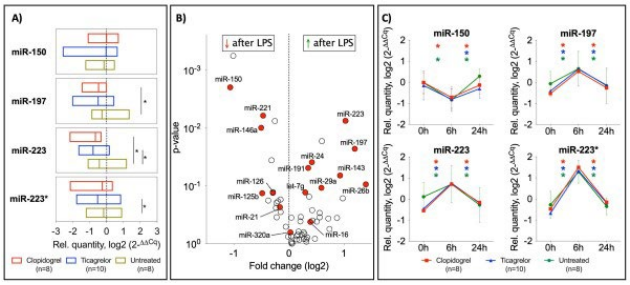

FIGURE 2

Results:

1. Levels of platelet-enriched miR-197, miR-223 and miR-223* were significantly lowerafter one week of antiplatelet therapy, whilst leukocyte-enriched miR-150 was not affected (Figure 2A).
2. Endotoxin administration markedly reduced levels of leukocyte-enriched miR-150, whilst the opposite effect was seen for platelet-enriched miR-197, miR-223 and miR-223* (Figure 2B).
3. Neither of these endotoxin-mediated effects on miRNAs were altered by $P2Y_{12}$ inhibitors. MiRNA levels returned to baseline within 24 hours (Figure 2C).

Conclusions:

1. Downregulation of miR-150 in response to endotoxin is in line with previous observations in critically ill sepsis patients, where miR-150 negatively correlates with survival.
2. Circulating levels of platelet-associated miRNAs are responsive to antiplatelet therapy, but $P2Y_{12}$ inhibitors fail to attenuate the rise of platelet miRNAs in response to low dose endotoxemia.
3. Further studies in sepsis patients may reveal whether circulating miRNAs can aid the prediction of clinical outcomes.

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PB0466 | Profiling of Inflammatory Biomarkers and Coagulation Factors in End-stage Renal Disease

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Background: Early diagnosis of chronic kidney disease (CKD) is necessary in preventing complications that develop into kidney failure and vascular complications. Currently, the detection of early stages of CKD is lacking predictive values, delaying the application of specific diagnostics and therapeutic interventions. End-stage renal disease (ESRD) patients are at risk for cardiovascular complications and vascular thrombosis, exhibiting numerous coagulation and fibrinolytic abnormalities.

Aims: Profiling inflammatory biomarkers and coagulation factors in ESRD patients may allow early onset detection and severity of kidney damage, furthering the understanding of the pathogenesis of this disease.

Methods: Citrated blood samples were collected from 95 confirmed ESRD patients undergoing maintenance hemodialysis under an IRB approved protocol. Biomarkers of inflammation and coagulation, including PAI-1, TAFI, CRP, MMP-9, MP, D-Dimer, Factor VII, IX, X, and XIII were measured using commercially available ELISA methods. Normal controls were comprised of commercially available male and female citrated plasma (George King Biomedical). All results were compiled as mean ± SEM, and percent change in comparison to normal human plasma.

Results: Biomarkers including CRP (7ug/ml ± 0.6, 183%), MMP-9 (88ng/ml ±8, 58%) D-Dimer (1059ng/ml±138), Factor IX (124%±3, 23%), and Factor XIII (104%±1, 27%) showed significant increase when compared to normal plasma. Elevated CRP indicates severe inflammatory response and elevated D-Dimer indicates endogenous fibrin formation. MP and PAI-1 suggest endothelial dysfunction.

Conclusions: These results indicate that the upregulation of inflammatory responses and hemostatic defects contribute to the multifactorial pathophysiology of ESRD. Profiling of these biomarkers may be helpful in the prognostic management of these patients.

PB0467 | LBP as a Novel Plasma Protein Associated to Venous Thromboembolism

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Background: Venous thromboembolism (VTE) is a multifactorial disease with environmental and genetic risk factors. Current laboratory techniques do not identify all individuals at high risk. Therefore, it is necessary to identify new specific biomarkers of VTE.

Aims: To identify novel plasma proteins related to VTE.

Methods: We performed an untargeted proteomic analysis in plasma samples of 18 VTE patients and 18 controls using SWATH-LC-MS/MS and identified a total of 522 proteins. We obtained a set of 28 proteins able to distinguish between patients and controls, 16 of them were not directly related to thrombotic pathology. We selected commercial ELISA kits to validate 4 selected proteins at a detectable concentration levels according to bibliography (LBP, RBP4, NGAL and PLA2G7), in a new cohort of plasma samples of 60 VTE patients and 60 controls.

Results: We were able to determine one of the selected proteins. Levels of LBP were measured in the plasma of patients and controls using a commercial ELISA kit. Results showed that there were significant differences between both groups ($p< 0.001$). A ROC analysis was then carried out to determine the cut-off value of the concentration of LBP that allows discriminating between patients and healthy controls, as well as determining the diagnostic sensitivity and specificity of the trial. Setting a sensitivity value of 85% and a specificity of 97%, LBP values of 24.66 ug/mL allowed to discriminate patients and controls with an AUC of 0.93 (95% CI: 0.87–0.97).

Conclusions: LBP is a glycoprotein involved in the acute phase of the immune response in infections caused by bacteria, promoting cytokine release and could act as a link in the immunothrombosis process. Our next aim will be to validate these findings in a larger population to corroborate our results, as well as ascertain the implications of LBP in the coagulation system. ISCIII-FEDER(PI14/00079,PI14/00512,FI14/00269,PI17/00495,CPII15/00002),Generalitat Valenciana(ACIF/2017/138),IIS La Fe(2014/0421,2014/0718,2016/0820),ProteoRed(PRB2-PT13/0001,PT13/0001).

PB0468 | Development of a Microfluidic Pillar Device to Study Hemostasis in vitro

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Background: Hemorrhages occur in many situations, including disease- or drug-associated hemostasis failure, vessel diseases, and coagulopathies. Understanding the differences between the biological mechanisms of hemostasis and thrombosis is critical to the discovery of treatments to promote the former without adversely enhancing the latter. Current in vitro flow chamber-based models are adequately designed to study thrombosis yet not the biophysics of hemostasis.

Aims: Develop a microfluidic device to study the physical biology of hemostasis.

Methods: A polydimethylsiloxane-based microfluidic device was fabricated using photolithography consisting of two orthogonal channels: one for blood inlet (inlet channel) and the other (bleeding channel) to mimic the extravascular space that is exposed during vascular injury. A series of 3 pillars spaced 10 microns apart at the intersection of the channels acts as a model of endothelial barrier function. Only the bleeding channel is coated with extracellular matrix (ECM) proteins. Whole human recalcified blood was perfused

through the device at a constant flow rate and time to occlusion, platelet deposition and fibrin formation were quantified.

Results: The bleeding channel occluded as a function of time, shear and ECM protein coating. Platelets aggregated at or behind the pillars in the bleeding channel as a function of shear rate. Blocking the common pathway of coagulation prevented the formation of hemostatic plug in the bleeding channel.

Conclusions: We are focused on characterizing the spatial dynamics and molecular mechanisms of the two essential hemostatic blood systems, thrombin generation and platelet activation, during hemostatic plug formation. Unique to our approach is the study of these processes in the context of the fluid dynamics relevant to hemostasis, spanning from theoretical to applied in vitro and in vivo approaches. Future work will involve developing a comprehensive model of blood transport during hemostasis through percolation theory of fluid dynamics.

PB0469 | Familial Thrombophilia of Unknown Origin (TUO) Is Associated with Low Activated Protein C (APC) Response to *in vivo* Thrombin Formation in Stimulated Hemostasis Activity Pattern Evaluation (SHAPE)

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Background: Recently, we have shown that low APC response to *in vivo* thrombin formation increases the thrombotic risk of factor V Leiden (FVL) carriers. *In vivo* thrombin formation in this approach, termed SHAPE, was induced by low-dose administration of recombinant activated factor VII (rFVIIa) followed by hemostasis biomarker monitoring.

Aims: To extend this approach to patients with TUO, with a positive self-history of unprovoked VTE (VTE+) as well as a positive family history, in whom no established thrombophilic risk factor was detectable.

Methods: Blood samples were collected before and during a period of 8 hours following injection of 15 µg/kg rFVIIa from patients with TUO (n=21), VTE+ patients with FVL or prothrombin 20210G>A mutation (n=27), and healthy controls (n=29). APC levels were measured using an oligonucleotide-based enzyme capture assay (OECA). Prothrombin activation fragment 1+2 (F1+2), thrombin-antithrombin complex (TAT), and D-dimer were measured additionally.

Results: D-dimer levels remained within the reference range. F1+2 levels increased after rFVIIa injection without showing significant differences between cohorts. A comparable increase of TAT was observed in both TUO patients and VTE+ mutation carriers, from a median of 21.3 to 51.1 pmol/L (P=0.002), and from 21.3 to 43.9 pmol/L, respectively ($P< 10^{-4}$) (Fig. 1A). In the controls TAT increased from 21.3 to 30.89 pmol/L (P=0.024). APC increased from 0.80 to 3.84 pmol/L (P=0.001) in the TUO cohort, from 1.14 to 5.82 pmol/L

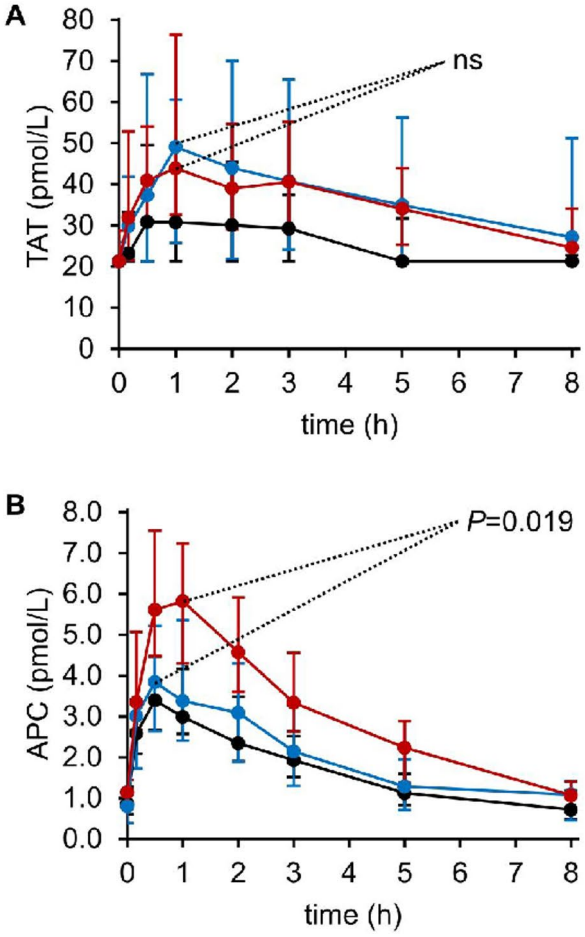

Fig. 1. In-vivo plasma concentrations of A) thrombin-antithrombin complexes (TAT) and B) activated protein C (APC). After t=0 recombinant activated factor VII (15 µg/kg) was administered to patients with thrombophilia of unknown origin (blue symbols, n=21), to thrombophilic patients carrying a factor V Leiden or a prothrombin 20210G>A mutation (red symbols, n=27), and to healthy controls (black symbols, n=29). Data are shown as median and interquartile range. ns, not significant.

FIGURE 1

($P< 10^{-4}$) in VTE+ mutation carriers, and from 0.79 to 3.18 pmol/L (P=0.001) in the control group. The increase of APC levels was significantly smaller in the TUO cohort than in the VTE+ mutation carriers (P=0.019) (Fig. 1B).

Conclusions: A low APC response to an increased *in vivo* thrombin formation is a frequent finding among patients with thrombophilia of unknown origin, suggesting that a low APC response might be an independent risk factor of VTE.

PB0470 | Cross-sectional Study of Thrombin Generation and Overall Hemostatic Potential in a Cohort Clinically Investigated for Thrombophilia

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Background: Global haemostatic assays are emerging as general biomarkers of thrombotic risk but are not in common clinical use. The Innovance® Endogenous Thrombin Potential (ETP) is an automated global thrombin generation assay. An increased thrombin generation in this assay has been identified as a marker of thrombotic risk. The Overall Hemostasis Potential (OHP) is a plasma-based global fibrin generation assay. Increased fibrin generation and decreased fibrinolytic potential have been demonstrated by OHP in thrombotic populations such as thrombophilia, pregnancy and after venous thromboembolism (VTE).

Aims: Assessment of the prevalence of prothrombotic results in the plasma-based global haemostatic assays ETP and OHP, in a cohort clinically investigated for thrombophilia.

Methods: The study was approved by the Stockholm ethical board. All patients analyzed for thrombophilia between 2014–2016 were invited to participate (n = 2500). Written informed consent was obtained in 480 patients, but only patients with strict clinical indication of thrombophilia testing were included. These were 78 with VTE before the age of 50, 72 with a family history of VTE and 19 with both inclusion criteria. ETP and OHP results were normalized against pooled normal plasma (PNP). Results were compared against 95th percentile reference ranges for ETP and 90th percentile reference ranges for OHP.

Results: Increased generation of thrombin (high ETP AUC and ETP C_{max}), fibrin (high OFP and OCP) or decreased fibrinolysis (low OFP) was demonstrated in 5–27% of patients depending on parameter, see table 1. In a healthy reference population without thrombophilia, the proportion of pathological results would be expected to be 2.5–5.0%. The three clinical subgroups had similar ETP and OHP result dispersions.

Conclusions: Analysis of plasma based global haemostatic assays in patients undergoing a clinically warranted thrombophilia investigation yields up to 27% of results outside normal range. A clinical characterization of the patients outside normal range remains.

TABLE 1 Samples were considered as prothrombotic if high for ETP AUC, ETP Cmax, OFP, OCP and if low for OFP

Assay	Mean (IQR)	Proportion with prothrombotic results	Normal range
ETP AUC (%)	100 (87–115)	0.09	83–127
ETP Cmax (%)	97 (84–108)	0.09	80–115
OCP (%)	123 (100–145)	0.27	88–143
OHP (%)	127 (85–159)	0.17	65–174
OFP (%)	100 (93–108)	0.05	93–116

PB0472 | Molecular Pathogenesis of Bone Degenerative Disease and Associated Inflammatory and Thrombotic Processes

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Background: Osteoarthritis is one of the most common forms of arthritis, which is a result of the degeneration of protective cartilage at the end of bones, resulting in friction in the joints. During this process, several mediators of inflammation thrombogenesis and matrix degradation are formed.

Aims: The aim of this study is to profile various biomarkers of inflammation and hemostatic activation in osteoarthritis patients undergoing total joint replacement.

Methods: Seventy-four EDTA plasma samples were collected from osteoarthritis patients prior to their surgery. The control comprised of a pool prepared from normal male and female EDTA plasma. Such coagulation markers as D-Dimer, vWF, MP-TF, PAI-1, TAFI, and fibronectin were performed using ELISA techniques. Matrix metallopeptidase 9 (MMP-9) and C-reactive protein were also measured using ELISA methods. All results were compiled as group means and compared with EDTA controls. Appropriate statistical methods were used to analyze the data.

Results: The circulating level of D-Dimer was much higher on average in the patient samples (9178 ± 3.56 ng/ml) in comparison to the control samples (115 ± 85 ng/ml) $p< 0.05$. The concentration of vWF was higher (125 ± 28%) than in the control sample (104 ± 7.4%) $p< 0.05$. There was not a significant difference between the MP-TF or TAFI concentrations in the total joint samples vs the controls $p< 0.05$. There was no correlation among any of the tested biomarkers.

Conclusions: This data shows that osteoarthritis patients exhibit marked elevation of inflammatory and hemostatic activation biomarkers. These results suggest that multifactorial processes are involved in the pathogenesis of osteoarthritis. Profiling of inflammatory and thrombotic biomarkers may be helpful in the risk stratification and risk management in these patients.

PB0473 | Defining Acetylsalicylic Acid Responsiveness: Understanding the Relation between Thromboxane B_2 Inhibition and Aggregation Induced by Arachidonic Acid

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Background: Inhibition of serum thromboxane (TxB_2) and arachidonic acid-induced platelet aggregation (AA-PA) are direct indicators of platelet COX-1inhibition by acetylsalicylic acid (ASA). It is widely believed that >95% TxB2 inhibition from baseline is essential for clinical efficacy of ASA.

Aims: To evaluate the relation between AA-PA, and serum TxB2, urinary 11-dehydro thromboxane B2 (u11-dh TxB2), and ASA and salicylic acid (SA) levels.

Methods: ASA effect was assessed serially over a 24-hour period at 10 timepoints in seventeen healthy volunteers either receiving a single dose of 162 mg chewed /swallowed or 50–100 mg inhaled ASA. AA-PA was measured using light transmittance aggregometry, serum TxB2 by liquid chromatography-mass spectrometry (LC-MS), u11-dh TxB2 by ELISA, and plasma concentrations of ASA and SA were measured by LC-MS. Receiver operator characteristic (ROC) analysis was used to develop criterion for each test measure using the definition for complete ASA inhibition (AA-induced PA of < 5%).

Results: Baseline variability was more pronounced with serum TxB2 (5–95% CI, 31–680ng/mL) as compared to AA-PA (65–81%) and u11-dh TxB2 (1556–4440 pg/mg).

AA-PA of < 5% was reached within 2 min with inhaled ASA and 30 min with chewed/swallowed ASA. Based on ROC analysis both u11-dh TxB2 and ASA level were outstanding (AUC>0.9) whereas serum TxB2 inhibition was excellent (AUC 0.8–0.9) (Table 1). ROC based criterion for serum TxB2 to indicate complete ASA inhibition is >49% and for u11-dh TxB2 is 1520 pg/mg.

Conclusions: Our study results suggest that the definition of >95% inhibition of serum TxB2 to indicate platelet COX-1inhibition for clinical efficacy may be overestimated and should be re-considered in future investigations.

TABLE 1 ROC analysis of Test Measures

Test Measure	Criterion	AUC	Sensitivity	Specificity	p-value
Serum TxB2 Inhibition (%)	>49	0.82	96.4	64.3	<0.001
Serum TxB2 (ng/mL)	<100	0.76	86.2	67.7	<0.001
u11-dh TxB2 (pg/mg)	<1520	0.92	76.0	100	<0.001
Acetylsalicylic acid (ng/mL)	>264	0.92	87.7	93.5	<0.001
Salicylic acid (ng/mL)	>263	0.83	68.1	93.6	<0.001

PB0474 | The Prognostic Role of Red Cell Distribution Width in Venous Thromboembolism and Related Mortality

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Background: Red cell distribution width (RDW) is a novel prognostic marker that may reflect an underlying inflammatory state. Recent meta-analyses demonstrated this measurement could be strong predictor in various conditions. Nevertheless, there is no meta-analysis evaluating the role of RDW in prediction of the occurrence of venous thromboembolism (VTE), and related mortality.

Aims: To investigate the association of RDW and VTE including deep vein thrombosis (DVT) and pulmonary embolism (PE) and related mortality.

Methods: Major medical databases were systematically searched for observational study which assessed the association of RDW and VTE published until January 2020. The databases were searched with predefined protocol without language restriction based on PRISMA guideline. The analysis was performed in RevMan 5.3 to provide pooled measures for Risk Ratio (RR) and weighted mean difference (WMD).

Results: Twenty-two studies were enrolled including 6,718 cases and 58,651 controls. Higher RDW was indicated as independent predictors for VTE compared to lower RDW (RR=1.82, p< 0.00001), comprising DVT and PE (RR=1.52, p< 0.00001, and RR=2.05, p=0.002, respectively). There was also an association between higher RDW and risk of mortality after PE episode with RR=1.31 (p=0.001), involving short- (30-day) and long-term (>1 year) mortality (RR=1.79, p=0.04, and RR=1.15, p=0.03, respectively). Compared to lower RDW, higher RDW was also revealed a significant association with unprovoked VTE (RR=2.18, p=0.02). VTE-group had a higher mean of RDW compared to control with WMD=1.13% (p< 0.00001), consisting of PE-group (WMD=0.99%, p< 0.00001) and DVT-group (WMD=1.07%, p< 0.00001). Among PE patients, PE-related mortality group had also higher mean of RDW in comparison to survivor group with WMD=2.04% (p< 0.00001).

Conclusions: Higher RDW value was associated with the occurrence of VTE, including DVT, PE, unprovoked VTE, and PE-related mortality. Therefore, the use of the potential role of RDW should be underlined since it is low-cost and availability, even in the low-resource setting.

PB0475 | Acquired Antithrombin III Deficiency Is a Risk Factor for Venous Thromboembolism after Severe Injury

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Background: Up to 20% of severely injured patients on prophylactic anticoagulation experience venous thromboembolism (VTE). Our previous work shows that acquired antithrombin III (ATIII) deficiency [ATIII< 80%] occurs in approximately 20% of trauma patients upon admission and drives poor responsiveness to enoxaparin. However, changes in ATIII over time and its association with VTE remain unknown.

Aims: To determine the relationship between acquired ATIII deficiency and VTE in severely injured patients.

Methods: A secondary analysis of the Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR) clinical trial was performed. Demographics, mechanism and severity of injury, transfusions, and outcomes were compared between patients who did and did not develop VTE. Kruskal Wallis and Chi square tests analyzed continuous and categorical data, respectively. Logistic regression analyses were performed to identify significant contributions to VTE risk, controlling for ATIII deficiency (over first 72 hours), age, gender, race, and injury severity. Cox proportional hazards modeling determined the contribution of ATIII deficiency to the cumulative incidence of VTE.

Results: Of the 680 patients enrolled in PROPPR, 87 (13%) developed VTE. The median time to VTE was 6 days (IQR 3, 13). No differences in demographics, injuries, or transfusions were identified. Multivariable modeling demonstrated that ATIII deficiency at 72 hours post-admission was independently associated with the development of VTE. Patients who experienced ATIII deficiency at this time point had a 2.5 fold increased risk of VTE [p< 0.01; 95% CI 1.28–4.71]. Lastly, patients who developed VTE had worse outcomes as displayed by significantly fewer hospital-free days compared to non-VTE patients [0 (0, 8) vs 4 (0, 18), respectively].

Conclusions: Acquired ATIII deficiency is an important risk factor for VTE in severely injured patients. These data indicate that intervening, perhaps through ATIII supplementation, in the first three days after injury could mitigate the risk of VTE and improve patient outcomes.

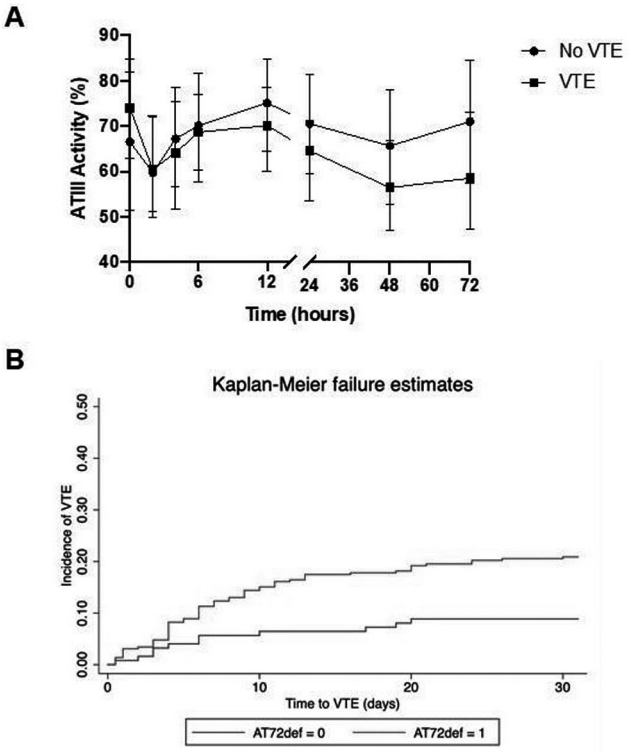

FIGURE 1

PB0476 | Evaluation of Thrombomodulin Levels in Women with Breast Cancer Undergoing Doxorubicin Treatment

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Background: Thrombomodulin is a membrane glycoprotein present on the surface of endothelial cells, which is often increased in response to diffuse endothelial damage.

Aims: To investigate thrombomodulin levels in women with breast cancer treated with doxorubicin (DOXO) and the association of this glycoprotein with molecular subtypes of breast cancer.

Methods: Our study included 80 breast cancer patients under treatment with DOXO attended at the Hospital Alberto Cavalcanti (FHEMIG). The study was approved by the Ethics Committee of the Federal University of Minas Gerais and FHEMIG, according to Declaration of Helsinki. All participating previously signed the informed consent form. Thrombomodulin levels in serum samples taken before (T0) and after the last cycle (T1) of chemotherapy were measured by Multiplex immunoassay. Patients were grouped according to molecular classification: luminal, HER2 positive (HER2+) and triple negative. A statistical analysis was performed in SPSS v.17.0 software.

Results: In all patients, the median thrombomodulin was 3.81 ng/mL (IQR 1.98) at T0 and 4.80 ng/mL (IQR 2.86) at T1 (p>0.05). The most frequent molecular classification was HER2+, n=33 (41.8%). At T0, median levels of thrombomodulin was 4.08 ng/mL (IQR 3.59) for luminal, 3.68 ng/mL (IQR 1.72) for HER2+ and 3.65 ng/mL (IQR 2.13) for triple negative. At T1, median levels of thrombomodulin was 4.93 ng/mL (IQR 3.31) for luminal, 4.45 ng/mL (IQR 2.52) for HER2+ and

4.97 ng/mL (IQR 1.92) for triple negative. There was no difference between the two times and the subtypes of breast cancer for thrombomodulin levels (p> 0.05).

Conclusions: The assessment of thrombomodulin cannot contribute to the evaluation of endothelial injury post-DOXO treatment in women with breast cancer and is not associated with the molecular subtype of tumor.

Acknowledgments: CNPq and FAPEMIG.

PB0477 | RMean Platelet Volume to Lymphocyte Ratio and VTE Recurrence

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Background: It has been reported that mean platelet volume (MPV), platelet-to-lymphocyte ratio (PLR), mean platelet volume-to-lymphocyte ratio (MPVLR), and neutrophil-to-lymphocyte ratio (NLR) may play a role in the diagnosis of venous thromboembolic disease (VTE). These parameters are easy to obtain from a complete blood count.

Aims: Analyze the association between these parameters and the recurrence of VTE.

Methods: An observational, retrospective study was conducted. Inclusion criteria: Patients with a first venous thromboembolic event (deep vein thrombosis and/or pulmonary thromboembolism), who had a complete blood count within the first 7 days after the event.

The values of MPV, PLR, MPVLR, and NLR that the patients had at the time of diagnosis were analyzed.

The patients were divided according to whether they had recurred or not after the first event. The association between the previously named parameters and the recurrence was analyzed.

Results: 71 patients were included. MPV (8.5 vs. 9 fl, p = 0.43), PLR (147 vs. 216, p = 0.22), MPVLR (0.0048 vs. 0.0076, p = 0.094) and NLR (3.73 vs. 4, 15, p = 0.66) had higher values in the recurrence group, although this difference was not statistically significant. Given the result a ROC curve analysis of MPVLR for predicting recurrence in VTE was performed which found a cut-off value of 0.0047 for MPVLR, an area under curve of (0.688, 0.51–0.87), p = 0.049, with a sensitivity of 64% and specificity of 62%.In the provoked VTE event group of patients, MPVLR was higher in the recurrence group (0.0053 vs 0.0083, p = 0.058) and in the cancer associated thrombosis (CAT) group the difference in the MPVLR was statistically significant (0.006 vs 0.011, p = 0.013).

Conclusions: MPVLR could a potential marker for recurrence in VTE, especially in the CAT group. Larger and prospective studies about the subject are needed.

PB0478 | Panel of Prothrombotic-inflammation Genetic Markers and Thrombin Generation Assay for Evaluation of Thromboprophilaxis and Thrombosis Risk in Thrombophilia Patients

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Background: Among prognosis biomarkers of thrombosis risk, the polymorphisms FV Leiden (FVL) and Prothrombin G20210A (F2GA) cause primary thrombophilia. Vascular stasis results from venous insufficiency and a vascular proinflammatory-state might be associated to the risk-allele D of the Angiotensin Converter Enzyme, ACE (I/D) gene. The thrombin generation assay using the Calibrated Automated Thrombogram (CAT) method is an effective predictor of thrombosis risk and might be useful in evaluating thromboprophilaxis, including novel oral anticoagulants (NOACs).

Aims: To evaluate the prognosis value of a panel of prothrombotic-inflammation markers for predicting thrombosis risk and the usefulness of the CAT assay in thromboprophilaxis.

Methods: After their consent to participate, 18 patients (12 females, 6 males; aged 15 to 66 yr) who had developed at least one thrombosis (5 arterial, 11 venous, 2 both) and were under various thromboprophilaxis schemes (NOACs, Vitamin-K antagonists, antiplatelet drugs or without anticoagulant), have been followed after their diagnosis: 1 FVL-homozygous, 3 FVL-heterozygous, 5 F2GA-heterozygous, 2 FVL/F2GA double-heterozygous, and 7 secondary thrombophilia. In all patients, we evaluated venous insufficiency, ACE genotype, thromboprophilaxis status and thrombosis-risk. To assess the latter, we collected blood by venopuncture in Sodium-citrate 0.19M tubes to obtain the PPP that was frozen at −80°C until the CAT assay was performed. The patients' thrombograms were compared against a pool of normal plasma to establish the risk threshold of ETP at 124% of normal (Luna-Záizar et al., Thromb Res 136:1291;2015).

Results: Among the studied variables, only familial thrombosis history was significant (p=0.002). Venous insufficiency and proinflammatory risk ACE (D) were predominant (11/18 and 14/18 patients, respectively). The CAT assay defined the adequate hemostatic level or risk according to the ETP value.

Conclusions: The CAT assay showed better control in NOAC anti FXa than FIIa, and a risk value of ETP was found in 2/4 patients treated by antiplatelet therapy.

BLOOD COMPONENTS AND MANAGEMENT

PB0479 | Administration of Fibrinogen vs. Cryoprecipitate Stored out to 10 Days in Microfluidic Models of Coagulopathies in Hemorrhage

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Background: Fibrinogen replacement is critical for hemorrhaging patients. Cryoprecipitate (cryo) and fibrinogen concentrates (FC) are sources of fibrinogen used clinically. Differences in their hemostatic effects are unclear.

Cryo contains additional proteins, including von Willebrand factor (vWF), FVIII, and FXIII, which may augment hemostasis. It is stored frozen and must be used within 6 hours of thaw, which limits clinical use due to logistical burden and waste.

Aims: The objective of this study was to examine the hemostatic rescue effects of cryo and FC in induced coagulopathies in a microfluidic model of hemorrhage.

Methods: The microfluidic model of hemorrhage comprised a native lumen and a tissue-factor- and collagen-coated injury space (Fig 1A).

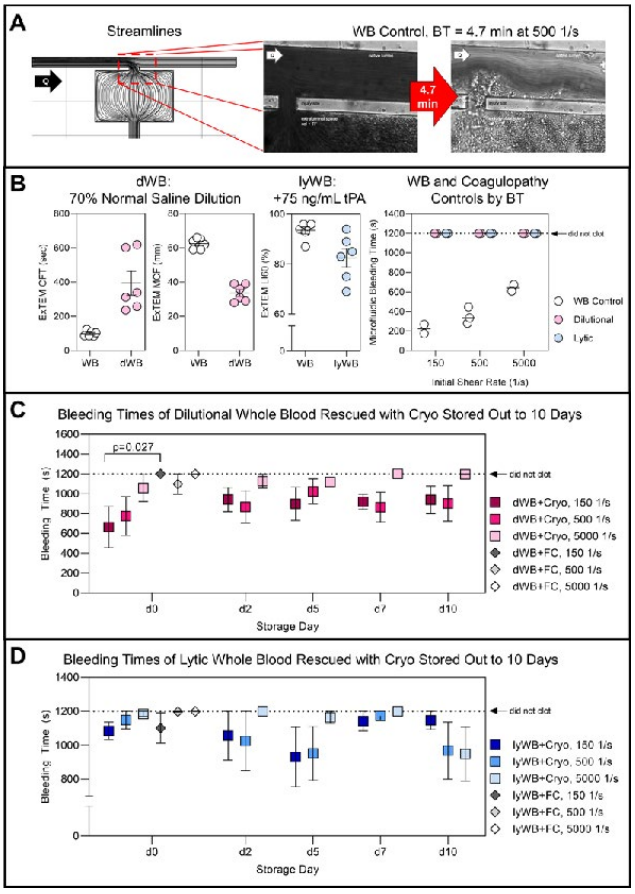

FIGURE 1 Microfluidic model (A), coagulopathy induction (B), and results (C-D)

Healthy human donor whole blood (WB) was either diluted or tissue plasminogen activator (tPA)-treated to induce dilutional or fibrinolytic coagulopathy, respectively. Dosages were based on clinically-relevant targets (Fig 1B). Coagulopathic blood was mixed with cryo (n=6) or FC (n=6) and perfused at an initial shear rate of 150, 500, or 5000 1/s. Cryo was tested on day 2 (d2), d5, d7 and d10 post-thaw. The FC dose was normalized to d0 cryo fibrinogen concentration (900 mg/dL). The primary endpoint was time to cessation of flow out of the lumen (bleeding time, BT).

Results: The mean BT of WB was 3.7 min at 150 1/s, 5.9 at 500, and 10.7 at 5000 (Fig 1A). Coagulopathic blood did not clot within a maximum of 20 min at any shear rate (Fig 1B). Cryo provided better rescue over FC for dilution (Fig 1C), but there were no differences observed for fibrinolytic rescue (Fig 1D). There was no loss in function of cryo out to 10 days post-thaw.

Conclusions: Cryo provides flow-dependent rescue of dilutional coagulopathy better than FC in a microfluidic model of hemorrhage. These differences occurred despite fibrinogen normalization. No loss in cryo function was observed up to 10 days post-thaw.

PB0480 | Improved Functional and Biochemical Characteristics of Buffy-Coat Platelet Concentrates Stored in Citrate-Free PAS-III Additive Solution for 7 Days

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Background: Deterioration in the quality of platelet concentrates (PCs) during storage results from the appearance of lesions affecting their hemostatic properties and platelet survival after transfusion. These lesions depend on the methods used for their preparation and pathogen inactivation, the duration of storage and the type of additive solutions present in the storage bags.

Aims: To assess the effects of the citrate contained in the PAS-III additive solution on storage lesions in buffy-coat PCs with or without photochemical (amotosalen-UVA) treatment (PCT).

Methods: A pool-and-split method was used to obtain four study groups: PCT-PCs stored in commercial PAS-III solution containing 10 mM sodium citrate, untreated PCs stored in PAS-III, PCT-PCs stored in citrate-free PAS-III and untreated PCs stored in citrate-free PAS-III (n=3 per group). *In vitro* platelet quality and function were tested over 7 days (D).

Results: Platelet counts were conserved during storage in the four groups, while platelet volume was significantly reduced upon citrate removal, regardless of PCT. Glycoprotein (GP) IIb-IIIa and GPVI expression remained stable whereas GPIbα declined similarly in all groups during storage. Removal of citrate caused a highly significant decrease in glucose consumption, which largely countered a modest deleterious effect of PCT. Citrate removal also resulted in decreased lactate generation and better maintenance of pH during storage, while PCT had no impact on these parameters. Citrate-free storage

moreover significantly reduced P-selectin expression at all time points and phosphatidylserine exposure on D7, thereby abolishing the activating effect of PCT on both parameters. Finally, citrate removal benefited platelet aggregation, with an increased response to collagen up to D7, to ADP until D2.5 and to TRAP-6 on D7, whereas PCT had no impact on these aggregation responses.

Conclusions: Removal of citrate from PAS-III improved platelet storage, which should allow the transfusion of platelets with better and longer preserved functional properties.

PB0481 | An Audit of Fresh Frozen Plasma Transfusions: Justified or Not?

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Background: Fresh frozen plasma (FFP) has been used for management of various clinical disorders. Main indications are clotting factor deficiency, plasma exchange and reversal of warfarin. It is now evident that that most of the time this blood product is being used inappropriately and without any scientific rationale. It must be remembered that it is associated with potential risks to the recipient. Inappropriate use not only leads to a wastage of limited resources depriving more needy patients, but also leads to an increased healthcare cost and increased risk of transfusion related complications.

Aims: To determine the indications of FFP transfusions and to assess the practices according to the guidelines proposed by British committee of standards in Haematology (BCSH).

Methods: This audit was planned according to the BCSH guidelines for the FFP usage. Retrospective review of transfusion request forms and patients charts from October 2019 to December 2019. Patients demographics, indications and laboratory record was retrieved from LIMS system and was recorded.

Results: During the study period the total number of ffps arrangred were 259 and then 150 were transfused to 55 patients. The age range from 4 days to 65 years with male predominance. Majority of the request received from NICU (71.9%) followed by general ICU (19.2%) and then different wards (8.9%). Weight based dose (15ml/kg) was followed in all pediatric patients whereas only 4 adult patients received proper dose of ffp. 02 patients had no prior record of coagulation screening. Majority of patients received ffp for correction of INR (65%). Transfusion reactions were not observed in these patients.

Conclusions: FFP transfusions were not justified in majority of patients, showing poor compliance with the guidelines.

PB0482 | Thromboelastometry Identifies Coagulopathy Associated with Liver Failure and DIC Caused by Yellow Fever, Guiding Specific Hemostatic Therapy: A Case Report

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Background: Yellow fever is a viral sepsis which most commonly clinical manifestations include since sudden onset of fever and prostration to more serious illness associated with multiorgan dysfunction, worse outcome and high mortality. The severe form of the disease can be complicated by acute fulminant hepatitis, rapid deterioration of liver function and jaundice. Hepatic-induced coagulopathy may be associated with severe hemorrhagic manifestations. Liver failure and disseminated intravascular coagulation (DIC) are the main causes of bleeding. TEM score is a highly desirable goal designed to detect a hypocoagulable state in the advanced stages of DIC.

Thromboelastometry has been used for early diagnosis of coagulopathy guiding therapy by goals. Factor concentrates and hemostatic drugs can prevent the unnecessary use of blood components.

Aims: This a case report of a patient admitted to the ICU with diagnoses of yellow fever complicated by liver failure and DIC, in which thromboelastometry identified coagulopathy guiding hemostatic therapy with fibrinogen concentrate and vitamin K. No blood components transfusion was required, even in presence of thrombocytopenia.

Methods: Case report description.

Results: We report the case of a 23-year-old male admitted to the department of critical care with diagnosis of yellow fever associated with liver failure, and DIC. Laboratory tests showed thrombocytopenia and a tendency of hypofibringenemia, suggesting higher risk of bleeding. Unlike CLT, TEM identified specific coagulation disorder and thereby guided hemostatic therapy by goals. Both Fibrinogen concentrate and Vitamin K were administered, no blood components transfusion was required, even in presence of thrombocytopenia.

Conclusions: Thromboelatometry allowed early identification of coagulopathy, guiding hemostatic therapy by goals. Administration of

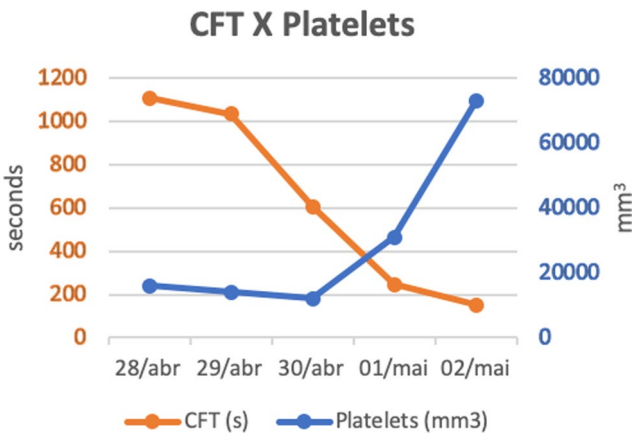

FIGURE 1 Results of thromboelastometry and platelet count

hemostatic drugs including fibrinogen concentrate and vitamin K could improve thromboelastometric parameters, correcting the complex coagulation disorder. Blood components transfusion were not performed, and there was no bleeding as well.

PB0483 | Apoptosis Inhibition during Cold Storage of Apheresis Platelet Concentrates Improves Cell Vitality without Affecting the Functionality

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Background: Transfusion of apheresis platelet concentrates (APCs) is an established therapy to treat bleeding. However, bacterial infection caused by storage at room temperature (RT) still remains a major problem. Recently, we reported that cold-stored APCs (C-APCs) are associated with better PLT functionality but faster PLT clearance caused by apoptosis activation.
Aims: To investigate the protective effect of apoptotic inhibitor during cold storage of APCs and its impact on PLT functionality.
Methods: APCs were stored at RT and 4°C with or without a caspase-3 inhibitor. PLT apoptosis was assessed measuring the mitochondrial membrane potential (MMP), phosphatidylserine exposure by flow cytometry and protein expression via immune blot. The PLT aggregation ability was analyzed performing an aggregation Assay.
Results:
In C-APCs a higher phosphatidylserine expression was detected compared to RT: (RT-APCs) (% apoptotic events mean±SEM: 13±1% vs. 5±1%, C- vs. RT-APCs, p=0.018). Interestingly, a reduction of MMP after cold storage compared to RT was observed (mean fluorescence intensity TMRE Mean±SEM: 6.13±1.89 vs. 18.53±3.64, C- vs. RT-APCs, p=0.038). Accordingly, the procaspase-3 protein level in immune blot was found to be decreased upon cold storage. These data, indicate the activation of the intrinsic apoptotic pathway during cold storage of PLTs. In the presence of a caspase-3 inhibitor a significant rescue of the cold-stored PLTs viability was observed (% alive cells mean±SEM: 74±3% vs. 22±3%, caspase-3 inhibitor vs. ionomycin, p=0.005) suggesting that the caspase inhibitor can prevent the cold-induced apoptosis. Importantly, the caspase-3 inhibitor doesn′t affect the PLT aggregation ability that is comparable to PLTs stored without the inhibitor (% maximal aggregation in response to TRAP mean±SEM: 76.24±5.63 vs. 66.91±16.06 without vs. with caspase-3 inhibitor, p=0.638).
Conclusions: These results demonstrate that cold-induced apoptosis can be prevented by a caspase-3 inhibitor without affecting PLT aggregation capability. Consequently, cold storage may become an efficient strategy for APC storage in combination with apoptotic inhibitors.

PB0484 | Study of the Stability of the Main Parameters of Hemostasis Over Six Hours

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Background: Hemostasis is the set of mechanisms that aim to stop bleeding.
The coagulation tests are influenced by the delay between the sampling and their accomplishments. For this, it is recommended by most guidelines to carry out these tests within 4h after the sampling.
Aims: To determine the stability of the main hemostasis parameters at 6h of sampling.
Methods: Our study, which took place at CHTS Mustapha CHU on 40 apparently healthy subjects, evaluated the stability of TP, TCK, Fg, D-dimers, FVII and PC on citrated whole blood and on citrated plasma after less than 2 h, 4h and 6h sampling at 23 ° C.
Results: Conservation of whole blood up to 4 and 6 hours resulted in significant changes in TP and TCK, whereas < 25% of samples had a variation
> 10% which makes it possible to preserve the stability of the studied parameter.
Among the remaining parameters, only the FVII showed a significant difference after 6 h, and 30% of the samples had a variation> 10% having a percentage average of variation> 10%, which does not allow to maintain the stability.
In citrated plasma, only TP, TCK and Fg have significant differences after 4h and 6h, gold < 25% of the samples show a variation> 10.
Conclusions: TP, TCK, Fg, D-dimers and PC are stable up to 6 h in whole blood and in citrated plasma. The stability of FVII in whole blood is 4 h, and 6 h in citrated plasma.

PB0485 | The Use of Platelet Transfusions during Extracorporeal Membrane Oxygenation in a Large Tertiary Centre

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Background: Bleeding, thrombocytopenia and platelet dysfunction occur frequently with Extracorporeal Membrane Oxygenation (ECMO). However, there is limited data on prevalence, indication and outcomes in patients receiving platelet transfusions during ECMO.
Aims: To review the outcomes of patients requiring platelet transfusions during ECMO.
Methods: A single centre retrospective analysis of patients receiving veno-venous ECMO between 01/01/2011 and 31/12/2017 was performed using electronic patient data software. Platelets were transfused at clinician discretion during bleeding and to maintain a

platelet count > 50x10^9/L. Platelet transfusion prescriptions, clinical outcomes were obtained. Data was analysed using R software with Chi-squared and Wilcoxon rank tests.

Results: Over a 7-year period, 402 patients received ECMO with a total of 1348 platelet transfusions given. 225 [56%] of patients did not receive platelet transfusion and 177 [44%] received at least one unit, giving a mean of 0.2 platelet transfusions/patient/day of ECMO. The incidence of transfusion reduced linearly over the first 28 days with a slope of −0.013 platelet transfusions/patient/day (95% CI: −0.011, −0.016). Patients who received platelets were older (47 vs 43 years, p< 0.05), with higher APACHE II scores (19 vs 17, p< 0.05) and longer duration of ECMO support (10 vs 7 days, p< 0.05). Survival to decannulation (200/225 [89%] vs 128/177 [72%], p< 0.05) and discharge from intensive care was lower in those receiving platelet transfusions. 712 [53%] of platelet transfusions were given to those with a preceding platelet count < 50x10^9/L. 83 of 177 patients [47%] received platelet transfusions in the context of a major haemorrhage (>/=4 units red cells unit in 24 hours).

Conclusions: Platelet transfusions were administered to patients with greater critical illness severity and with an increased short-term mortality rate. There was heterogeneity in the transfusion thresholds due to a mixture of asymptomatic patients and those with significant bleeding.

PB0486 | The Functional Profiles of Platelets Collected from Blood Diversion Pouches and Apheresis Concentrates Are Comparable to the Ones from Platelets Isolated through Standard Methods

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Background: Blood collection systems have been designed to divert the first flow of blood into a diversion pouch (DP) that lacks anticoagulants. These systems help reduce blood contamination from skin pathogens. Blood collection is a critical preanalytical variable to control in preparation for functional testing of platelets. Having access to samples from healthy blood bank donors offers the possibility to study diverse aspects of platelet biology such as age, sex, or race from a vast number of individuals.

Aims: To determine if the functional profiles of platelets isolated from the diversion pouch (DP) as well as from apheresis (Aph) concentrates are representative of platelets isolated from venipuncture with immediate anticoagulation (VP).

Methods: Whole blood from the DP was collected into citrated tubes within two minutes of collection. Platelet-rich plasma (PRP) and washed platelets (WP) were isolated following standard protocols.

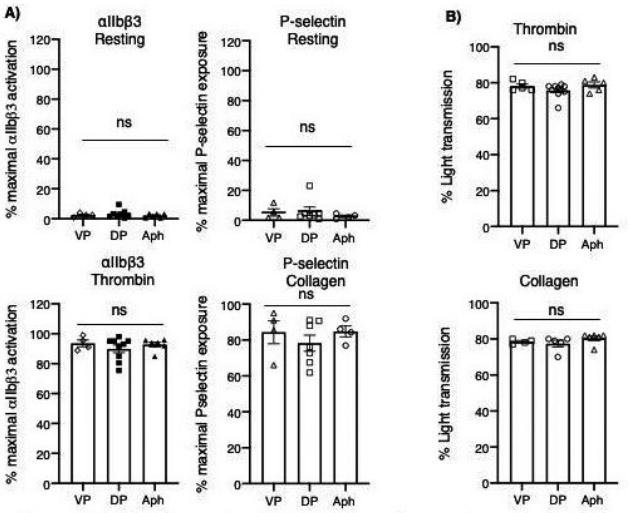

Figure 1- Platelet activation profiles of samples obtained by venipuncture (VP), diversion pouch (DP) and Apheresis (Aph) concentrates are similar. (A) flow cytometry and (B) Light transmission aggregometry (LTA) analyses. Platelet-rich plasma was used for both, flow cytometry and LTA. Activation with thrombin 0.1 IU/ml and collagen 2 μg/ml.

FIGURE 1

For a subset of donors (N=6), platelets from the DP were also compared to Aph concentrates isolated on the same day and from the same individual. Baseline (resting) and activation (thrombin [0.1 IU/ml], ADP [5μM], and collagen [2 μg/ml]) profiles were determined by flow cytometry using anti-P-selectin and PAC-1 antibodies. Platelet aggregation was assessed by light-transmission aggregometry (LTA) of PRP using thrombin and collagen. Ongoing platelet metabolome analysis is pending.

Results: Platelets from blood isolated through standard VP, DP, and Aph exhibited no differences in P-selectin and PAC-1 signal at baseline. Similarly, all groups exhibit the same degree of activation by LTA and flow cytometry for all the agonists (Figure 1).

Conclusions: Platelets isolated from blood collected from the diversion pouch and apheresis exhibit a similar activation profile as platelets isolated per standard methods and are suitable options for functional testing analysis.

PB0487 | Deciphering the Protein Secretome of Leukocyte-platelet Rich Fibrin

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Background: Leukocyte-platelet rich fibrin (L-PRF) is extensively used in the dentistry field and other clinical scenarios due to its

regeneration properties. Active platelets and leukocytes secrete growth factors and other proteins that contribute to wound healing.

Aims: The goal of the present study was to identify the proteins secreted by L-PRF membranes cultured in-vitro and differences in the secretome over time.

Methods: L-PRF membranes were obtained after centrifuging blood samples from healthy volunteers, in the absence of anticoagulants, following an established protocol. Membranes were cultured in DMEM and the secretome was collected at days 3, 7 and 21. Following precipitation, protein samples were separated by SDS-PAGE and analysed by LC-MS/MS. Initially, the secretome at day 3 of culture was analyzed. A SWATH-based quantitative differential proteomic analysis of the L-PRF secretome over time was also carried out.

Results: Overall, 705 proteins were identified in the secretome of L-PRF membranes after 3 days of culture, including growth factors and proteins related to platelet and neutrophil degranulation. Clathrin-mediated endocytosis was the top canonical pathway related to the proteins identified. Moreover, 202 differentially secreted proteins were quantified by SWATH when comparing secretomes at days 3, 7 and 21. The majority of them (187) were enriched at day 3. Some proteins that were also found in the initial qualitative analysis (CO3, TSP1 and MMP9) were validated by western blotting confirming an up-regulation at day 3. Other proteins such as fibrinogen and cathepsin S, which increased over time, were also validated corroborating the proteomic results. A quantitative growth factor analysis showed higher levels of PDGFA and EGF at day 3 versus day 7.

Conclusions: In summary, this is the most detailed proteome analysis of the L-PRF secretome to date. Proteins and growth factors identified, and their kinetics, provide novel information to further understand the wound healing properties of L-PRF.

PB0488 | Six Years of Experience with Plasma Fractionation Industries at a Private Super Speciality Hospital in South India: Renaissance in Usage of Excess Plasma in Blood Banks

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Background: According to World Health Organization (WHO) each country must plan for its safe and consistent supply of blood and plasma products for regular clinical needs as well as in case of any disaster. Self-sufficiency ratio is the key parameter to assess the ability of a country to meet its need for plasma derived medicines, through utilization of locally collected plasma. It also helps in proper usage of surplus plasma in blood banks.

Aims: This study aims at evaluating the impact of plasma fractionation industries as an add on in proper utilization of plasma components in the current Indian scenario.

Methods: A retrospective observational study was conducted to analyze the data of 6 years in providing excess plasma for plasma fractionation (PF) industries at Blood Bank, Yashoda Hospitals, Hyderabad, India.

Results: A total of 25361 whole blood donations were collected from qualified blood donors between January 2013 and December 2018. Of which, 23448 plasma units were prepared from the donations which were negative for Transfusion Transmittable Infections (TTIs). Only 54.7% (12832) of the plasma units were transfused to various patients and 0.79% (187 units) was discarded. The remaining 44.3% (10301) of the plasma units were sent to PF companies at regular intervals where they are used for preparing varied plasma derived medical products.

Conclusions: Six years of shipping plasma to PF interdicted wastage of 44.3% (10301) TTI negative plasma units. Implementation of usage of plasma components for transfusion to patients along with shipping excess plasma for PF periodically improves the effective utilization of blood components as well as preparation of medical products in large numbers by PF industries. However, the blood donation process and plasma supplied should meet the specifications as per Drugs and Cosmetics Act, 1940 and Rules made thereunder.

PB0489 | Overdosing of Protamine during Cardiopulmonary Bypass May Increase Use of Unnecessary Blood Product Administration when Monitoring with ROTEM

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Background: The rotational thromboelastometric (ROTEM) parameters INTEM clotting time (CT) and HEPTEM-CT are used perioperatively in cardiopulmonary bypass (CPB) surgery to monitor heparin. Higher protamine-to-heparin dosing ratios (PH-ratio) are associated with paradoxical increases in both INTEM and HEPTEM-CT values, with HEPTEM-CT frequently exceeding INTEM-CT (I/H-CT ratio < 1.0). Normal protamine dosing occurs at PH-ratio of 0.8–1.0 (>1.0 is considered high-dose). Protamine itself has anticoagulant effects by inhibiting platelet function, FVa, and thrombin generation.

Aims: Investigate the PH-ratio and use of plasma post-CPB in bleeding patients with HEPTEM-CT > INTEM-CT.

Methods: We reviewed ROTEMs performed at our institution between March 2018-March 2019. Forty patients undergoing CPB were found to have I/H-CT ratios < 1.0. Of those, twenty-eight were temporally associated with protamine administration. Data regarding total protamine and heparin dose, the PH- and I/H-CT ratios, and intraoperative plasma use were collected.

Results: Among the twenty-eight patients with IH/CT-ratio < 1.0 temporally associated with protamine, twenty-one (75%) received high-dose protamine (PH-ratio ≥1.0). The mean PH-ratio was 1.26 (median 1.25), while the mean I/H-CT ratio was 0.91 (median 0.93). Twelve patients (43%) received plasma resulting from ROTEM

TABLE 1

Patients with I/H-CT Ratio* < 1.0 (n=28)	
PH-Ratio† ≥ 1.0	FFP given based on ROTEM results
21 (75%)	12 (43%)

*IH-CT Ratio (INTEM:HEPTEM clotting time ratio) = INTEM CT divided by HEPTEM CT;
IH-CT Ratio > 1.0 reflective of heparin effect
IH-CT Ratio < 1.0 implies HEPTEM CT prolonged compared to INTEM CT
†PH-Ratio (protamine-to-heparin ratio) = protamine sulfate administration (IU) divided by total heparin administration (IU)
<0.8 = low-dose protamine
0.8-1.0 = normal dose protamine
> 1.0 = high-dose protamine

abnormalities, receiving a mean of 2.17 units (447 mL) plasma in the period immediately following protamine.

Conclusions: Most patients with I/H-CT ratio < 1.0 received high-dose protamine, reinforcing the association between increasing PH-ratio and prolongation of HEPTEM-CT compared to INTEM-CT. As HEPTEM-CT should be equal to INTEM-CT in the absence of heparin, aberrant results such as those seen here may be misinterpreted as a coagulopathy and result in additional protamine administration and/or transfusion of unnecessary plasma for protamine induced coagulopathy/bleeding. In our institution, 43% of patients with IH/CT-ratios of < 1.0 received additional FFP as a direct result of ROTEM results. Understanding protamine's effects on ROTEM and the adoption of more modest protamine dosing regimens may result in fewer unnecessary transfusions.

CELLULAR THERAPIES

PB0490 | Enhancing Transfusable Platelets Using mRNA Therapy to Produce Exogenous Proteins

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Background: Platelets are transfused therapeutically for hemostasis, and are an integral part of hemorrhage management. However, transfusions can be ineffective in the most severe cases of hemorrhage. In addition to hemostasis, platelets are a potential cell therapy in other applications, but development has been hindered by inadequate methods to control which proteins are expressed by platelets. For example, there are no methods to express exogenous proteins in transfusable platelets, which would expand their use to help treat the diseases they modulate. A method is therefore needed to modify transfusable platelets, and thus enhance their protein composition for specific applications.

Aims: To produce engineered, transfusable platelets to enhance their natural coagulability and functional repertoire by directly

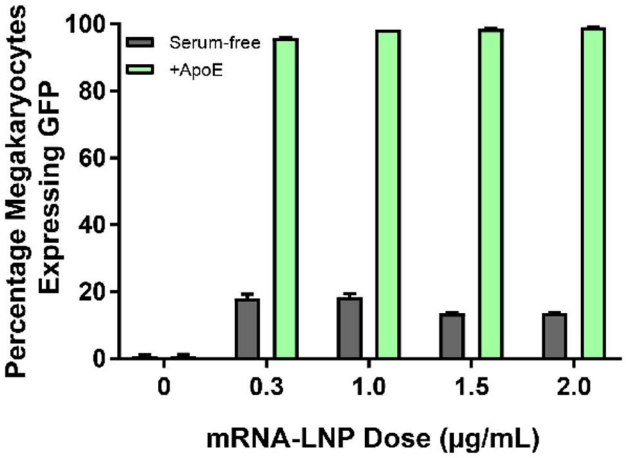

FIGURE 1 Megakaryocyte transfection efficiency with mRNA-LNPs under serum-free conditions or supplemented with ApoE. Error bars represent SEM

transfecting donor-derived platelets and megakaryocytes with mRNA via lipid nanoparticle (LNP)-mediated delivery. LNPs have already demonstrated clinical safety and efficacy for gene therapy, and cultured megakaryocytes provide an alternative source of cells that can be engineered to produce modified platelets.

Methods: As a proof-of-concept, megakaryocytes cultured from cord-blood hematopoietic stem cells were treated with GFP-encoding mRNA-LNPs at varying mRNA doses and with the addition of apolipoprotein E (ApoE) to facilitate LNP uptake in culture. Donor-derived platelets were also incubated with the same mRNA-LNPs or LNPs incorporating the lipophilic tracer DiI. GFP expression and LNP uptake were assessed following 24 hours via flow cytometry and confocal microscopy.

Results: Both the megakaryocytes, when supplemented with ApoE (Figure 1.), and platelets (Figure 2.) demonstrate GFP expression, with over 95% and 30% transfection efficiency in megakaryocytes and platelets, respectively. In addition, nearly 100% of platelets appear to take up the LNPs.

Conclusions: Megakaryocytes and platelets can readily be transfected with LNPs to produce exogenous protein. Further optimization can eventually lead to the creation of a platform technology that in the long-term will allow platelets to deliver therapeutic proteins and yield more effective platelet products.

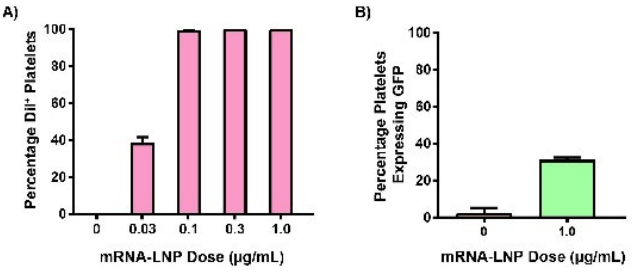

FIGURE 2 Donor-derived platelet mRNA-LNP uptake (A) and transfection efficiency (B) after 24 hours. Error bars represent SEM

PB0491 | Gene Therapy Model by Modification of *F9* Gene through CRISPR/Cas9 for Functional Factor IX Production in Mesenchymal Stem Cells: Preliminary Results

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Background: Recent advances of clinical trials for gene therapy in hemophilia B (HB) through viral vectors have reached great results in the long-term transgene expression of therapeutic factor IX (FIX). However, liver toxicity as the targeted tissue and the drop off of FIX in plasma are still limiting. Innovative gene therapy through CRISPR/Cas system has been implemented on HB with promising results in experimental models. The CRISPR/Cas system applied on mesenchymal stem cells, which show high transfection capacity and differentiation potential, could be a plausible model to correct any mutation in *F9* gene from mesenchymal cells of HB patients.

Aims: Modify the *F9* gene on human mesenchymal cells through CRISPR/Cas9 system to produce functional human coagulation FIX.

Methods: Guide sequences (sgRNA) for flanking the promoter-exon 1 region and production of recombination template (CMV.FIX.IRES.EGFP) were designed for *F9* gene edition. Recombination template, Cas9 enzyme and sgRNAs will be transfected on cells using EGFP expression like reporter. FIX production will be quantified by ELISA assay.

Results: Based on several software, guide sequences of high scores were obtained for flanking the target region of *F9* gene. As initial test, HEK293 cells were transfected with the plasmid template (prrl.CMV.FIX.IRES.EGFP), to evaluate the transfection through expression of EGFP protein by fluorescence microscopy; however, a low expression was obtained, probably due to a deficient transfection efficiency. Human FIX secreted to culture medium was quantified by ELISA (36 a 64 ng/ml), and was frozen to evaluate the clotting activity. Human mesenchymal stem cells cultures (StemPro™ Human Adipose-Derived Stem Cells) were established, develop, and cryopreserved for future assays.

Conclusions: Bioinformatics programs facilitate the design of relevant guide sequences for gene editing with CRISPR/Cas9 system. The low expression of EGFP protein in HEK293 cells, confirms that further tests are necessary to improve the transgene expression.

EPIGENETICS, OMICS AND BIOINFORMATICS

PB0492 | Quantitative Mass-Spectrometry-Based Targeted Proteomics of Plasma from Colorectal Cancer Patients with Venous Thromboembolism

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Background: Cancer patients have a four to sevenfold increased risk of developing venous thromboembolism (VTE). Current prediction models for cancer-associated thrombosis (CAT) perform suboptimal.

Aims: To discover novel plasma biomarkers to improve VTE prediction in cancer patients.

Methods: Plasma samples were used from 142 stage III/IV colorectal cancer patients initiating chemotherapy who were included in a multinational, prospective cohort study. The primary outcome was objectively confirmed symptomatic or incidental deep vein thrombosis or pulmonary embolism during a 6-month follow-up period. Quantitative mass-spectrometry-based targeted proteomics with internal standards was used to measure a panel of 269 proteins including most coagulation and fibrinolysis proteins. The Mann-Whitney U test was performed to compare groups.

Results: Of the 142 patients, 12 (8.5%) developed CAT. Multiplexed targeted proteomics allowed for quantification of 114 peptides in plasma in the qualitative analysis. 38 peptides were measured below the quantitative range in at least in 1 sample, and were therefore excluded from the quantitative analysis. Four proteins were upregulated in patients who developed VTE compared to patients who did not: angiotensinogen (525.2 nM vs. 388.0 nM), apolipoprotein B100 (272.5 nM vs. 202.9 nM), CD5 antigen-like (326.2 nM vs 248.4 nM) and immunoglobulin heavy constant mu, detected by two different peptides (4132.2 nM vs. 2915.6 nM for peptide GFPSVLR and 4143.2 nM vs. 2906.4 nM for peptide VSVFVPPR).

Conclusions: This study identified four plasma proteins that associate with the development of CAT in patients with advanced CRC. Upon validation in an independent cohort, these biomarker candidates may be incorporated in risk models to improve CAT prediction. This could allow for identification of cancer patients who benefit most from thromboprophylaxis.

PB0494 | Interest of Improved Z-Score Bivariate Analysis: Application to a ECAT DDimer Survey

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Background: Laboratories must participate in an interlaboratory comparison program, such as external quality assessment (EQA) that substantially fulfills the relevant requirements of ISO/IEC 17043. In regard to ISO 17043, it is satisfactory if z-scores are in the range [−2; +2] for each EQA sample, questionable if in the range [−3; −2 or +2; +3] and unsatisfactory if < −3 or > + 3. The ISO did not propose a bivariate analysis of z-scores, although this would be contributive to identifying any defect in accuracy or precision.

Aims: Taking an ECAT DDimer survey as an example, the present study aimed to explain the usefulness of improved bivariate z-score analysis to statistically identify any problem of accuracy: i.e. negative bias with respect to the specific peer group on an analyzer in a set of 8 devices.

Methods: The Lyon Hospitals Board's multisite hemostasis laboratory has a set of 8 analyzers belonging to the peer group ACL TOP family IL HemosIL HS 500 in the ECAT DDimers program. In this survey, there were 2 ECAT samples measuring very close to the deep venous thrombosis (DVT) exclusion cut-off value, at respectively 440 and 580 mg/l FEU. One of the 8 analyzers showed z-scores of respectively −2.60 and −1.65 on the 2 ECAT samples. Bivariate z-score analysis, taking account of the correlation between the EQA results, was used to determine whether the device in question tended to return significantly lower DDimer rates than the others, thus being unable to rule out DVT.

Results: The elliptic acceptability limits taking account of correlation phenomena showed that the device presented no statistical bias jeopardizing patient care (figure 1).

Conclusions: The forthcoming implementation of this improved bivariate z-score analysis in ECAT reports will be of great benefit for laboratories.

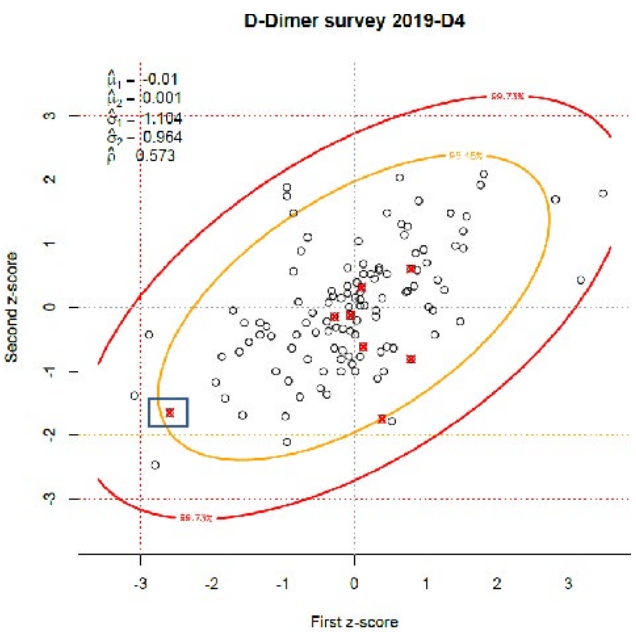

Figure 1: Z-score bivariate analysis, indicating that the bivariate z-score for the device shown in the blue rectangle was acceptable.

FIGURE 1

PB0495 | Physicochemical Changes by Missense Mutations Influences in Hemophilia A Determination

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Background: Hemophilia A (HA) is a X-linked bleeding disorder caused by Factor VIII deficiency. A wide range of mutation types are responsible for this disease, being the missense mutation the more prevalent of them. The functional impact prediction of these mutations is a challenging task, since they could be pathogenic or completely innocuous.

Aims: Identify properties involved in the protein substitutions effects, to elucidate the physicochemical alterations arising from amino acid substitutions of missense mutations that determine the disorder manifestation. The presented abstract brings forward the A1 domain mutations of a more broad study we performed.

Methods: A total of 16 A1 domain mutations were selected from the factor VIII mutation database. Structural models for each mutated and wild sequence were generated using Phyre-2 software in a modeling approach. Electrostatic potential (EP) distance values from mutation models in comparison to the wild structure were extracted using the webPIPSA software. Other attributes as hydrophobicity, disulfide-bond disruptions, and solvent surface-accessible and exposure areas were assessed using Chimera interface resources. The values of each attribute were extracted and compiled altogether with amino acid replacement indexes in a Hierarchical clustering analysis using the R Studio platform with pvclust package.

Results: In electrostatic terms, 11/16 mutations show a distance from Wild Structure (WS) (table 1). All variants show hydrophobicity and surface differences to WS. The hierarchical clustering analysis separates every mutation from WS, based on the features cited and there is a separation of phenotypes in clusters (only one case grouping severe with a mild one (p.Lys67Glu with p.Glu162Lys)(Figure1).

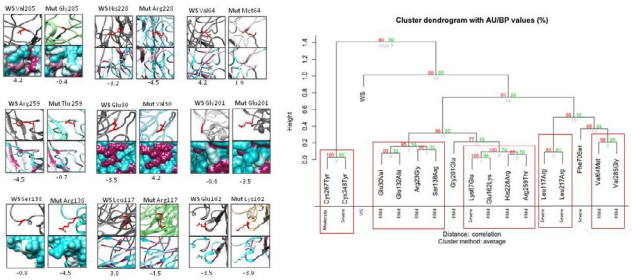
Conclusions: Every substitution has altered one or more features indicating its role in the mutations' separation and importance to explain the current disorder. None of the aspects can explain the

TABLE 1 Substitutions and its information about phenotype and physicochemical properties

Mutation[1]	Phenotype	EP distance from WS[2]	Hydrophobicity modular value[3] comparing to WS	Surface changes (surface solvent-acessible area and surface	Mutation[1]	Phenotype	EP distance from WS[2]	Hydrophobicity modular value[3]	Surface changes (surface solvent-acessible area and surface
p.Arg23Gly	Mild	0.07	4.1	changes	p.Glu162Lys	Mild	0.16	0.4	changes
p.Glu30Val	Mild	0.1	7.7	changes	p.Gly201Glu	Severe	0.07	3.1	changes
p.Val64Met	Mild	0	2.3	changes	p.Leu217Arg	Severe	0.04	8.3	changes
p.Lys67Glu	Severe	0.12	0.4	changes	p.His228Arg	Mild	0.07	1.3	changes
p.Phe70Ser	Severe	0	3.6	changes	p.Arg259Thr	Mild	0.08	3.8	changes
p.Leu117Arg	Severe	0.07	8.3	changes	p.Cys267Tyr	Moderate	0	3.8	changes
p.Glu132Ala	Mild	0.08	5.3	changes	p.Val285Gly	Mild	0	4.6	changes
p.Ser138Arg	Mild	0.07	3.7	changes	p.Cys348Tyr	Severe	0	3.8	changes

[1] Sequence changes at protein level following the Nomenclature for Description of Genetic Variations approved by Human Genome Variation Society (HGVS). The mutations and phenotype information were recovered from Factor VIII (gene) F8 variant database of EAHAD (European Association for Hemophilia and Allied Disorders) located on the domain: f8-db.eahad.org/. [2] Electrostatic potential distance values obtained from the webPIPSA generated epogram.[3] The hydrophobicity value is a modular difference between wild and mutated residues. The original values varies between −4.5 (more hydrophilic) to 4.5 (more hydrophobic).

disorder alone, but their combined use proved to be a good method to separate mutations from normal.



Representative hydrophobicity alterations and Hierarchical clustering analysis (HCA) from A1 Factor VIII domain. Changes in atomic conformation and hydrophobicity from the mutated residues (Mut) related to wild type (WS) were specified in schemes containing the values of a hydrophobicity scale that varies from −4.5 (more hydrophilic residues) to 4.5 (more hydrophobic ones). The maroon pigment were applied for more hydrophobic residues (those with positive values) are depicted with the maroon color, while white and cyan colors represents neutral and hydrophilic (negative values) respectively.

FIGURE 1

PB0496 | Protein Expression Profiling Suggests Relevance of Non-Canonical Pathways in Isolated Pulmonary Embolism

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Background: Whole-body imaging studies have demonstrated that in over half of pulmonary embolisms (PE) there is no evidence for a causative deep vein thrombus (DVT). In addition, epidemiological studies have shown that patients with isolated PE have a reproducibly distinct clinical profile that suggests a non-embolic pathogenesis, e.g. disproportionate comorbidity with pulmonary disease.

Aims: We hypothesized that isolated PE is characterized by a distinct molecular pathophysiology as well.

Methods: To test this hypothesis, data from 532 individuals from the Genotyping and Molecular Phenotyping of Venous ThromboEmbolism (GMP-VTE) Project, a multi-center prospective cohort study with extensive biobanking, were analyzed. All patients with confirmed PE received whole-leg color Doppler ultrasonography

to rule out concomitant DVT. Targeted, high-throughput proteomics technology, machine learning, and bioinformatic methods were applied to contrast the plasma proteomes of acute isolated PE patients (n=96) against those of patients with acute DVT-associated PE (n=276) or acute isolated DVT (n=160).

Results: This resulted in the identification of an isolated pulmonary embolism-specific signature comprising five proteins (IFN-γ, GDNF, PADI2, GALNT3, IL-15Rα).

Conclusions: This signature, and auxiliary protein-protein interaction network analysis, implicate non-canonical pathways more commonly associated with respiratory disease in the acute pathophysiology of this phenotype, lending further credence to the de novo hypothesis.

PB0497 | Development of an Accessible Open Source Code in Python for High-Resolution Melting Data Analysis from Patients Diagnosed with Hemophilia A in Bogotá, Colombia

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Background: Hereditary bleeding disorders are the most frequent orphan diseases in the Colombian population. The main ones include: Hemophilia A (HA), Hemophilia B (HB) and von Willebrand disease (VWD). Currently, diagnosis relies on multiple clinical laboratory assays to assign a phenotype, a fact that can lead to an incomplete diagnosis or confuse one disease with another. In these cases, genetic studies reinforce the clinical diagnosis, assist the detection of carriers, promote early genetic counseling and predict the likelihood of inhibitor development.

In a previous study we characterized the molecular basis of 11 HA patients by sequencing the *FVIII gene*. Recruited patients had a clinical diagnosis made by their treating physician, with phenotypes that included joint and mucosal bleeding, low factor VIII (FVIII) levels. 40 variations were found in HA patients: 13 novel nonsynonymous benign variations, 10 novel nonsynonymous damaging variations, 1 insertion polymorphism, and 3 reported SNPs.

Aims: To validate the High-Resolution Melting (HRM) gene-scanning technique as a diagnostic approach and to develop an accessible open source code in Python for data analysis.

Methods: Disease causing variations found were used as positive controls for validation of the High-Resolution Melting (HRM) gene-scanning technique, from which 26 samples could be validated. For data analysis we developed an accessible open source code in Python that allows different laboratories to analyze HRM data correctly with better sensitivity and without bias when using different HRM equipment. This code also allows the analysis of amplicons with a length greater than 300 bp, by implementing an analysis by denaturation domains.

Results: –

Conclusions: HRM is a low-cost genetic diagnosis method that has been proposed to be incorporated in developing countries. This approach is useful in order to get real data of the population affected with bleeding disorders, given the great socioeconomic impact of these diseases on the different countries health systems.

PB0500 | Exome-array Analysis of Plasma Hemostatic Factors D-dimer, tPA and PAI-1 Identifies Novel Genes

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Background: Use of targeted exome-arrays with common, rare variants and functionally-enriched variation has led to discovery of new genes contributing to population variation in risk factors. Plasminogen activator-inhibitor 1 (PAI-1) and tissue plasminogen activator (tPA), and the plasma product D-dimer are important components of the fibrinolytic system.

Aims: There have been few large-scale genome-wide or exome-wide studies of PAI-1, tPA and D-dimer. We sought to discover new genetic loci contributing to variation in these traits using an exome-array approach.

Methods: Cohort level analysis and fixed effects meta-analysis of PAI-1 (n=15,063), tPA (n=6,605) and D-dimer (n=15,306) from 12 cohorts of European ancestry were conducted using the seqMeta package.

Results: Twelve variants (7 for D-dimer, 5 for tPA) achieved genome-wide significance (P< 5E-08) including 9 rare variants (allele frequency < 1%). Replication was sought for 57 well-imputed variants with P< 1E-4 in the discovery using an independent cohort. Replication was observed for 6 variants including one SNP in the known Factor V locus (*F5*) and 2 SNPs in the fibrinogen coding locus, all for D-dimer association. We observed and replicated association of an uncommon (0.013 allele frequency) coding variant Trp256Leu in *FGL1* (fibrinogen like 1) with increased plasma D-dimer levels, replicating a prior study. Novel signals were replicated for a rare coding variant (rs201393961-T) in *MTFR1L* associated with increased tPA, and for an intronic SNP (rs11057830-A) in *SCARB1* associated with

increased D-dimer levels. Mouse models of *Scarb1* deficiency previously showed roughly doubled plasma cholesterol levels, increased platelet reactivity, arterial occlusion and venous thrombosis. Human GWAS studies showed association of the same *SCARB1* variant allele rs11057830-A with increased coronary artery disease risk.

Conclusions: Utilizing a targeted exome approach, we found common and rare variation associated with tPA and D-dimer plasma levels, including newly discovered and replicated loci linked to thrombosis.

PB0501 | The CDC Hemophilia A Mutation Project (CHAMP) *F8* Mutation List: 2020 Update

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Background: The Centers for Disease Control and Prevention (CDC) Hemophilia A Mutation Project (CHAMP) *F8* Mutation List was established in 2011 to provide a resource for researchers and clinicians to identify previously-reported hemophilia A (HA)-causing *F8* variants and their characteristics. CHAMP, available in Excel format at http://www.cdc.gov/hemophiliamutations, has recently been updated.

Aims: To describe the variants in *F8* reported to cause HA in the literature and in publicly-available databases through 2019.

Methods: Variants were collected from 325 publications and databases identified by systematic literature review. Each was reviewed and uniquely identified using the Human Genome Variation Society nomenclature for coding DNA and predicted protein changes, as well as traditional nomenclature based on the mature, processed protein. Each was given a single listing, including severity classified by ISTH criteria from factor levels provided, reported severity, and inhibitor history with links to original publications. Known benign variants, multiple possible causative variants in a single patient, and those reported only in women were excluded.

Results: Preliminary analysis showed a total of 3782 variants, an increase of 1245 (49%) over the first CHAMP publication in 2013. Proportions of variant types reported (Figure 1) were largely unchanged, as were the proportions of each type reported with inhibitors. Almost one-half of variants were missense. Inhibitors were reported most often with large structural changes (60.5%) (Figure 2). CHAMP has 770 more non-benign variants listed than the next largest *F8* variant database and can be used as a source for variant curation efforts. As it contains only the original report of each variant, it cannot be used to describe variant frequencies in the HA population.

Conclusions: The number of variants reported to cause HA continues to expand without significant change in their characteristics. CHAMP currently provides the most complete list of these variants.

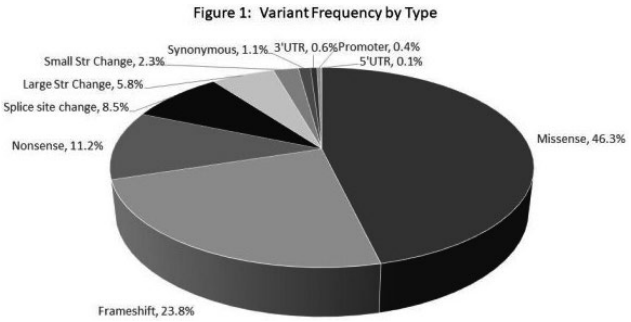

FIGURE 1 Variant frequencies by type of variant in the CDC Hemophilia A Mutation Project (CHAMP)

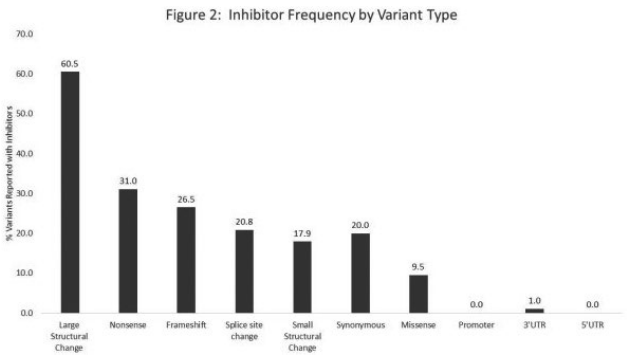

FIGURE 2 Percentage of variants reported with inhibitors by variant type in the CDC Hemophilia A Mutation Project (CHAMP)

PB0502 | Von Willebrand Disease Type 1 Is Associated with Number of Rare Nonsynonymous Variants in the *VWF* Gene

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Background: Approximately 35% of patients with Type 1 von Willebrand Disease (VWD) do not have a known pathogenic variant in the *VWF* gene. While genome-wide association studies (GWAS) have successfully identified several non-*VWF* loci, they do not detect associations with rare variants.

Aims: Determine whether the number of rare (< 1%) variants and the predicted pathogenicity of those variants in low-VWF and VWD patients is associated with VWF levels and type of VWD.

Methods: 709 low-VWF and VWD subjects, as well as normal subjects were recruited from 35 centers across the United States. *VWF* sequencing was performed and VWF antigen levels (VWF:Ag) assayed at a central laboratory. All subtypes of Type 1 (except 1C), 2 and 3 were combined to increase power. CADD score >20 was used as a measure of predicted pathogenicity.

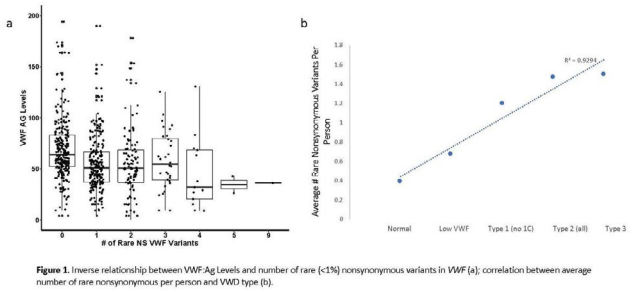

FIGURE 1 Relationship between VWD type, number of rare nonsynonymous variants and VWF:Ag levels

Results: The number of rare nonsynonymous *VWF* variants was a significant predictor of VWF levels, independent of VWD type ($p=3.4x10^{-7}$). There was an association between average number of rare nonsynonymous *VWF* variants with VWD Type 1 ($p=1.5x10^{-10}$) and to a much lesser extent low-VWF (p=0.01), with Type 1 subjects possessing on average a two-fold increase over those with low-VWF and three times as many as normal subjects (1.2 vs 0.67 vs 0.39). A variant with CADD score > 20 significantly predicted VWF:Ag levels even when controlling for number of rare nonsynonymous variants.

Conclusions: The number of rare nonsynonymous variants in *VWF* as well as the presence of a variant with CADD >20 are both significantly associated with VWF levels, even when number of rare nonsynonymous variants is included in the model. This suggests that differences in VWF levels in VWD are due in large part to the number of rare variants, as an increase in rare variants likely increases the probability that one of them is pathogenic.

LABORATORY DIAGNOSTICS

PB0505 | Severe Haemophilia A Plasma Artificially Spiked with Emicizumab and Recombinant Factor VIII Concentrates: Measurement by One-Stage and Chromogenic Factor VIII Assays

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Background: Emicizumab (Roche Chugai) prophylaxis reduces bleeding in patients with severe Haemophilia A (SHA) but laboratory measurement of additional factor VIII (FVIII) treatment may be necessary during traumatic bleeding or peri-operatively.

Aims: To measure emicizumab concentration or FVIII activity (FVIII:C) by different one-stage (OSA) or chromogenic FVIII assays (CSA) in SHA plasma spiked with combinations of emicizumab and recombinant FVIII (rFVIII).

Methods: Emicizumab at a calculated potency of 25, 50 or 75 µg/mL was added to SHA plasma then 0.5 IU/mL or 1.0 IU/mL of Novo8, Refacto AF (RAF), Elocta or Advate was added. Emicizumab concentration was measured by modified OSA (mOSA) using 8 APTT reagents and CSA FVIII:C using 6 assays.

Results: At 25µg/mL emicizumab, addition of 0.5 IU/mL rFVIII increased the measured emicizumab concentration by maximum of 23.7µg/mL (Pathromtin/Novo8). At 50µg/mL, the rFVIII increased by maximum of 26µg/mL (CK Prest/Advate). At 75µg/mL, the increase was maximum 35.7µg/mL (CK Prest/Elocta). Addition of 1.0 IU/mL to 25, 50, 75µg/mL emicizumab increased the measured emicizumab concentration by maximums of 35.1µg/mL (Pathromtin/Elocta), 40.9µg/mL (Pathromtin/Novo8) and 46µg/mL (CK Prest/Elocta) respectively. Overall, at 0.5 and 1.0 IU/mL rFVIII, mOSA varied according to reagent (Table 1 shows data for samples containing 0.5 IU/ml FVIII).

At 0.5 IU/mL rFVIII, CSA FVIII:C ranged from 0.44 IU/mL (Precision Biologic/RAF) to 1.10 IU/mL (Hyphen/Novo8) (figure 1). At 1.0 IU/mL rFVIII, mean FVIII:C ranged from 1.03 IU/mL (Siemens/RAF) to 1.93 IU/mL (Technoclone/Elocta). Hyphen CSA detected the presence of both emicizumab and rFVIII; as the emicizumab concentration increased, the Hyphen CSA FVIII:C increased.

Conclusions: mOSA measures both emicizumab and rFVIII present in the plasma and a cumulative effect was observed at increasing rFVIII concentration however this was reagent dependant. CSA containing bovine FIXa were insensitive to the presence of emicizumab and despite variability between reagents, can be used to measure rFVIII in the presence of emicizumab.

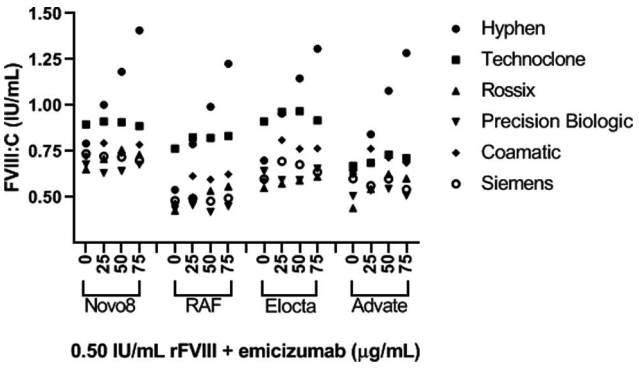

FIGURE 1 FVIII:C of SHA spiked with 0.5 mL rFVIII and emicizumab at 0–75 ug/ml

TABLE 1 emicizumab (emi) concentration (μg/mL) measured by mOSA in SHA spiked with rFVIII and 25–75 ug/mL emicizumab

	Actin FS (μg/mL)	Actin (μg/mL)	Actin FSL (μg/mL)	Pathromtin SL (μg/mL)	Synthasil (μg/mL)	Synthafax (μg/mL)	APTT SP (μg/mL)	CK Prest (μg/mL)
emicizumab only; 25, 50, 75 μg/mL	22.8, 60.5, 80.1	23.9, 64, 82.9	22.3, 63.7, 80.8	21.6, 61.7, 81.1	31, 60.4, 83.8	28.6, 60.1, 77.5	28.6, 62.2, 80.3	29.8, 69.4, 78.4
Novo8 + 25, 50, 75 μg/mL emi	37.2, 70.3, 88.9	37.9, 70.3, 91.8	43.8, 79.9, 99.2	45.3, 81.6, 99.2	42.5, 72.2, 97.9	32.5, 65.2, 82.9	36.1, 67.7, 83.6	46.3, 82.1, 86.9
RAF + 25, 50, 75 μg/mL emi	33.8, 69.5, 85.4	35.3, 68.8, 88.9	40.2, 78.1, 96.4	38.7, 77.9, 97.6	38.8, 69.6, 90.8	32.0, 62.5, 80.4	32.5, 63.0, 81.2	42.3, 77.4, 106.2
Elocta + 25, 50, 75 μg/mL emi	37.2, 71.6, 89.0	37.6, 71.7, 90.2	43.8, 82.3, 99.2	44.1, 84.1, 98.3	39.8, 70.3, 93.1	32.3, 62.6, 80.7	32.5, 63.0, 81.2	44.0, 81.1, 114.1
Advate 25, 50, 75 μg/mL emi	35.3, 70.7, 88.2	36.9, 69.8, 90.0	40.7, 81.3, 96.6	40.8, 80.7, 95.0	39.6, 75.5, 94.0	33.0, 61.9, 78.1	32.9, 65.8, 90.1	43.2, 95.4, 107.4

PB0506 | The use of an Automated Thrombin Generation Assay for the Measurement of Emicizumab and Recombinant Factor VIII in Spiked Plasma

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Background: Emicizumab is in use in patients with haemophilia A with and without anti-factor VIII (FVIII) inhibitors. Some patients may require additional recombinant FVIII (rFVIII) therapy to cover bleeding or surgery.

Aims: This study assessed the cumulative effects of rFVIII and emicizumab on thrombin generation (TG) using an automated TG instrument, ST Genesia® (Stago, France) in SHA plasma spiked with four rFVIII concentrates and increasing emicizumab concentrations.

Methods: SHA plasma (HRF, USA) was spiked with Novo8, Refacto AF (RAF), Elocta or Advate to approximately 0.5 or 1.0 IU/mL by labelled potency. Emicizumab was then added at 3 concentrations (25, 50, 75 μg/mL). STG-BleedScreen (Stago, France) was used to assess TG on the ST Genesia system. Lag time (LT), Peak thrombin (Peak), Time to peak (TTP), endogenous thrombin potential (ETP) and velocity index (VI) were measured.

Results: SHA plasma and emicizumab increased LT, Peak, TTP, ETP but not VI. Addition of 0.5 IU/mL rFVIII (no emicizumab) increased

TABLE 1 TG of SHA plasma spiked with 0.5 IU/mL rFVIII and 0–75 ug/mL emicizumab

rFVIII (0.5 IU/mL)	Emicizumab concentration (μg/mL)	Peak Thrombin (nM)	Endogenous thrombin potential (ETP, nM.min)	Velocity Index (nM/min)
SHA plasma	0	55.0	590.2	15.8
No rFVIII (emicizumab only)	25, 50, 75	52.0, 80.5, 102.1	678.8, 1015.4, 1067.1	10.1, 12.1, 15.6
Novo8	0, 25, 50, 75	159.1, 138.1, 163.9, 205.1	1078.3, 1053.5, 1175.7, 1292.6	66.7, 58, 67.6, 87.0
Refacto AF	0, 25, 50, 75	137.6, 121.5, 143.8, 178.3	1077.4, 1057.9, 1159.3, 1261.2	43.4, 35.3, 44.1, 61.2
Elocta	0, 25, 50, 75	112.7, 101.3, 125.9, 161.0	1019.0, 1004.8, 1133.7, 1246.7	31.1, 24.7, 30.4, 41.6
Advate	0, 25, 50, 75	138.8, 120.5, 136.8, 173.3	1019.1, 942.2, 1099.7, 1267.6	46.8, 39.2, 50.6, 58.1

TABLE 2 TG of SHA spiked with 1.0 IU/mL rFVIII and 0–75 ug/mL emicizumab

rFVIII (1.0 IU/mL)	Emicizumab concentration (μg/mL)	Peak Thrombin (nM)	Endogenous thrombin potential (ETP, nM.min)	Velocity Index (nM/min)
SHA	0	55.0	590.2	15.8
No rFVIII, (emicizumab only)	25, 50, 75	52, 80.5, 102.1	678.8, 1015.4, 1067.1	10.1, 12.1, 15.6
Novo8	0, 25, 50, 75	254.1, 216.8, 245.9, 267.8	1271.6, 1240.0, 1286.3, 1393.2	140.4, 103.5, 130.5, 146.0
Refacto AF	0, 25, 50, 75	209.3, 163.3, 188.3, 223.7	1199.6, 1176.5, 1208.4, 1292.4	88.1, 56.1, 71.1, 93.0
Elocta	0, 25, 50, 75	169.2, 132.6, 161.6, 188.4	1221.1, 1140.0, 1231.4, 1321.1	57.4, 38.7, 52.1, 64.8
Advate	0, 25, 50, 75	224.3, 169.0, 203.3, 225.8	1249.4, 1137.6, 1271.1, 1280.6	108.0, 66.2, 89.6, 107.8

Peak thrombin 2–3 fold from 55 nM (SHA plasma) to 112.7–159.1 nM (Elocta and Novo8) and doubled ETP from 590.2 nM.min to 1019–1078.3 nM.min (Elocta and Novo8) (table1). Addition of 1.0 IU/mL rFVIII increased the Peak to 169.2–254.1 nM (Elocta and Novo8) and ETP to 1199.6–1271.6 nM.min (RAF and Novo8). SHA plus 1.0 IU/mL rFVIII and 75 µg/mL emicizumab further increased the Peak to 188.4–267.8 nM (Elocta and Novo8) and ETP to 1280.6–1393.2 nM.min (Advate and Novo8) (table2).

Conclusions: The addition of rFVIII to SHA increased TG compared to SHA alone; however differences between rFVIII products were observed. Adding emicizumab at 25 or 50 µg/mL to these samples containing rFVIII had only a minor impact on thrombin generation. A clear increase in TG was observed when 75 µg/mL emicizumab was combined with rFVIII compared to rFVIII alone.

PB0507 | Diagnosing and Categorizing Acquired Fibrinolysis Activity with Global Hemostatic Assays

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Background: Quantitative diagnosis of the fibrinolysis is not so well developed and not routinely practiced as compared to the coagulation system.

Aims: This study aimed to assess the utility of thromboelastogram (TEG® 5000) and Thrombin Generation Assay (TGA) by TEG in diagnosing and categorizing the fibrinolysis activity.

Methods: All Citrated Kaolin TEG done during study period were classified into primary fibrinolysis (group1), secondary (group 2) and without fibrinolysis (group 3) based on TEG algorithm. Clinical management were decided upon TEG and clinical outcome were compared to understand the utility of TEG and TGA. Parameters analysed were TEG, TGA, blood transfusions, antifibrinolytics, anticoagulants, length of hospital stay and complications.

Results: A total of 436 patients included with a male to female ratio of 2.17 and a mean age of 43.64 (SD18.67). Common indications were trauma, CLD, DIC, Sepsis and Post partum hemorrhage. Fibrinolysis was observed in 192 (44.03%) patients, of these 59.3% was primary, 40.6% was secondary and remaining were group 3 (n=244). Antifibrinolytic was given in 62.5% of primary fibrinolysis. Transfusion was avoided in sepsis induced coagulopathy (n=11, 46.3%) even with prolonged PT or aPTT and started on heparin. Both TEG and TGA had a positive correlation with fibrinolysis (r=0.662, P< 0.0001) All parameters except angle was significantly associated with fibrinolysis categories (P < 0.001 by multiway ANOVA). Among TEG and TGA parameters, G, Ly 30 and Total lysis had significant association for fibrinolysis categories (Beta of −0.438, −0.331, −0.196 respectively, P< 0.002). ROC curve showed Ly30 and L (AUC = 0.989, 0.946) differentiated presence of fibrinolysis meanwhile CI and TG were significantly differentiated primary and secondary (AUC=0.992, 0.951).

Conclusions: Differentiating primary & secondary fibrinolysis may not be possible with routine investigations and since management of both the conditions are different, incorporating TEG into the decision algorithm will improve the patient care.

PB0508 | Negative Efect of Pre Pre-Analytical Error Sources on the APTT Test

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Background: Laboratory tests represent an integral part of modern medicine, influencing patient management during early diagnosis, appropriate treatment and monitoring of anticoagulation therapy. The APTT (activated partial thromboplastin time) test is recommended for monitoring unfractionated heparin therapy, a shortened result possibly reflecting an increased risk of thrombosis.

Aims: The aim of this study is documentation and awareness of the errors that can lead to a false shortening of APTT test, in order to guarantee the safety and the full quality of the laboratory services.

Methods: From August 2018 to January 2019, in the Department of Hematology of "St. Spiridon" from Iasi, it was performed identification of the possible error sources that could cause faulty shortening of APTT values.

Results: 201 tests with diminished values were recorded. The study showed that the possible erroneous (Figure 1) reduction for 8 APTT (3.98%) was determined by insufficiently sampled volume, *in vitro* hemolysis was observed in 3 specimens (1.49%) and for 86 APTT (42.78%) resampling was recommended due to clot highlighting post-analytical in the sediment, subsequent to transvasation procedure (Figure 2). All the results were accompanied by comments reporting the non-compliance, suggesting thus clinicians the possible interference with the determination.

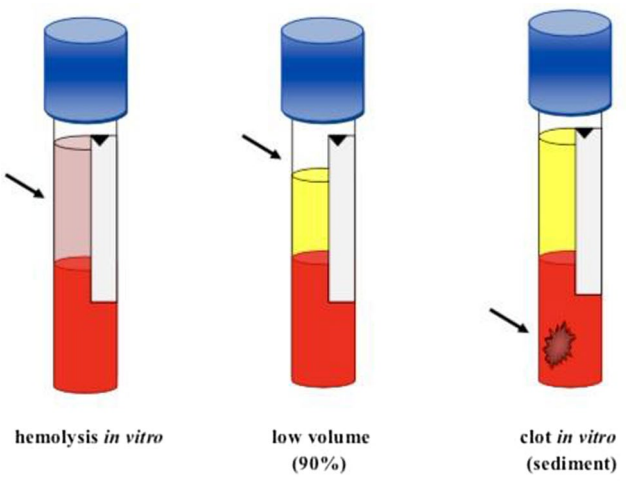

FIGURE 1 Possible errors sources

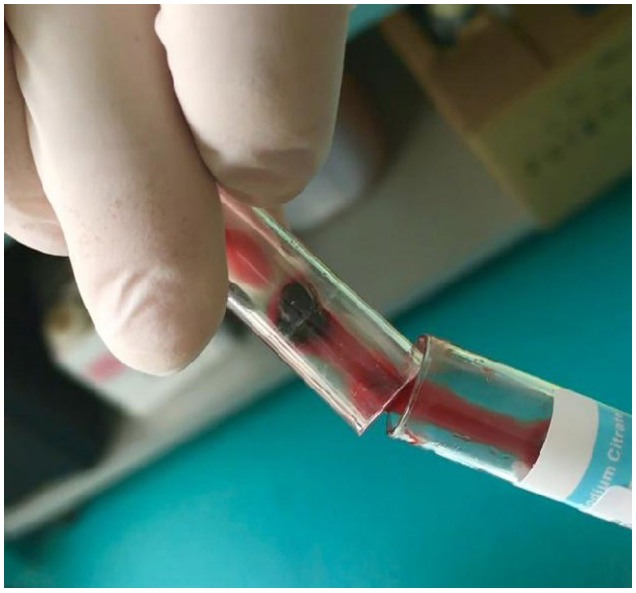

FIGURE 2 Post-analytical clot detection by transvasation

Conclusions: Quality indicators are important tools for quality system improvement in a clinical laboratory and patient care. The severity of the consequences of false test results leads to undesirable clinical outcomes in hemostasis determinations, as these investigations are often considered "diagnostic".

PB0509 | Rotational Thromboelastometry - A Point of Care Tool to Assess Bleeding Risks in Hemophilia A and B

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Background: Hemophilia A and B are inherited bleeding disorders caused by deficiencies of factor VIII (FVIII) and IX (FIX), respectively. Hemophilia treatment is based on the prophylactic replacement of the deficient factor. The most common method used for measuring FVIII and FIX is the one-stage clotting assay (OSCA) based on the activated partial thromboplastin time (APTT). Rotational thromboelastometry (ROTEM) is an alternative to assess hemostasis *in vitro*. Unlike APTT, which requires trained personnel, the ROTEM *sigma* is a fully automated point-of-care (POC) instrument.
Aims: The main aim of this study was to assess whether deficiencies of FVIII and FIX can be detected by ROTEM *sigma*.
Methods: Contact activation (formerly known as intrinsic) pathway activated ROTEM (INTEM) and APTT were assessed in plasma samples with different FVIII and FIX concentrations (ranging from 0% to 50%). INTEM and OSCA (using HemosIL SynthASil) were performed on a ROTEM *sigma* and ACL TOP 550 instruments, respectively (Instrumentation Laboratory, Bedford, MA).
Results: For FVIII, using Spearman's analysis, a very strong correlation was detected between results of APTT and INTEM clotting time (CT), r=0.986 (p< 0.0001). There was also a very strong correlation between APTT and INTEM CT results for FIX deficient plasma r=0.978 (p< 0.0001). Both INTEM and APTT were not sensitive for FVIII and FIX levels below 0.5%.
Conclusions: In conclusion, ROTEM *sigma* can be used as a POC tool to assess FVIII and FIX activity in hemophilia patients. This approach may facilitate the management of hemophilia patients in outpatient settings. Moreover, compared to conventional methodologies, ROTEM provides more information on a patient's hemostatic status, including platelet and fibrinogen contribution to clot firmness and clot stability. Further studies are needed in hemophilia to determine the feasibility of ROTEM *sigma* for routine monitoring of clinical effectiveness.

PB0510 | Effect of Dabigatran versus Enoxaparin on Blood Coagulation using the Viscoelastic Coagulation Test

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Background: A rapid and reliable assessment of the anticoagulation effect is desirable in anticoagulated treated patients following uncontrolled bleeding or before surgery. The changes that occur in blood viscosity during clotting could be used as the basis for a test that monitors coagulation function.
Aims: The aim of this study was to evaluate the effect of Dabigatran and Enoxaparin on blood coagulation using a viscoelastic coagulation test.
Methods: A total of thirty patients with Acute Coronary Disease were sampled in three different times. All patients were on treatment for 5 days with dabigatran, washout of 30 days and underwent enoxaparin treatment for another 5 days. Viscoelastic coagulation test FOR (ReoRox G2®, MediRox, Nyköping, Sweden) was performed on the three moments. Analyses were run at 37 °C within 1 h after blood collection.
Results: Compared to basal values dabigratan increased the blood viscosity (p=0.03), the coagulation time (COT) (p <0.001) and clot formation time (CLOT) (p <0.01) (Table 1). On the other hand enoxaparin besides increasing the blood viscosity (p=< 0.01) and CLOT (p=< 0.01) also changed the maximum strength of the clot (GMAX) (p= 0.020).
The comparison between the results of the 2 anticoagulants showed significant differences in viscosity (p=0.015), COT (p <0.001) and GMAX (p=0.027) values.

TABLE 1 Comparison among the parameters of viscoelastic coagulation test

	Baseline (T0)	Dabigatran (Td)	Enoxaparin (TXa)	T0 vs Td	T0 vs TXa	Td vs TXa
	(n=30)			p		
Viscosity (mPa.s) ‡	140 (110–230)	162 (366–729)	127.5 (85–165)	0.031	<0.01	0.015
Coagulation Time- COT (s)*	31 ±8	95 ± 35	30.7 ± 8	<0.001	0.741	<0.001
Clot Formation Time -CLOT (mPA)*	12.9 ±5.2	17.7±10	15.7±5.4	<0.01	< 0.01	0.590
Maximum Strength of the Clot-GMAX (Pa)*	1707 ± 491	1796 ± 386	1999 ± 527	0.377	0.020	0.027
Clot Strength Reduction- CLOT SR (%)*	14 ± 9	15±5	17±10	0.455	0.250	0.468

*Mean±SD ‡Median (25th–75th percentiles)

Conclusions: Viscoelastic coagulation changes according to the anticoagulation treatment. The main difference between these anticoagulants was on the clot formation time (Viscoelastic and COT).

PB0511 | Low-Angle Light Scattering for the Analysis of the Platelet Functions: Preanalytical Variables and Evaluation in Pediatric Patients

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Background: Light transmission aggregometry (LTA) is a highly informative method, but has some limitations. A recently developed assay evaluates platelet aggregation in a diluted sample using low-angle light scattering, which has low sample volume requirement, provides additional information about aggregate structure and platelet shape change depending on the scattering angle, and uses standardized buffer as a medium.

Aims: Validation of the low-angle light scattering method for measurement of platelet aggregation.

Methods: Platelets were diluted by modified HEPES buffer to 10,000 platelets/ µl, stimulated by 800 nM ADP, and the initial velocity and amplitude of aggregation at 1.5° and 12° were evaluated. The study included healthy adults (n = 32, 21–40 years old) and healthy children (n = 20, 1–18 years old), pediatric patients with Glanzmann's thrombasthenia (TG, n = 2), hypo- and dysfibrinogenemia (n = 1), as well as patients with bleedings (n = 19).

Results: We obtained reference intervals for healthy adults and children (Table 1). There were no significant differences in the parameters characterizing platelet aggregation and shape change between these control groups. The aggregation amplitudes obtained by low-angle light scattering method and LTA moderately correlated both in the group of healthy children and patients (Spearman's correlation coefficient 0.47 and 0.44 respectively, $p < 0.05$). For patients with TG, hypo- and dysfibrinogenemia, there was shape change but no aggregation in response to ADP stimulation. The intake of a fat meal or physical activity before blood sampling, as well as the blood: citrate ratio did not affect the results of the study.

TABLE 1 Reference intervals for healthy adults and healthy children

	Initial velocity at 1.5°, AU	Amplitude of aggregation at 1.5°, AU	Amplitude of aggregation at 1.5°, AU
Adults	0.94–1.98	414–1159	1.88–2.63
Children	1.05–2.06	378–1008	1.72–2.53

Conclusions: Our results show that the low-angle light scattering method can be used to characterize platelet aggregation: the influence of preanalytical variables has been evaluated; reference intervals were determined for healthy adults and children; the method has been tested on patients with bleeding.

PB0512 | Lidocaine Effects on Coagulation Assessed by Whole Blood Rotational Thromboelastometry

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Background: Lidocaine is used in vascular graft preservation solutions, having endothelial-protecting and antithrombotic effects. Lidocaine blocks membrane ion channels, leading to platelet membrane stabilization and platelet function inhibition, however the complex effects of lidocaine on blood coagulation and clot formation kinetics are not completely elucidated.

Aims: To study the effect of increasing lidocaine concentrations on coagulation parameters and clotting kinetics using the velocity

TABLE 1 Coagulation parameters and parameters derived from the ROTEM velocity curve of clot formation

Test	Control	Lidocaine 0.3%	Lidocaine 0.6%	Lidocaine 0.9%	P value
CT	655.8 (±188.1)	821.0 (±227.2)*	1043.2 (±342.1)*	1315.33 (±414.8)*	<0.001
t-MaxVel	765.3 (±238.7)	972.6 (±293.1)*	1295.9 (±418.3)*	1707.4 (±580.1)*	<0.001
MaxVel	7 (5; 11)	8 (6; 11)	4 (3; 5)*	2 (1.1; 4)*	<0.001
MCF	54.8 (±5.1)	55.6± (6.3)	39.1 (±6.7)*	14.6 (±5.7)*	<0.001
AUC	5588.2 (±506.4)	5732.2 (±568.9)	3939.8 (±646.8)*	1508.8 (±562.5)*	<0.001
MCE	124 (±26.4)	129.6 (±35)	66.4 (±19)*	17.9 (±8.3)*	<0.001
LI30	100 (100; 100)	100 (100; 100)	99 (96; 100)*	100 (99; 100)	<0.001
LI45	98 (96; 99)	100 (99; 100)*	92 (88; 97)*	97 (92; 100)	<0.001
LI60	94 (92; 95)	98 (97; 99)*	86 (82; 90)*	94 (89.5; 100)	<0.001

curves of clot formation obtained from rotational thromboelastometry (ROTEM).

Methods: In this in vitro study, whole-blood samples without lidocaine and with 3 different lidocaine concentrations (0.3%, 0.6% and 0.9%) were analysed using non-activated ROTEM. Clot amplitudes were used to calculate the clot elasticity at different time points and the velocity of clot formation based on changes in clot amplitude and elasticity.

Results: Whole blood from 15 healthy volunteers was used (mean age±SD was 37±5.64 years, 40% male). Lidocaine induced statistically significant differences in haemostasis as revealed by RMANOVA or Friedman tests.

0.3% lidocaine concentration prolonged the initiation phase of clotting, without significant differences in the propagation phase or in clot stability and inhibited clot lysis compared to control group. Higher lidocaine concentrations prolonged both initiation and propagation phases, decreased clot stability and maximum clot elasticity compared to control group. Lysis was increased only in the 0.6% group compared to control. Velocity traces of clot formation showed all phases of clotting impaired and increased lysis in lidocaine 0.6% samples and feeble clot strength and elasticity in lidocaine 0.9% samples.

Conclusions: While lidocaine 0.3% only delayed coagulation initiation, lidocaine 0.6% inhibited all phases of haemostasis and increased clot lysis compared to control, while lidocaine 0.9% resulted in very weak clot formation with low lysis visible on ROTEM. More research is needed to explain the effects of lidocaine on clot formation and lysis, as other mechanisms besides platelet inhibition might be involved.

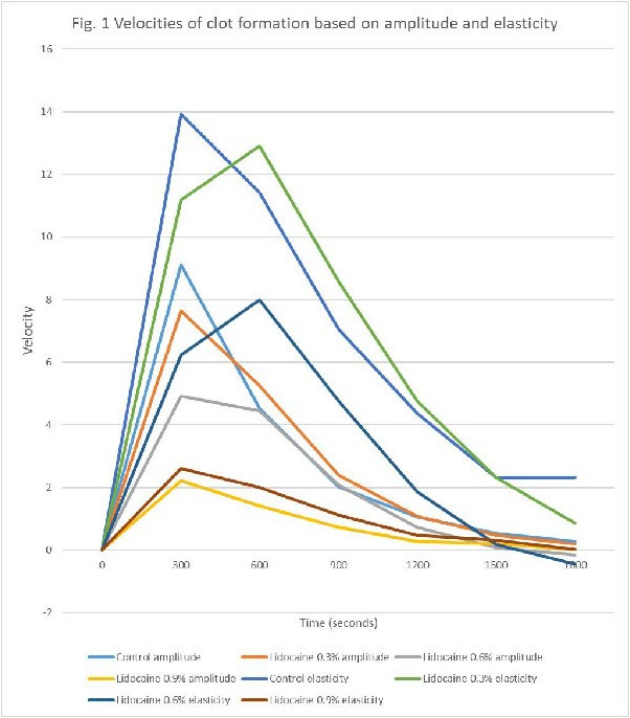

FIGURE 1 Velocities of clot formation based on amplitude and elasticity

PB0513 | The Sensitivity of Two New APTT Reagents to Factors VIII, IX and XI

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Background: The APTT is used as a screening test for deficiencies of factors VIII, IX and XI. A prolongation to the APTT should be observed when the clotting factor is below 30 IU/dL. APTT reagents may demonstrate different sensitivities to these clotting factors because of their composition.

Aims: To compare the APTT sensitivity to FVIII, IX and XI of two new APTT reagents from Horiba (Kyoto, Japan), APTT 4 and APTT Liq 4, with 6 commonly used APTT reagents.

Methods: FVIII deficient plasma (Siemens), FIX and FXI deficient plasma (Precision Biologic) was spiked with normal plasma (CCNRP, Precision Biologic) to obtain plasmas with factor concentrations of 0–90 IU/dL. APTT were performed with 8 APTT reagents on 5 different days. Local reference ranges were generated for each reagent. The intersection of the upper limit of the APTT reference range and concentration of clotting factor determined the reagent sensitivity.

Results: Sensitivity to FVIII ranged from 31.5–67.3 IU/dL (Synthasil, Actin FS), to FIX from 15.9–36.1 IU/dL (APTT SP, Synthasil) and to

TABLE 1 The senstivity of 8 APTT reagents to factors VIII, IX and XI

APTT Reagent	Composition	FVIII Sensitivity (IU/dL)	FIX Sensitivity (IU/dL)	FXI Sensitivity (IU/dL)
Actin FS	Ellagic acid, purified soy phosphatides	67.3	25.5	49.3
Actin FSL	Ellagic acid, soy/ rabbit brain phosphatides	46.7	22.6	28.3
STA-PTTA	Silica, rabbit brain phospholipid	47.6	29.0	35.7
APTT 4	Micronised silica, rabbit brain phospholipid	40.9	26.8	43.3
APTT LIQ 4	Ellagic acid, rabbit brain phospholipid	37.4	29.7	48.9
SYNTHASIL	Silica, synthetic phospholipids	31.5	36.1	49.0
SYNTHAFAX	Ellagic acid, synthetic phospholipids	62.8	27.5	55.2
APTT SP	Silica, synthetic phospholipids	38.4	15.9	35.6

FXI from 28.3–55.2 IU/dL (Actin FSL, Synthafax). Interassay CV was less than 5.5% for all reagents and factors. Sensitivity to FVIII and FXI was greater than 30 IU/dL with all reagents, including APTT 4 and APTT Liq 4, with the exception of Actin FSL and FXI (28.3 IU/dL). Sensitivity to FIX was greater than 30 IU/dL with Synthasil only. **Conclusions**: A normal APTT does not always indicate normal haemostasis. The sensitivity to FVIII and FXI of most of those APTT reagents included in this study was considered acceptable however sensitivity to FIX was lower. The source of activator did not appear to effect sensitivity but the phospholipid source, type and concentration may well influence the ability to diagnose reduced factor activities, particularly FIX.

PB0514 | Establishment of a Novel Mixing Test Using Activated Partial Thromboplastin Time (aPTT)-Based Clot Waveform Analysis

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Background: The differentiation of coagulation disorders with prolonged aPTT is important for appropriate treatment. Mixing test is useful for screening coagulation factor deficiencies, inhibitors, and lupus anticoagulants (LA), but this assay is a complicated work and requires 2-hour incubation. In addition, interpretation of the results is sometimes difficult.

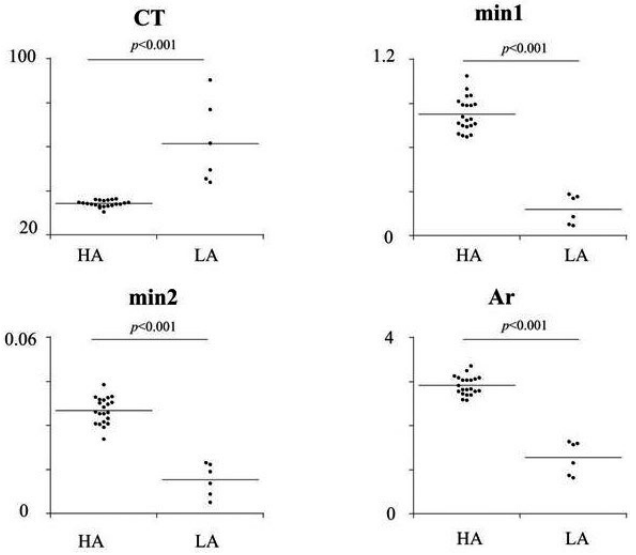

FIGURE 1 ratio of 4 parameters (clotting time, min1, min2, Ar). * : p<0.01, ** : p<0.001

Aims: To differentiate factor deficiencies, inhibitors, and LA-positive cases by a short-time incubation and the numerical parameter index using aPTT-based clot waveform analysis (CWA).

Methods: aPTT-CWA was performed with CP3000™ apparatus using Coagpia APTT-N®. FVIII-deficiency (n=20), LA-positive (n=6), and FVIII inhibitor plasma (n=48; 0.6–302 BU/mL) from patients and commercial plasma were used. Pooled normal plasma (PNP) and samples were mixed (1:1) and aPTT waveform were obtained immediately (0 min-incubation) and after 12 min-incubation. The differentiation was performed in 2 steps. To first differentiate FVIII inhibitor, ratio of 12 min/0 min of 4 parameters (clotting time, min1, min2, Aspect ratio (Ar:presentated in ISTH2019, ID PB1302) were assessed. Following, to differentiate LA and FVIII-deficiency, the above parameters were assessed.

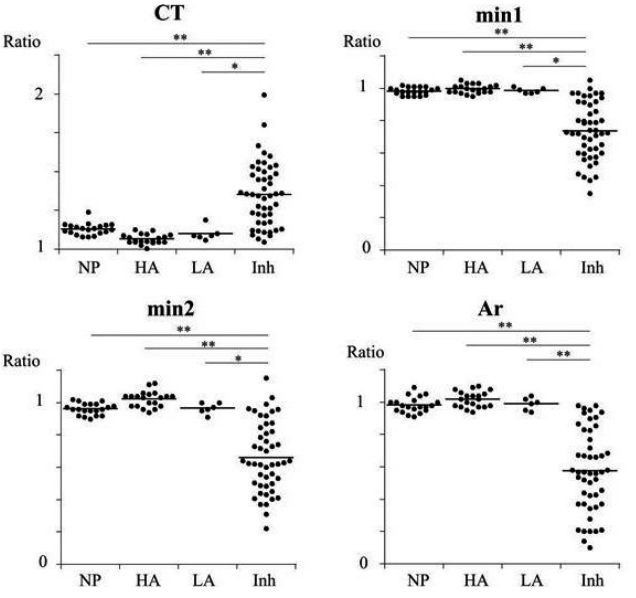

FIGURE 2 Parameters for differentiation between LA and Haemophilia A (HA)

Results: In CWA with FVIII inhibitor sample, the clotting time ratio was increased and the other ratios were decreased significantly ($p<0.01$) compared to the ratios in factor-deficiency and LA-positive (Figure 1). By setting the different mixing ratios including further 2 patterns (sample:PNP = 9:1, 1:1, 1:9), the sensitivity of differentiation in inhibitor with lower (< 2 BU) and higher titer (>100 BU) was improved, although only clotting time ratio in cases with lower inhibitor titer showed no significant difference. The 4 parameters between LA-positive and factor-deficiency cases showed significant differences ($p<0.01$, Figure 2).

Conclusions: We successfully established a novel mixing test based on aPTT-CWA to precisely differentiate FVIII-deficiency, FVIII inhibitors, and LA-positive in a short-time incubation. This method will greatly contribute prompt diagnosis of prolonged aPTT samples.

PB0515 | Influence of Anticoagulants on the Biomechanical Phenotype of Platelets

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Background: Biomechanical properties of platelets are highly relevant for platelet function and are emerging as diagnostic markers for disturbed platelet physiology.

Aims: To assess the impact of different anticoagulants on cytoskeleton dependent biomechanical properties of platelets by high-throughput real-time fluorescence and deformability cytometry (RT-FDC).

Methods: Blood was collected in BD Vacutainer® Tubes containing acid citrate dextrose solution A (ACD-A), 3.2% buffered sodium citrate (Citrate), Lithium-Heparin (Li-Heparin), Hirudin and dipotassium ethylenediaminetetraacetic acid (K_2EDTA). PRP was obtained by centrifugation at 120xg. Mechano-phenotyping of platelets in PRP was performed using RT-FDC (Figure 1a,b and c). Platelets were labeled with anti-CD61-PE and anti-CD62P-AlexaFluor 647 was used to quantify platelet activation by TRAP6. Confocal fluorescence microscopy and flow cytometry were used to quantify changes in F-actin amounts in platelets using phalloidin labelling.

Results: Unstimulated platelets show higher deformation (i.e. softer platelets) of 0.1225 (in ACD-A); 0.123 (in Citrate); and 0.102 (in Hirudin) (Figure 1d). Intriguingly, platelet deformation was lower (i.e. stiffer platelets) 0.0694 (in Li-Heparin) and 0.0338 (in K_2EDTA). TRAP-6 stimulation decreased deformation (i.e. stiffened platelets) by three fold to 0.0434 (in ACDA-A), 0.044 (in Citrate) and 0.0421 (in Hirudin). In contrast, minor differences in platelet deformation where observed in Li-Heparin (0.0434) and K_2EDTA (0.0314) upon TRAP-6 stimulation. Basal F-actin content in unstimulated platelets was up to threefold higher in K_2EDTA, Li-Heparin and Hirudin in

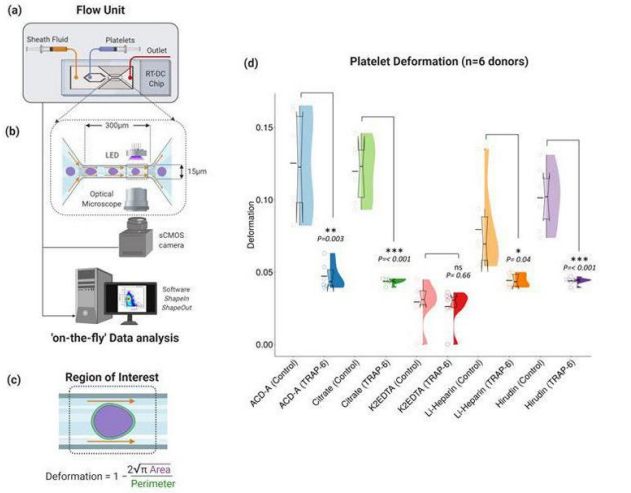

Figure 1: Schematics of RT-FDC microfluidic chip (a) consist of inlets for sheath fluid and for cells/platelets, which combine into to a channel with narrow constriction zone (b) where cell/platelets undergo deformation due to hydrodynamic compression brought about by sheath fluid. A high- powered light emitting diode (LED) is used to illuminate the samples. Images are recorded by a scientific complementary metal–oxide–semiconductor (sCMOS) camera working at high frame rates (≈ 2000 images/second) that is synchronized with the frequency of illumination time. This combined together with microfluidic flow, RT-FDC allows for mechanoprofiling of >1000 unique platelets per second. Platelet deformation (c) is determined on-the-fly in real time by computational image processing taking into account the area and perimeter of the deformed platelet as it passes through the region of interest. (d) Comparison of viscoelastic deformation of single platelets in platelet rich plasma (PRP) measured by RT-FDC in the presence of different anticoagulants and upon stimulation with platelet agonist TRAP-6. The half-violin plots show distribution of platelet population based on their deformation, notched-box plot represent median deformation and 95% CI while horizontal line shows mean deformation. n=6 donors, >1500 single platelets analyzed for each donor.

FIGURE 1 Effect of anticoagulants on platelet biomechanics

comparison to ACD-A and Citrate. TRAP-6 resulted in F-actin fold increase of 4.56 (in ACD-A); 4.53 (in Citrate); and 2.9 (in Hirudin) while minor changes in total F-actin content of 1.86 (in Li-Heparin), 1.183 (in K_2EDTA) were observed in comparison to unstimulated platelets.

Conclusions: Anticoagulants K_2EDTA and Li-Heparin influence platelet biomechanics by decreasing human platelet deformability (i.e. increase stiffness) by an increase in actin polymerization resulting in higher F-actin content in unstimulated platelets. These observations are relevant for further exploration of human platelet biomechanical properties.

PB0516 | Evaluation of the Newly-developed Collagen Induced Platelet Aggregation Level (CPAL) System in Aggregometer on Automated Coagulation Analyzer

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Background: Light transmission aggregometry (LTA) is the gold standard for platelet function assessment. The scoring system,

calculated from two results by different concentration agonist on the semi-automated analyzers, is commonly used to confirm the effect of antiplatelet drugs in Japan. Last year, we published a new parameter for ADP-induced platelet aggregation in Clin. Lab., and more recently a new parameter "Collagen induced platelet aggregation Level (CPAL)" was developed and equipped on the automated coagulation analyzer from SYSMEX. CPAL is calculated as a score from 0.0 to 10.0 based on platelet aggregation patterns with 1.0 and 5.0 μg/mL of collagen.

Aims: We evaluated the basic performance of the new CPAL system and comparison with the maximum aggregation rate of 1.0 mM arachidonic acid (AA-MA) and result of VerifyNow Aspirin assay, ARU.

Methods: The within-run precision was calculated by conducting five replicate analyses of the platelet-rich plasma (PRP) from healthy volunteers and 1.0 mM of aspirin-spiked PRP. The effect of the aspirin was evaluated using several concentrations of it. A comparative study was performed using 63 PRP samples from patients who received antiplatelet therapy.

Results: The coefficient of variation in within-run precision was within 5% for CPAL. CPAL showed excellent correlation to the scoring system in Japan ($r = 0.81$, $p < 0.001$). CPAL gently decreased depending on aspirin concentration, while AA-MA was sharply decreased. A significant correlation was observed between CPAL and AA-MA ($r = 0.64$; $P < 0.001$). However, a weak or no correlation was observed between CPAL and ARU ($r = 0.21$; $P = 0.102$).

Conclusions: The newly developed CPAL has acceptable performance. CPAL showed good correlation with AA-MA and changed according to slight difference of aspirin concentration. CPAL is a new platelet aggregation scoring system with the potential to monitor the effect of aspirin on the automated coagulation analyzer.

PB0517 | Heparin-Insensitive Chromogenic Measurement of Activated Factor XI

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Background: Measurement of activated FXI (FXIa) has been implemented to confirm the absence of thrombosis-generating agents in human immunoglobulin G (IgG) preparations used for the treatment of primary immunodeficient patients. Unexpectedly, heparin was found to heavily interfere with the commercially available chromogenic FXIa assay (Rossix). This inhibition, observed even at low levels of heparin, could be reversed by protamine sulphate, however requires exact titration because excess protamine sulphate interfered with the assay.

Aims: To overcome these limitations, heparin degradation by heparinase was implemented before running the chromogenic FXIa assay.

Methods: Inhibitory effects of heparin and protamine sulphate on the chromogenic FXIa assay were determined for fixed FXIa concentrations, both in buffer and in a human IgG preparation. The optimal heparinase concentration was determined in buffer and confirmed in an IgG sample, spiked with heparin and purified FXIa. The feasibility of the method was confirmed by the measurement of IgG samples, spiked with 1 IU heparin/mL and low levels of purified FXIa.

Results: Incubation of heparinized IgG samples with heparinase at concentrations of 5 and 10 U/mL fully reversed heparin's inhibitory effect. Even at the maximum heparin concentration investigated (3 IU/mL), FXIa recoveries, spiked at 8 mIU/mL, were 92.7% and 90.9% for heparinase concentrations of 10 and 5 U/mL, respectively. IgG samples spiked with 1 IU heparin/mL demonstrated FXIa recoveries within an acceptable 100 ± 11% range. Intra-run precision ($n = 6$), determined for these samples, ranged from 6.6% to 7.1%.

Conclusions: Heparinase pretreatment was added to the commercially available chromogenic FXIa test. This pretreatment, having no effect on assay performance, fully removed the inhibitory effect of heparin. FXIa spiked at low levels to the IgG preparation was found to have acceptable recoveries even in the presence of 3 IU heparin/mL.

PB0518 | UK NEQAS for Blood Coagulation D-Dimer Point of Care Testing Programme: A Review of Data

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Background*:*

In May 2014 UK NEQAS BC introduced a POCD-dimer programme. n= 106: (Roche Cobas h232=67 and Quidel Triage=39).

Aims: To provide external quality assessment (EQA) to POC D-dimer testing.

Methods: Samples are lyophilised citrated plasma with a pre measured volume of diluent and a disposable pipette such that no laboratory equipment is needed. A patient scenario is also provided and users are required to choose either "no further investigation required" or "further investigation required" based on their local test result. This post analytical aspect is scored if there is a consensus of 80% or more. Peer group performance assessment is undertaken. Median values are calculated and the furthest 10% from the median (both higher and lower) are considered "outwith consensus". If a centre has 3 outwith consensus surveys consecutively they will be classified as "persistently outwith consensus" and receive contact from the programme director.

Results: Results are shown in tables 1 and 2.A small number of centres have given interpretations that were inappropriate based on their results. One centre had a result of 0.74ug/ml FEU and stated "no further investigation", manufacturers stated cut off is 0.5ug/ml FEU. For samples that were provided with a patient scenario of a Wells score of 3 or 4 a small number of centres stated no further investigation required. ie they had results of below the cut off but did not take into account the provided high Wells score.

TABLE 1 Data shown for the last 2 years for Triage users

Table 1 Survey	Triage median ng/ml FEU	Triage interpretation	% Outwith consensus results	% Outwith consensus interpretation	CV%
16	296	89.5% no further investigations	8.7	10.5	17.4
17	443	Not scored	14.7	not scored	13.2
18	402	Not scored	16.1	not scored	23.4
19	1810	100% Further investigations	18.9	0.0	25.2
20	597	88.9% Further investigations	15.1	2.0	18.1
21	100	93.3% no further investigations	0.0	6.7	Not applicable
22	357	Not scored	15.2	Not scored	27.4
23	1400	100% Further investigations	17.1	0.0	17.5

TABLE 2 Data shown for the last 2 years for Cobas h232 users

Survey	Cobas h232 median ug/ml FEU	Cobas h232 interpretations	% Outwith Consensus results	% Outwith Consensus interpretation	CV%
16	0.25	89.9% No further investigations	19.6	10.2	20.1
17	0.25	100% No further investigations	14.5	0.0	17.3
18	0.23	Not scored	9.3	not scored	17.1
19	1.52	98% Further investigations	16.7	2.0	23.4
20	0.36	92% No further investigations	14.0	8.0	15.6
21	0.1	89.4% No further investigations	1.9	10.6	31.5
22	0.37	91.5% No further investigations	17	8.5	24.1
23	0.995	98% Further investigations	18.3	2.0	19.7

Conclusions: EQA is essential for D-dimer testing whether in the laboratory or in a POC setting. The interpretations of these results overall was good but some centres did not take into account the patient scenario provided. We have also seen results which are over the manufacturers stated cut off which have been deemed by the user to not require further investigation.

PB0519 | Laboratory Test Ordering Practices for Suspected Heparin-Induced Thrombocytopenia

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Background: Heparin induced thrombocytopenia (HIT) is caused by antibodies to platelet factor 4 (PF4)-heparin complexes that can cause thrombocytopenia and life-threatening thrombosis. HIT laboratory testing includes immunoassay (IA) screening for HIT antibodies, which has relatively low specificity, and the limited availability serotonin release assay (SRA), which has high sensitivity and specificity. The 4Ts clinical scoring system determines pre-test probability and, when low, is a reliable method to exclude HIT. Optimal HIT evaluation incorporates the 4Ts score, which can direct appropriate testing.

Aims: This study aims to evaluate lab utilization for HIT testing with 4Ts score and HIT antibody latex immunoturbidometric assay (LIA) implementation.

Methods: Our laboratory implemented measures to encourage appropriate HIT test ordering by: 1) application of improved turn-around-time HIT LIA and 2) order prompts for 4Ts score calculation. We then reviewed laboratory orders for the HIT IA and SRA for six months before and after HIT LIA assay and for 3 months of 4Ts score implementation.

Results: Before LIA, our lab performed 272 enzyme IA and 39 SRA. After LIA implementation, 230 LIA and 38 SRA were performed in a similar 6 month period. The 4Ts score order prompt was implemented in 77 cases to date, with 18 (23.4%) low; 52 (67.5%) intermediate; and 7 (9.1%) high probability scores. Of cases with a low probability 4Ts score, 17/18 had a negative LIA and no case had a positive SRA. The SRA was positive in 3/12 (25%) cases with intermediate or high probability 4Ts score and positive LIA. The SRA was negative in all cases with negative LIA.

Conclusions: This study demonstrates that implementation of rapid turn-around time LIA and 4Ts have not yet resulted in fewer SRA tests which are still ordered despite negative LIA. Strategies to optimize HIT IA and SRA utilization will be discussed.

PB0520 | Comparison of the Biophen® Heparin LRT with the HemosIL® Liquid Anti-Xa Assay for the Measurement of Apixaban, Edoxaban and Rivaroxaban

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Background: Chromogenic anti-Xa activity assays with substance-specific calibrations are used for quantitative measurement of apixaban, edoxaban, and rivaroxaban. Recently, a novel hybrid calibrator setting was developed for the Biophen® Heparin LRT assay, which only requires one single calibrator set for the measurement of apixaban, edoxaban, and rivaroxaban.

Aims: Aim of this study was to perform an analytical evaluation of the Biophen® Heparin LRT assay using this novel hybrid calibrator setting as well as a method comparison with the HemosIL® Liquid Anti-Xa assay.

Methods: All measurements with the Biophen® Heparin LRT as well as the HemosIL® Liquid Anti-Xa assay were performed on the same ACL Top 750® analyser. For the method comparison study 159 patients on anticoagulation treatment (n=80 for apixaban, n=23 for edoxaban, and n=55 for rivaroxaban) were measured in parallel with the respective the Biophen® Heparin LRT and the HemosIL® Liquid Anti-Xa assays.

Results: The detection limit for the Biophen® Heparin LRT apixaban, edoxaban, and rivaroxaban assays were 6.6 ng/ml, 7.5 ng/ml, and 9.7 ng/ml, respectively. Within-run and total coefficients of variation were ≤3.8% and ≤5.6% for the apixaban, ≤5.2% and ≤5.6% for the edoxaban, and ≤5.4% and ≤6.1% for the rivaroxaban assay. The method comparison study showed a very strong correlation between the Biophen® Heparin LRT and the HemosIL® Liquid Anti-Xa assays for apixaban (r_s=0.996; p< 0.001), edoxaban (r_s=0.994; p< 0.001), and rivaroxaban
(r_s=0.986; p< 0.001). Passing and Bablok regression analyses revealed only a rather small, clinically not relevant constant bias as well as proportional difference between the two methods.

Conclusions: The Biophen® Heparin LRT assay using the novel hybrid calibrator setting for measurement of apixaban, edoxaban, and rivaroxaban meets the needs of quality specifications of laboratory medicine. The test results of the Biophen® Heparin LRT assays and the HemosIL® Liquid Anti-Xa assays were comparable for apixaban, edoxaban, and rivaroxaban.

PB0521 | Estimation and Analysis of Measurement Uncertainty Acceptability: The Interest of Bayesian Inference

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Background: According to the ISO 15189 standard, section 5.5.1.4, "The laboratory shall determine measurement uncertainty (MU) for each measurement procedure in the examination phase used to report measured quantity values on patients' samples. The laboratory shall define the performance requirements for the measurement uncertainty of each measurement procedure and regularly review estimates of measurement uncertainty." Section 5.5.1.2 of the standard also specifies that "The laboratory shall obtain information from the manufacturer/method developer for confirming the performance characteristics of the procedure without modification." From this point of view, Bayesian predictive inference is an especially well-adapted approach for IQC management, as several reports have made clear.

Aims: Taking factor-II assay as an example, the present study aimed to explain the usefulness of Bayesian inference applied to IQC results analysis, as a means both of calculation and of assessing the acceptability of estimated MU.

Methods: The Bayesian approach provides a predictive distribution of the future observable. This sequentially updated distribution can be used to determine a region where the future observable will (most likely) be, as long as no special causes are present. Using the predictive distribution, a 100(1-a) % Highest Predictive Distribution (HPrD) region, defined by the lower and upper PCC limits (figs 1 and 2), estimates MU with 95% probability if the chosen a value is 5% (Tsiamyrtzis et al., Blood Coagulation and Fibrinolysis 2015; 26:590–6).

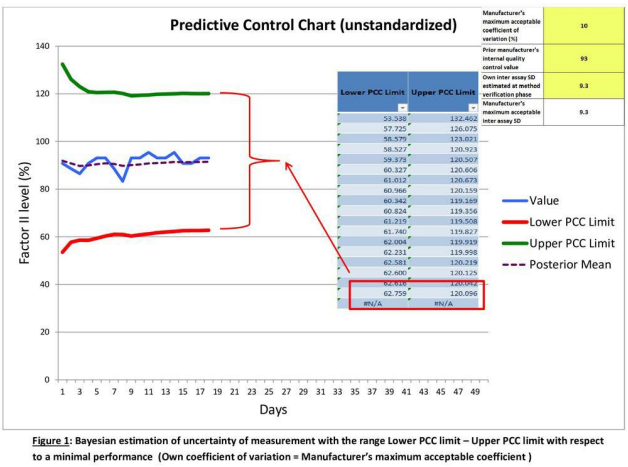

FIGURE 1 Bayesian estimation of uncertainty of measurement with the range Lower PCC limit - Upper PCC limit with respect to a minimal performance

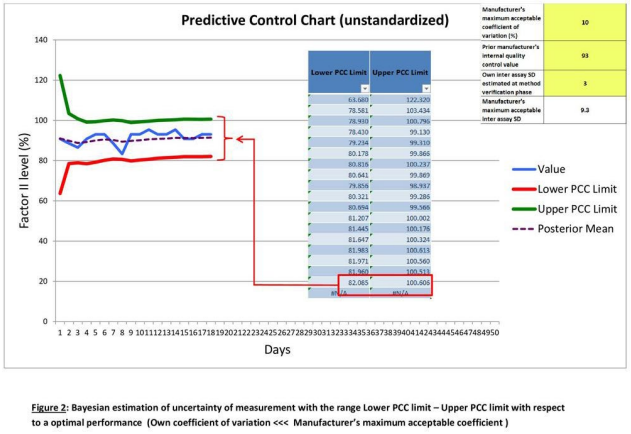

FIGURE 2 Bayesian estimation of uncertainty of measurement with the range Lower PCC limit - Upper PCC limit with respect to a optimal performance

Results: The manufacturer's maximum acceptable coefficient of variation defines the maximum acceptable MU in terms of lower and upper range limits (fig. 1). In truly efficient laboratories, MU is significantly lower (fig. 2).

Conclusions: Bayesian inference is an appropriate means of estimating measurement uncertainty for methods not subject to modification.

PB0522 | Long-Term Internal Quality Control Management: The Interest of Bayesian Inference

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Background: On the conventional internal quality control (IQC) management approach, it is not easy to deal with two major practical issues that may jeopardize the analytic quality of the Results long-term revision of control chart parameters, and managing reagent batch change-over and the accompanying recalibration of control chart target values.

Aims: The present study seeks to demonstrate the reliability of using three Bayesian tools in long-term IQC management with respect to ISO 15189 standard requirements for optimal patient care. Alongside the predictive control chart (PCC), previously described, two other tools are used to detect any insidious persistent analytic trends during both preliminary and long-term IQC management phases.

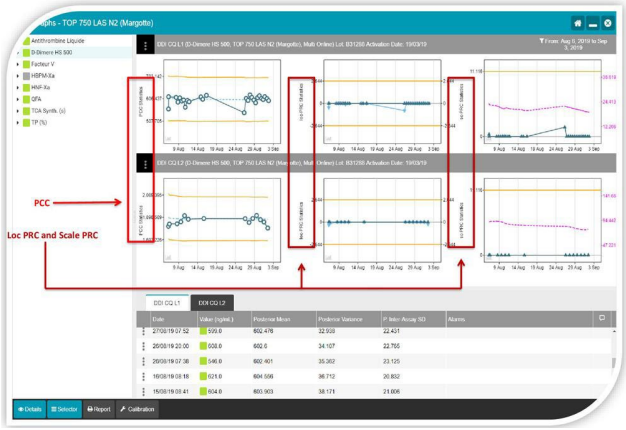

FIGURE 1 The three Bayesian tools for long term IQC results management

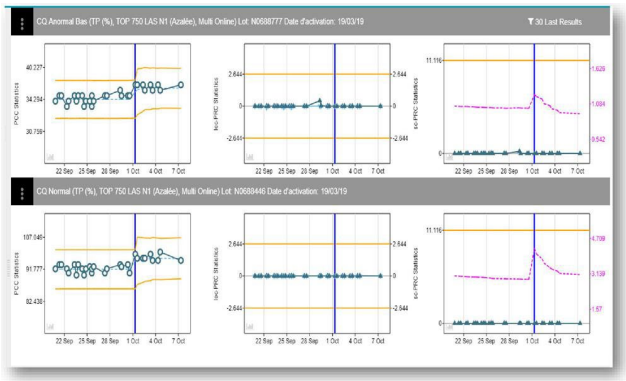

Figure 2

FIGURE 2 A reagent batch switch management with the three Bayesian tools

Methods: These Bayesian tools utilize the CUSUM (cumulative sum), which, much like the EWMA (exponentially weighted moving average), is a powerful tool for detecting trends. The Location Predictive Residual CUSUM (Loc PRC) and the Scale Predictive Residual CUSUM (Scale PRC) are dedicated to detecting even small persistent shifts in systematic and random error, respectively.

Results: Figure 1 shows PCC functioning under Loc PRC control regarding shift in control chart target values and under Scale PRC control for shift in method variance. Figure 2 shows the same controls during reagent batch changeover.

Conclusions: The association of these 3 Bayesian tools enables efficient longitudinal management of IQC results, avoiding the need for a preliminary phase in IQC reagent batch changeover. Bayesian inference, which considers the target value as a random variable, also avoids having to revise parameters on the control chart.

PB0524 | The Effect of RBCs on Different Platelet Count Enumeration Methods in Patients with Hemoglobinopathies

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Background: Platelet count is measured by automated cell counters by various principles including impedance, optical and fluorescence. Variations have been noted between the different methodologies. We undertook this study to determine whether changes in RBC size or structure influence platelet count variation in patients with hemoglobinopathies.

Aims: 1. To determine the percentage difference between platelet values measured by impedance (PLT-I) and fluorescence-optical (PLT-F) in patients with Beta-Thalassemia, HbE and HbE/Beta-Thalassemia (HbE-BT). 2. To determine whether the calculated percentage difference between PLT-I and PLT-F showed any correlation with type of hemoglobinopathy, Micro-RBC% or FRC%.

Methods: 40 patients with either Beta-Thalassemia, HbE or HbE/B-T detected by high performance liquid chromatography in our hospital between April 2019 and January 2020 whose samples were run in both PLT-F and Reticulocyte Mode on the Sysmex XN 1000 were included in the study. PLT-F, PLT-I, RBC count, Mean Cell Volume (MCV), Micro RBC%, FRC% and Hemoglobin F% data was collected. Statistical analysis was performed.

Results: HbE/B-T showed the maximum percentage difference (27.81%) between PLT-F and PLT-I while HbE showed the least difference (8.12%). The mean HbF% was 4.85% and the mean FRC% was 1.45%. Statistically significant positive correlation was seen between PLT-I and PLT-F percentage difference and HbF as well FRC% ($p < 0.05$) by Pearson correlation test. The mean MCV was 64.5fl, while the mean Micro RBC% was 47.59%. No significant correlation was seen between PLT-I and PLT-F percentage difference and MCV, Micro RBC% or RBC count in these patients.

Conclusions: Significant correlation between PLT-I and PLT-F percentage difference and HbF and FRC% in patients with Beta-Thalassemia, HbE and HbE/Beta-Thalassemia suggest that RBC and hemoglobin structure may play a role in automated platelet measurement interference in hemoglobinopathies.

PB0525 | Inter-Centre and Inter-Observer Agreement of Determination of DOACs in Urine Samples of Patients by Means of DOAC Dipstick Test

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Background: In emergency situations, detection of direct oral anticoagulants (DOACs) needs to be fast, but this is not always feasible. With the recent availability of a accurate dipstick point-of-care device, DOACs can be rapidly detected in urine. However, the inter-observer agreement of this visual analysis requires evaluation.

Aims: This study aims to define the inter-centre and inter-observer agreement of the analysis of DOACs in urine samples of patients treated with factor Xa inhibitors or thrombin inhibitor dabigatran by means of DOAC Dipstick test.

Methods: The inter-centre agreement was analysed from the results of a prospective, open-label, controlled, non-randomized, multicentre study performed in Germany including subjects treated with an oral direct factor Xa inhibitor (DXI) or the oral thrombin inhibitor (DTI) dabigatran. The true positive and true negative rate of the factor Xa inhibitor and thrombin inhibitor DOAC Dipstick test was compared with the results obtained by liquid chromatography tandem mass spectrometry (LC-MS/MS) from urine samples of subjects. The inter-rater reliability was analysed from data obtained from an international laboratory study including 13 centres and 10 DOAC samples of patients treated with apixaban, rivaroxaban and dabigatran and controls without anticoagulants.

Results: Eight hundred and eighty of 904 included subjects were evaluable from 18 German centres for analysis. The rate of false results for the specific DXI and DTI pads were 2.8% and 0.7%, respectively. The difference between centres was not significant for DXA and the number of false results with DTI pads was insufficient to perform statistical analyses. The international study showed an

inter-observer agreement with kappa values of 0.94 for apixaban and rivaroxaban and of > 0.95 for dabigatran.

Conclusions: The present study shows that DOAC Dipstick test is determined with a very high agreement between centres and between observers encouraging further multinational evaluation.

PB0526 | Discrimination between Acquired Hemophilia A and Lupus Anticoagulant Using Rotational Thromboelastometry (ROTEM)

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Background: Patients with prolonged activated partial thromboplastin time (aPTT) present with opposing phenotypes of either excessive bleeding or thrombotic tendency. However, there is currently no rapid and reliable way to distinguish coagulation factor inhibitors from lupus anticoagulant (LA).

Aims: To verify the usefulness of rotational thromboelastometry (ROTEM) in distinguishing acquired hemophilia A (AHA) from LA.

Methods: Retrospective analysis was performed on ROTEM data acquired from patients with either AHA (n = 6) or LA (n = 10) because of prolonged aPTT, who were treated at Tokyo Medical University Hospital from April 2018 to June 2019. ROTEM was performed according to standard procedures 30 minutes after blood collection, using four channels simultaneously in the (Non-Activated TEM) NATEM mode. Parameters were calculated based on the median and interquartile range (IQR) of the data obtained across all four channels, and the Mann-Whitney U test was used for comparisons between groups. Of the six AHA patients, four had already initiated prednisolone therapy. The two untreated AHA patients with a marked bleeding tendency were removed from the analysis because no clot formation was detected by ROTEM.

Results: The median quantity of anti-factor VIII antibodies in the four AHA patients was 15.8 (IQR 5.4–55.7) BU/mL. The median values for aPTT, clotting time (CT), and maximum clot firmness (MCF) for the AHA group vs the LA group were 62.5 (IQR 60.2–66.6) s vs 49.4 (IQR 46.4–53.5) s, 1718 (IQR 1559.5–1964.3) s vs 707.5 (IQR 503.5–744.0) s, and 59.5 (IQR 51.6–63.8) mm vs 57.0 (IQR 53.5–59.5) mm, respectively (Figure 1).

Conclusions: The CT for the AHA group was significantly longer (p = 0.0047) than that for the LA group. Thus, CT, as determined by ROTEM in the NATEM mode, may have the potential to clearly distinguish AHA from LA.

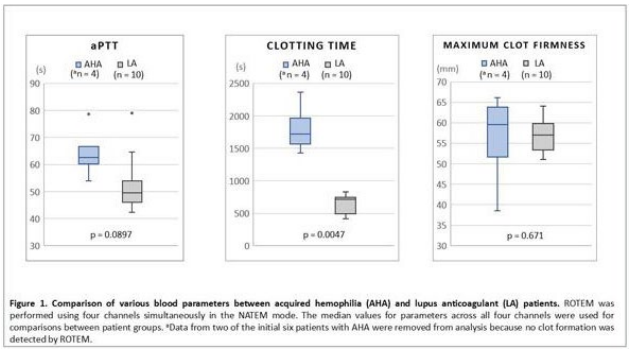

FIGURE 1 Comparison of various blood parameters between acquired hemophilia (AHA) and lupus anticoagulant (LA) patients

PB0527 | Development of a Logistic Regression Model for Prediction of Conversion Risk from Mild Cognitive Impairment (MCI) to Alzheimer's Disease (AD)

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Background: MCI is an intermediate picture between normal cognition and dementia. Although high, the rate of conversion from MCI to dementia, particularly AD, has been poorly studied. Thus, a tool for this purpose is desirable.

Aims: To develop a logistic regression model based on hemostatic and vascular parameters to investigate the risk of converting from MCI to AD based on a 3-year follow-up.

Methods: 38 patients diagnosed with MCI had their levels of hemostatic parameters (generation of thrombin [TG], protein S [PS], tissue factor inhibitor [TFPI], von Willebrand factor [FvW], plasminogen activator inhibitor 1 [PAI-1], D-dimer [D-Di], factor VIII [FVIII], ABO classification) and vascular injury markers (intercellular adhesion molecule 1 [ICAM-1], homocysteine [Hcy], platelet microparticles [PMPs], conventional and nonconventional lipid profile) determined. These data were used to build a logistic regression model ("R" software) to estimate the probability of a patient evolving from MCI to AD after three years of follow-up, when the same patients had a new cognitive assessment.

Results: Of the 38 patients with MCI, 13 (34.2%) progressed to AD and 25 (65.8%) did not over 3 years. Through the logistic regression model, the covariables age, sex, increased both generation of thrombin (Peak) and homocysteine (Hcy) were independently associated with the conversion from MCI to AD. Through these covariables, an algorithm was built to predict the risk of conversion from MCI to AD (sensitivity = 100%; specificity = 84%), as fig. 1.

Conclusions: For elderly male patients, it is recommended to monitor and control serum homocysteine levels and hemostatic status, whose alterations are potentially modifiable in order to reduce the chances of progressing from MCI to AD.

Acknowledgments: FAPEMIG, CNPq and CAPES.

PB0528 | XIIa-Dependent Fibrinolysis in Patients with Ph-Negative Myeloproliferative Neoplasms (MPN)

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Background: Ph-negative myeloproliferative neoplasms (MPN) such as polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are characterized by thrombotic complications. Currently there is not general diagnostic laboratory method for the development of thrombosis. Disorders in the system of fibrinolysis are one of the factors that contribute to the progress of thrombosis. The time of XIIa-dependent fibrinolysis can extend with these patients and represent a change of the balance between blood coagulation and fibrinolysis.

Aims: Aim of this study to estimate time of XIIa-dependent fibrinolysis in patients with Ph-negative myeloproliferative neoplasms.

Methods: The study involved 73 patients with MPN (28 with PV, 17 with ET and 28 with PMF) and 38 healthy controls. XIIa-dependent fibrinolysis (minutes) was tested by "renam" (Russia). STATISTICA 12.0 was used. The results are given as median (Me) with 95% confidence intervals (CI). Mann-Whitney test was used to compare the studied groups. P< 0.05 was considered statistically significant.

Results: The data are shown in the table (*-p< 0.05 compare to controls,**-p< 0.05 differences between PV and PMF, ***-p< 0.05 differences between ET and PMF).

XIIa-dependent fibrinolysis is significantly higher in the whole group of patients with MPN and PMF. The most expressed changes in fibrinolysis (prolongation of time XIIa-dependent fibrinolysis) are identified in patients with PMF, while this parameter in patients with PV and ET is not differ from the control but it has a tendency to both decrease and increase. This fact can be considered as a sign of disbalance of coagulation in patients with Ph-negative myeloproliferative neoplasms and require further study.

Conclusions: In patients with PMF are detected significant prolongation of time of XIIa-dependent fibrinolysis. These changes indicate inhibition of fibrinolysis. Fibrinolytic system disfunction can lead to imbalance in hemostatic system with prothrombotic states development in MPN.

PB0529 | Multicentre Performance Evaluation of the Protein C Chromogenic Assay on the Cobas t 711 Analyser

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Background: The Protein C chromogenic assay (Roche Diagnostics) is intended for in vitro quantitative determination of protein C activity in citrated plasma to aid diagnosis of inherited and acquired protein C deficiencies.

Aims: Evaluate the analytical performance of the Protein C chromogenic assay on the cobas t 711 analyser.

Methods: The study was performed at four sites (Austria, Germany, Netherlands, United Kingdom; July 2017-April 2018). Analytical performance was evaluated using anonymised residual samples from routine testing. Repeatability/reproducibility were tested using two control and five human plasma pools (HPPs) according to CLSI EP05-A3 guidelines. For repeatability, one run (n=21 determinations) was performed from the same aliquot; for reproducibility, five aliquots of each control/HPP were tested over 5 days across three sites. Method comparison testing was performed using four sample sets (n≥120), versus the Berichrom Protein C assay on the Sysmex CS-5100 analyser (Siemens); Deming regression and Pearson's correlation were calculated. Lot-to-lot variability was assessed using the same sample sets (n≥120), and three reagent lots. Reference ranges were determined in 200 fresh samples (human citrated plasma) from apparently healthy blood donors.

Results: Across all sites, coefficients of variation (controls/HPPs) were: repeatability, 0.4–2.9% (mean Protein C, 7.43–124 IU/dL); intermediate precision, 0.5–4.8%; total reproducibility, 2.0–5.6% (mean Protein C for both, 8.22–117 IU/dL). The Protein C chromogenic assay showed good correlation with the comparator method, across all sites: slope, 0.931–1.023; r, 0.989–0.997; bias at 50 IU/dL (relative), −2.0–3.9. High correlation was shown for lot-to-lot comparisons, across all sites: slope, 1.001–1.035; r, 0.999–1.000; bias at

TABLE 1 XIIa-dependent fibrinolysis in patients with MPN (Me; 95% CI)

Parameter	MPN (n=73)	PV (n=28)	ET (n=17)	PMF (n=28)	Controls (n=40)
XIIa-dependent fibrinolysis, min	6.25* 3.75–20.00	6.17** 3.25–10.22	6.08*** 3.95–12.25	10.67* 5.33–29.83	5.29 4.16–7.33

50 IU/dL (relative), 0.2–2.7. Reference ranges (2.5–97.5th percentiles) were 80.5–150 IU/dL.

Conclusions: The Protein C chromogenic assay on the cobas t 711 analyser demonstrated good analytical performance and met all predefined acceptance criteria. Reference ranges in apparently healthy blood donors were established.

PB0530 | Performance Evaluation of Anysis-200 Platelet Function Analyzer in Cardiology Patients

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Background: Analysis of responsiveness to antiplatelet therapy is crucial in the management of patients with cardiovascular disease.

Aims: This study aimed to evaluate a new platelet function analysis system (Anysis-200) and to compare it with VerifyNow (Accumetrics, San Diego, CA) in cardiology patients.

Methods: A total of 125 citrated blood samples were collected from 85 cardiology patients who were referred for platelet function testing. In Anysis-200, platelet function was measured as blood migration distance (MD) until clogging of flow passage, which is comparable to aspirin resistance units obtained using VerifyNow. The two devices were simultaneously used and compared.

Results: The MDs before and after taking aspirin were 174±51 and 247±27 mm, respectively ($p < 0.0001$). Compared with VerifyNow (reference), the sensitivity and specificity of Anysis-200 was 91.5% and 75.5%, respectively (area under the curve, 0.829). Further, the true positive rate in patients newly taking aspirin was 85% for VerifyNow and 92.5% for Anysis-200, respectively. The Cohen's kappa coefficient between the two devices was 0.682, indicating a relatively high agreement.

Conclusions: Anysis-200, a novel system for assessing platelet aggregation, has accuracy and precision equivalent to that of, and significant agreement with, VerifyNow. Anysis-200 may be useful in screening patients with abnormal platelet reactivity and aspirin nonresponsiveness.

PB0531 | The Basic Evaluation of the Newly Developing Modified Diluted Prothrombin Time Reagent for Direct Oral Anticoagulants Measurements

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Background: Direct oral coagulants (DOAC) are commonly used for thrombosis prevention and treatment. Although DOAC is not routinely monitored, there are multiple clinical scenarios in which laboratory assessment of drug effect using its specific assay can be important.

Aims: We have developed the modified diluted prothrombin time reagent (mdPT) based on prothrombin time (PT) assay for DOAC measurements. The purpose of this study was to perform the basic evaluation of mdPT reagent.

Methods: The within-run precision was determined 10 times measurements in normal, abnormal, apixaban (101 ng/mL), edoxaban (112 ng/mL), and rivaroxaban (109 ng/mL) controls. The between-day precision was evaluated five times a day for five days. The comparative study was performed using 275, 257, and 243 patient samples with apixaban, edoxaban, and rivaroxaban against DOAC concentration, respectively. The correlations between DOAC concentration and four commercial PT reagents were also investigated, and compared with that of mdPT. The ratios (patient sample / normal sample) were calculated to normalize the values in both mdPT and PT reagents. The informed consent was obtained from all patients and the study was approved by the recognized medical ethics committees in all institutions.

Results: The coefficient of variation % (CV%) in within-run precision and between-day precision were within 0.6% and 1.4% in all controls, respectively. The correlation coefficients and the slopes between DOAC concentration and mdPT were 0.818, 0.914, and 0.814, and 0.0068, 0.0076, and 0.0072, and the slopes of four commercial reagents were less than 0.0023, 0.0038, and 0.0057 in apixaban, edoxaban, and rivaroxaban, respectively. mdPT showed higher slopes in all drugs.

Conclusions: mdPT showed good correlation against DOAC concentration and had a higher sensitivity than commercial PT reagents. It was considered that mdPT reagent was useful for DOAC measurements as specific assay of anticoagulants.

PB0532 | New Formula of "Ratio of Inhibited Thrombin Generation" Based on Modified Diluted Prothrombin Time Reagent Predicts Bleeding Risk of Patients with Low Coagulation Activity in Direct Oral Anticoagulants Therapy

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Background: Direct oral anticoagulants (DOAC) are widely used for treatment of thromboembolism. Although DOACs are not routinely monitored, the measurement of its activity can be needed in multiple clinical scenarios. We have developed the modified diluted prothrombin time reagent (mdPT) based on prothrombin time (PT) assay for DOAC measurement as global assay.

Aims: The purpose of this study was to investigate the characteristics of mdPT reagent against DOAC concentration.

Methods: The patient samples including 275 apixaban, 257 edoxaban, and 243 rivaroxaban plasmas were employed in the comparative study between mdPT and DOAC concentration based on anti-Xa assay. For the normalization, mdPT results were shown as Ratio of Inhibited Thrombin Generation (RITG) = (a-b) / b x 100, where a = the clotting time of patient sample, b = the clotting time of normal sample. The samples were divided into three groups based on the 99 percentile confidence interval, group 1; lower discrepancy than the 99 percentile, group 2; within the 99 percentile, group 3; upper discrepancy group than the 99 percentile. Factor II, V, VII, X activity were compared in these three groups. The informed consent was obtained from all patients and the study was approved by the recognized medical ethics committees in all institutions.

Results: The correlation coefficients were 0.818, 0.914, and 0.814 in apixaban, edoxaban, and rivaroxaban, respectively. The factor II, V, VII, and X activity in group 3 was significantly lower than that in group 1 for apixaban and edoxaban. In rivaroxaban samples, a significant decrease was recognized in only factor V activity. Overall, the upper discrepancy in group 3 was derived from lower coagulation activity.

Conclusions: mdPT reagent reflected not only DOAC inhibition but also coagulation factor activity. mdPT may predict bleeding risk in patients treated with DOAC at a low level of coagulation activity.

PB0533 | Extensive Experiments in (Un)Diagnosed Bleeding Patients with Prolonged Platelet Function Analyser Closure Times

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[1]Maastricht University Medical Center, Department of Hematology, Internal Medicine, Maastricht, the Netherlands; [2]Maastricht University, Department of Biochemistry, Cardiovascular Research Institute Maastricht [CARIM], Maastricht, the Netherlands; [3]Flowchamber, Maastricht, the Netherlands; [4]University of Cambridge, Cambridge, United Kingdom; [5]Maastricht University Medical Center, Central Diagnostic Laboratory, Unit for Hemostasis and Transfusion, Maastricht, the Netherlands; [6]University of Cambridge, Department of Haematology, Cambridge, United Kingdom

Background: Patients referred for evaluation of bleeding symptoms occasionally have unexplained prolonged platelet function analyser (PFA) results, without laboratory evidence for von Willebrand disease (VWD) or platelet function defect (PFD). This abnormal PFA might indicate shear stress-dependent abnormalities in VWF or platelet function.

Aims: To perform extensive experiments, including flowchamber and whole genome sequencing (WGS) in bleeding patients with unexplained prolonged PFA results.

Methods: Patients were included if they had unexplained prolonged PFA results. ISTH BAT score was assessed. Performed laboratory tests included PFA, light transmission aggregometry (LTA) with ATP release, VWF:RCo, VWF:ag, VWF multimers, collagen binding assay, flowcytometry, flowchamber analysis and WGS. For flowchamber experiments, collagen I and III surfaces were used at shear rates of 1000s^{-1} and 1600s-1 at 37°C. Formed thrombi were assessed for platelet surface area coverage; platelet aggregation; thrombus morphological score (MS), thrombus multilayer score (MT), thrombus contraction score (CS) at 3, 5 and 7 minutes and for P-selectin secretion, integrin $\alpha_{IIb}\beta_3$ activation and phosphatidylserine (PS) expression.

Results: 13 PFA-only patients were included. Mean ISTH-BAT score was 11 ± 5; mean PFA-ADP was 119 ± 28 s; mean PFA-EPI was 149 ± 43 s; no patient had abnormal VWF levels. LTA indicated a thrombopathy, not otherwise specified (NOS) in 1 patient. In 6/13 patients repeated unexplained prolonged PFA results were found. Flowchamber results of these patients showed decreased thrombus formation scores, PS expression and integrin activation. Furthermore, 4 patients with VWD and/or glanzmann's disease showed abnormal integrin activation and PS expression in the flowchamber. The patient with thrombopathy NOS, showed severely altered thrombus formation.

Conclusions: Unexplained prolonged PFA closure times, in patients with a bleeding tendency, can be confirmed by flowchamber experiments and cannot be ignored in the final diagnosis of these patients. WGS results are awaiting and might give more insight in the underlying genetic abnormality in these patients.

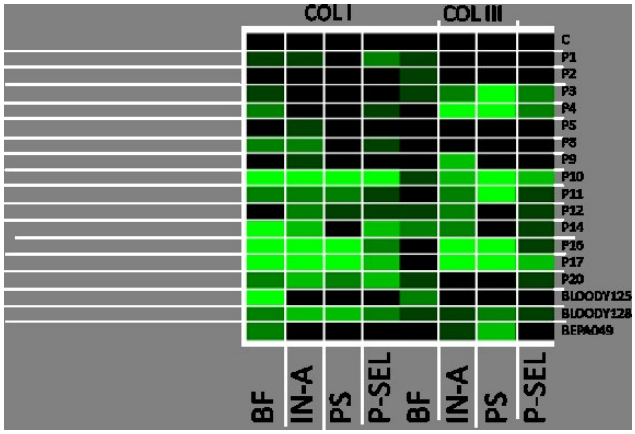

FIGURE 1 Heatmap Flowchamber Thrombus Formation

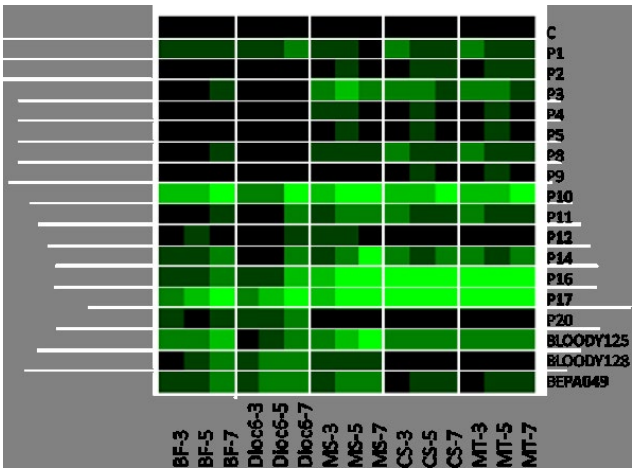

FIGURE 2 Heatmap Flowchamber labels at 1600s-1

PB0534 | Assessing the Quality of Interpretative Comments for Prolonged Activated Partial Thromboplastin Time and Mixing Tests

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Background: Activated partial thromboplastin time (APTT) is one of the basic tests for assessing hemostasis-related disorders or monitoring anticoagulation therapies. Prolonged APTT results may be caused by different situations (e.g. contamination with heparin, factor deficiency or presence of an inhibitor), which may trigger different medical actions. In these situations, mixing tests are simple but useful tools for further investigation of prolonged APTT results. The correct interpretation of coagulation tests may sometimes be challenging, and expert comments by laboratory professionals could help the physician in their clinical decisions.

Aims: Our aim was to assess the quality of interpretative commenting (IC) on two fictional patient samples in European laboratories.

Methods: Two lyophilized citrated plasma samples were distributed to 340 European laboratories alongside with their respective fictional case histories. Laboratories were asked to measure APTT, perform the pertinent mixing tests and interpret the results by means of IC. The quality of IC was evaluated using standard criteria for analytical data, interpretation and advice. Each comment was scored by expert pairs. Discrepancies were solved by consensus.

Results: A total of 270 laboratories completed the survey.

For case 1 (sample contaminated with heparin), 44.6% of laboratories presented the adequate analytical data, 64.4% correctly interpreted the results and 24.7% gave a useful advice to the physician. Wrong advices were given by 28.8% of laboratories.

For case 2 (coagulation factor VIII deficiency), 47.7% of laboratories presented the adequate analytical data, 64.1% correctly interpreted the results and 47.3% gave a useful advice to the physician. Wrong advices were given by 3.9% of laboratories.

Conclusions: A significant percentage of laboratories gave appropriate comments, including the interpretation of results and the advice to the requesting physician. Wrong advices given, especially in case 1, indicates educational need and poses risk for the patients.

PB0535 | APTT Clot Waveform Analysis: A Tool in Prediction of Pre-Analytical Errors in Short APTT- Retrospective Analysis

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Background: Apart from utility of APTT Clot Waveform Analysis (CWA) in Hemophilia and Sepsis, our experience in assessing its utility for existence of pre-analytical error has been evaluated. This is based on absence of proper CWA pattern or specific derivatives of CWA in too short APTT samples.

Aims: To Evaluate APTT CWA in short APTT samples for presence of various derivatives and compare with those in repeat samples received.

Methods: Citrate samples received in 3.2% Trisodium Citrate vaccutainers with 9:1 proportion were subjected to stringent rejection criteria established by laboratory. APTT of accepted samples was processed on ACL TOP 300/500 analyser using SynthASil. CWA was analysed in short APTT samples (less than 25 seconds). Samples with improper CWA were rechecked for absence of fibrin threads. Repeat samples were obtained and reprocessed. Changes in CWA were compared with original CWA.

Results:

Period of Evaluation: Jan 2018 to Dec 2019:

Citrates received: 52532:

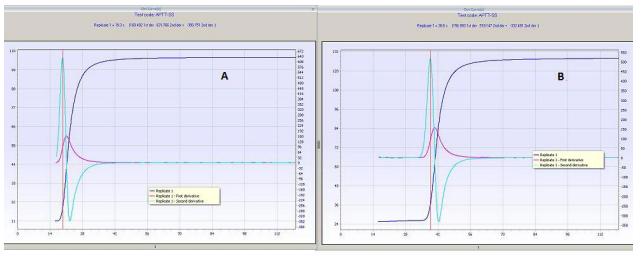

FIGURE 1 A: Short APTT with Missing Pre-Coagulation Phase in APTT CWA, B: Repeat Sample with proper APTT CWA

Citrates Rejected: 671 (1.27%):
Repeat Samples requested with short APTT: 178:
Samples with change: 146:
Samples without change: 32:
Samples with fibrin threads: 09:
Samples with Missing Pre Coagulation phase: 146:
2nd Derivative only: 76:
1st and 2nd Derivative: 64:
1st, 2nd and Clot curve: 06:
Median of APTT value (seconds):
1st Sample: 20.3:
Repeat sample: 26.7:

Conclusions: APTT CWA plays substantial role in identifying Preanalytical error in citrate samples which have been visually passed through rejection criteria. 2nd Derivative in CWA, which represents coagulation acceleration or Prothrombinase activity, is first to disappear in our samples. Difficult collection is common in ICU settings. Release of Tissue factor at the site of collection activates extrinsic tenase formation thereby initiation of clotting in vivo at site of puncture. This shortens APTT value and missing derivatives in CWA are observed. This may be as part of activated sample.

PB0536 | Paediatric APTT, PT and Fibrinogen Reference Intervals Using STA® - CK Prest, STA® - NeoPTimal and STA® - Liquid Fib

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Background: Previous studies in the setting of developmental haemostasis have established age-specific differences in the quantity, function of haemostatic proteins and functional assays. Routine and specialized haemostasis assays in the paediatric population should thus be undertaken with these differences in mind. Reagent, analyser and age-specific reference ranges should be established for all clinically utilised laboratory tests.

TABLE 1 Reference intervals for neonates, infants, children and adolescents

	NEWBORNS Birth to 1 month	INFANTS>1 Month–2 Years	CHILDREN>2 Years–12 Years	ADOLESCENTS>12 Years–21 Years
APTT (sec) meanreference interval	n=40 *37.629.9 –52.7	n= 4134.428.2 –96.5	n= 4432.026.9 –36.5	n= 4131.226 .2–38.7
PT (sec) meanreference interval	n= 44 *17.612.8 –24.9	n= 4514.112.6 –16.6	n= 4314.713.3 –16.8	n=4314.612 .8–15.8
Fibrinogen (g/L) meanreference interval	n= 44 *3.101.92 –4.81	n= 452.501.75 –3.34	n= 432.742.03 –3.77	n=432.721 .81–3.50

* P-value significantly different from adolescents (p=<0.05)

Aims: This study aimed to establish age-related reference intervals for activated partial thromboplastin time (APTT), prothrombin time (PT) and fibrinogen, using the STA R Max® analyser and Stago reagents, STA® - C.K. Prest®, STA® - NeoPTimal and STA® - Liquid Fib (Diagnostica Stago, France).

Methods: Citrated plasma samples were obtained via clean venepuncture from 44 healthy neonates and 132 healthy children undergoing elective surgery (e.g. circumcision). Results were divided into the following age groups: newborns (from birth to 1 month of age), infants (greater than 1 month to 2 years of age), children (greater than 2 to 12 years of age) and adolescents (greater than 12 to 21 years of age). Results were analysed using GraphPad Prism version 7.04, and are expressed as mean with intervals including 95% of the population (2.5th percentile - 97.5th percentile) in accordance with CLSI guidelines.

Results: Mean values and reference intervals according to age are presented in Table 1. The mean values for aPTT, PT and fibrinogen differ significantly between newborns and adolescents.

Conclusions: This study confirms the need to develop analyser and reagent specific reference intervals across the age spectrum, enabling accurate interpretation of coagulation tests and accurate diagnosis for newborns and children.

Other Items for Consideration: Written informed consent was obtained from parents of children. This study was approved by the Royal Children's Hospital Ethics in Human Research Committee (Reference number 34183). This study was funded by Diagnostica Stago, France.

PB0537 | Impact of Combination of Factor VIII-Deficient Plasma and Activator in One-Stage Clotting Assay on Potency Testing of Factor VIII Products

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Background: Factor VIII (FVIII) levels are measured by one-stage clotting assay (OSA) and FVIII OSA is based on APTT method using activator and factor VIII-deficient plasma (F8DP). A variation of the reagents and their combination has been an issue but was not fully evaluated for potency testing of FVIII products.

Aims: We aimed to evaluate the variation of OSA results in FVIII product-spiked plasma using various kinds of combination of activator and F8DPs.

Methods: FVIII OSAs were performed using following reagent combinations: two immunodepleted F8DP (F8DP-1/-2) and one of congenital F8DP (F8DP-3) was used in combination with APTT reagents; Actin (ACT), Actin FSL (FSL), Thrombocheck APTT-SLA (SLA) and Pathromtin SL (PSL). The combination of ACT/F8DP-3 and PSL/F8DP-3 showed poor internal control results and exclude in this study. Total of eleven combinations were evaluated. Octocog alpha, octocog beta (Bayer), rurioctocog alpha (Takeda), turoctocog alpha (Novo Nordisk), efraloctocog alpha (Sanofi)and rurioctocog alpha pegol (Takeda) were spiked into congenital FVIII-deficient plasma (George King Bio-Medical) to reach 0.80, 0.20 and 0.05 IU/mL based on the labelled vial potency.

Results: The mean FVIII activity (FVIII:C) and coefficient of variations (CVs) of spike recoveries were shown in Table 1. In all reagent combinations, higher CVs were shown in lower concentration of 0.05 IU/mL. The FVIII:C and CVs were similar in octocog alpha/beta, turoctocog alpha, and rurioctocog alpha pegol. The minimal CV was observed in turoctocog alpha-spiked plasma. Meanwhile, for comparison of APTT reagents, ACT and FSL showed higher FVIII:C than SLA and PSL. FSL/F8DP-1 showed the highest CV of 27.8% among FVIII products whereas the other reagent combinations resulted in CVs < 20%.

Conclusions: The CVs of spike recovery were within 25%. The FVIII:C variation due to OSA reagents was significant in lower FVIII concentration. The potency of FVIII depended on a combination of APTT reagent and F8DP.

TABLE 1 The mean FVIII activity and CVs of each product measured by eleven kinds of OSA. The FVIII:C (IU/mL) results were shown as mean±SD (CV%)

FVIII products	Nominal concentration (IU/mL)		
	0.80	0.20	0.05
Ocotocog alpha	1.17±0.21 (18.2)	0.31±0.05 (17.4)	0.09±0.02 (21.9)
Ocotocog beta	1.17±0.17 (14.4)	0.31±0.04 (14.2)	0.09±0.02 (19.2)
Rurioctocog alpha	0.96±0.14 (14.8)	0.25±0.03 (13.5)	0.07±0.01 (18.6)
Turoctocog alpha	1.10±0.13 (12.1)	0.29±0.03 (9.7)	0.08±0.01 (15.2)
Efraloctocog alpha	1.59±0.28 (17.9)	0.42±0.05 (12.8)	0.12±0.02 (16.7)
Rurioctocog alpha pegol	1.19±0.17 (14.4)	0.31±0.05 (17.2)	0.09±0.02 (22.2)

PB0538 | Establishing an External Quality Assessment Scheme (EQAS) for D-Dimer Assay in India: Results from a Pilot

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Background: The clinical laboratory is expected to provide results that are precise and accurate. External quality assessment schemes (EQAS) provide an opportunity to monitor the accuracy of the results. Starting in 2000, the Christian Medical College, Vellore provides EQAS for haemostasis testing in India and the region, with 993 laboratories enrolled basic and advanced modules comprising screening tests, selected factor assays and inhibitor studies.

D-Dimer assay is commonly used in the management of thromboembolic disease or disseminated intravascular coagulation. Several kit based tests are available in qualitative, semi-quantitative and quantitative formats based on a variety of principles.

Aims: To establish an EQAS program for D-Dimer test for clinical laboratories in India.

Methods: Through a prior survey, 25 participants who expressed willingness were included in the pilot program. Lyophilized materials were prepared from blood collected from consenting human donors and patients. Each participant was sent a pair of samples, with instructions for reconstitution and a result entry sheet to perform D-Dimer tests as per usual practice. Results were tabulated and analysed; robust statistics was used to remove outlier.

Results: Reports were received from 80% of laboratories to whom samples were sent. Majority (80%) of the participants used an

TABLE 1 Summary of Results

Qualitative Results	Parameter	Sample 1	Sample 2
	Expected result	Positive	Negative
	Number of results [n]	8	8
	Concordance [n(%)]	7 (87.5%)	8 (100%)
Quantitative Results	Number of results [n]	20	20
	Median D-Dimer (ng/ml) [CV %]	1675 [26.1%]	79.3 [46.1%]
Assessment	Results Within Consensus (%)	65	55
Interpretation	Acceptable	18 (90%)	18 (90%)
	Not acceptable	1 (5%)	1 (5%)
	Not assessable	1 (5%)	1 (5%)

automated platform, while the remainder used semi-automated methods. Qualitative method was used by 40%, in addition to quantitative method by all participants (100%).

Conclusions: There is variation in the quantitative assessment of results with a high CV, especially at the lower level of detection. Challenges to standardize D-dimer test includes use of different analytical techniques, reporting units, technical issues related to different monoclonal antibodies against the D-D Domain as well as reporting terminology. However, it is possible to offer proficiency testing for D-Dimer testing through locally produced samples in resource restricted circumstances. This is also an opportunity to standardize the reporting and interpretation of the D-Dimer test.

PB0539 | Multicentre Performance Evaluation and Reference Range Determination for a New Assay for the Quantitative Evaluation of Factor VIII in Citrated Plasma

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Background: Analysis of FVIII activity is important for diagnosis of FVIII deficiency, which is associated with bleeding. Conversely, raised levels of FVIII are a thrombotic risk. We conducted a multicentre evaluation of a new FVIII assay (Roche Diagnostics), intended for quantitative assessment of FVIII activity.

Aims: To evaluate the analytical performance of the FVIII assay on the cobas t 711 analyser.

Methods: Experiments were performed at three laboratories using 3.2% citrated, anonymised, ethically approved, residual or commercially purchased plasma samples. Five human plasma pools (HPP) and two controls were used for determination of assay repeatability (1 run per site; n=21 replicates per sample), intermediate precision and total reproducibility (5 aliquots/sample per day over 5 days); coefficients of variation (CV) and/or standard deviation (SD) were calculated. Lot-to-lot variability (3 reagent lots) and method comparison (versus Siemens Coagulation Factor VIII Deficient Plasma [in combination with Actin FS] reagent on Sysmex CS-5100 analyser) were evaluated by Passing-Bablok and Deming regression, respectively, and Pearson's r calculated. Assay-specific reference range was determined at one site using fresh plasma samples from apparently healthy individuals aged ≥18 years, not receiving anticoagulant medication.

Results: Across sites, CVs for repeatability ranged 0.9–3.8% for samples with >1.0 IU/dL FVIII activity; SDs ranged 0.0160–0.046 for low activity samples (≤1.0 IU/dL). Among samples with mean activity 0.344–133 IU/dL, high intermediate precision (SD, 0.020 for sample with 0.344 IU/dL activity; CV, 1.8–4.7%) and good total reproducibility (CV, 2.0–13.3%) were observed. The FVIII assay showed excellent lot-to-lot variability (Pearson's r, 0.999; Table 1) and good agreement with the comparator assay (Pearson's r, 0.993–0.996; Table 2). Among apparently healthy individuals (n=199), 2.5th and 97.5th percentiles for FVIII activity were 82.2 IU/dL and 218 IU/dL, respectively.

Conclusions: The FVIII assay demonstrated robust analytical performance on the cobas t 711 analyser, supporting use in routine laboratory practice.

TABLE 1 Lot-to-lot comparison for the Roche FVIII assay

Parameter	Freiburg	Sheffield	Vienna
Lot	A vs B	C vs A	B vs C
Sample, n	127	126	128
Slope	0.994	0.987	0.978
Intercept	0.254	−0.066	−0.210
Pearson's r	0.999	0.999	0.999
Absolute bias, IU/dL (activity range 0.2–2 IU/dL)	0.062	−0.037	−0.052
Absolute bias, IU/dL (activity range 1.5–10 IU/dL)	0.200	−0.040	0.340
Relative bias, % (activity range 50–70 IU/dL)	2.13	−1.47	−2.98

TABLE 2 Method comparison of the Roche FVIII assay versus the Siemens Factor VIII assay

Parameter	Freiburg	Vienna	Sheffield
Lot	A	B	C
Sample, n	126	127	120
Slope	0.919	0.944	0.989
Intercept	0.292	−0.438	2.15
Pearson's r	0.996	0.994	0.993
Absolute bias, IU/dL (activity range 1.5–10 IU/dL)	0.185	−0.390	0.470
Relative bias, % (activity range 50–70 IU/dL)	−7.89	−7.78	9.60

PB0540 | Correlation between the VerifyNow P2Y12 Assay and the Newly-Developed APAL System in Neuroendovascular Patients

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Background: Light transmission aggregometry (LTA) is the current gold standard method for assessing platelet function. Recently, a new parameter called the ADP-induced platelet aggregation level (APAL) was developed to aid interpretation of LTA results. APAL is a score from 0.0 to 10.0 calculated based on platelet aggregation patterns upon exposure to 1 μM and 10 μM adenosine diphosphate (ADP) and is determined using an automated coagulation analyzer.

Aims: We compared obtained APAL and P2Y12 reaction unit (PRU) values and % inhibition on VerifyNow P2Y12 assay for the patients with cerebrovascular disease.

Methods: 42 patients who have received antiplatelet therapy were studied. Platelet function tests were performed on CS-2400 for APAL and VerifyNow P2Y12 assay was used for PRU and % inhibition.

Results: Moderate correlations were observed between APAL and PRU ($r = 0.64$, $p < 0.001$), APAL and % inhibition ($r = -0.74$, $p < 0.001$), and PRU and % inhibition ($r = -0.83$, $p < 0.001$). The optimal threshold for APAL was 8.2 for PRU (threshold = 240) and 8.1 for % inhibition (threshold = 26%). The percentage of agreement between the above thresholds was 90.9%, 77.3%, and 84.1% between PRU and APAL, % inhibition and APAL, and PRU and % inhibition, respectively.

Conclusions: The newly-developed APAL system exhibits moderate correlation with PRU and % inhibition. All three results showed similar agreement rates. Moreover, since APAL is the result of LTA and can be measured at a lower cost than that required for VerifyNow, it is expected that the regular monitoring of antiplatelet therapy will be possible even in facilities where it has been difficult owing to cost.

PB0541 | FIBTEM Parameters Correlate Well with the Fibrinogen Concentration Measured by the Clauss Assay in Patients and Healthy Subjects

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Background: The Clauss assay is the assay most often used for measuring fibrinogen. However, also the FIBTEM-assay (determined using thromboelastometry (ROTEM)) can be used to estimate fibrinogen levels. A major advantage of the FIBTEM is that it can provide information about the fibrinogen levels within a few minutes, while the Clauss assay needs 30–60 minutes before results are available.

Aims: To investigate the correlation between results from the FIBTEM-assay and fibrinogen concentrations, as measured by the Clauss assay.

Methods: 116 patients for whom both ROTEM analyses and a fibrinogen measurement using the Clauss assay were available, acquired in May or June 2019 in the Erasmus Medical Center in Rotterdam, were included. This were mostly patients with major trauma or large surgery (cardiac surgery or liver transplantation). In addition, ROTEM and Clauss measurements from 75 healthy subjects were included. Spearman correlation was used to determine the association between both assays.

Results: The correlations between FIBTEM parameters and fibrinogen levels measured by the Clauss assay were strong in patients (Spearman's correlation coefficients (r_s) above 0.82 ($p < 0.001$) for

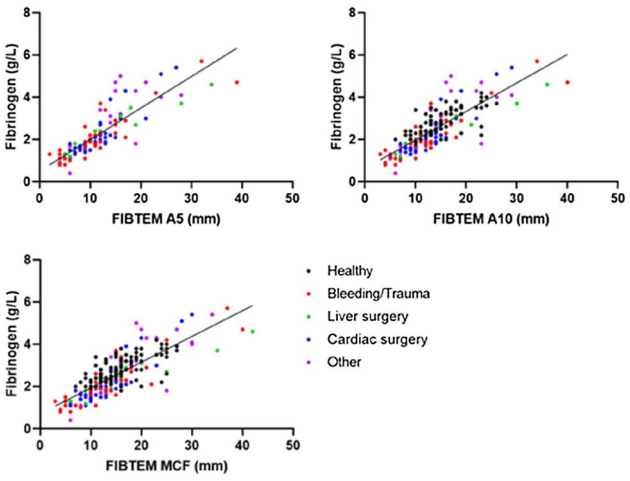

FIGURE 1 Correlation between fibrinogen concentrations measured by the Clauss assay and FIBTEM parameters

all subgroups) and moderate for healthy subjects (r_s=0.68, p< 0.001) (Figure 1). The correlation between early FIBTEM parameters (clot firmness at 5 or 10 minutes) and the maximum clot firmness (MCF) was almost perfect (r_s above 0.96).

Conclusions: Overall, strong correlations were found between the Clauss assay and several FIBTEM parameters, which suggests that early FIBTEM parameters can be used to determine fibrinogen levels in various patient groups. This indicates that FIBTEM parameters are usable for fast evaluation of fibrinogen levels in critical medical situations.

PB0542 | Performance of Thrombophilia Markers Assays Evaluated from Results of an External Quality Assessment Program

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Background: Deficiencies of the coagulation inhibitors, antithrombin (AT), protein C (PC) and protein S(PS) are associated with an increased risk for venous thromboembolism. Measurement of these markers requires reliable tests for comparability and transferability of results within and between laboratories.

Aims: This work evaluates AT, PC and PS main assay methods state-of-the-art from External Quality Assessment (EQA) results of the program provided by ProBioQual, a french proficiency testing association.

Methods: Participant laboratories (330 for AT,102 for PC,79 for PS in 2019) received 12 lyophilized human citrated plasmas for AT, 8 for PC and PS, at 3 or 4 different levels for each variable. Statistical evaluation was performed according to the ISO 13528:2015 guideline by applying Robust Algorithm A. The consensus value was calculated for all participants (AP) results and each peer group (PG). The imprecision of methods was evaluated by the inter-laboratory coefficient of variation (CV) for AP and each PG. Accuracy was quantified as median bias (bias50) and 90th percentile bias (bias90) of laboratory results from AP or PG consensus value.

Results: For AT activity, AP CVs increase from 5.6% at normal level to 10.4% at abnormal level; PG and AP bias90 are very close, around 9% at normal level and 16% at low level.

For PC activity, the PG CVs with chronometric assays (9 to 15%) are always higher than PG CVs with chomogenic assays (3 to 6%).

For PS clotting tests, PG and AP bias90 reach respectively 18.5% and 20.2% for a level of 30%. Free PS antigen performance is satisfactory: AP bias90 between 9.3% and 11.5%, and AP CVs between 6 and 7.5%.

Conclusions: Performance of AT, PC and PS assays depend on the methodology used (clotting, chromogenic, antigenic). Results obtained in EQA surveys can help to define required analytical quality specifications for routine practice.

PB0543 | Comparison of New Scoring System of Platelet Aggregation on Automated Coagulation Analyzer CN-6000 with CS-5100

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Background: The Sysmex CN-6000 analyzer utilizes light transmission aggregometry (LTA), the current gold standard method for assessing platelet function. The new parameters; adenosine diphosphate (ADP)-induced PAL (APAL) and collagen-induced PAL (CPAL) have been developed to aid with interpretation of LTA results. The APAL and CPAL parameters are scored from 0 to 10 and are able to be calculated based on platelet aggregation patterns upon exposure to 1 and 10 μM ADP and 1 and 5μg/mL Collagen. The CN-6000 has been developed to perform automated reagent/agonist dilution for platelet aggregation testing.

Aims: The aim of the study was to evaluate performance of platelet aggregation function of the new fully automated Sysmex coagulation analyzer CN-6000.

Methods: A comparative study between the CN-6000 and CS-5100 (SYSMEX) was performed using 85 platelet rich plasma (PRP) samples for ADP testing and 82 PRP samples for Collagen testing at different levels of aggregation by adding several concentration of the anti-platelet drug, Cangrelor (P2Y12 inhibitor) or acetylsalicylic acid (Aspirin) to healthy volunteer PRP samples.

Results: Excellent correlations were observed between CN-6000 and CS-5100. For maximal aggregation, y = 1.00x +3.80, r = 0.987 and y = 0.89x + 11.48, r = 0.957 for 1 and 10 μM ADP and y = 1.02x−0.89, r = 0.996 and y = 0.99x + 0.65, r = 0.973 for 1 and 5μg/mL Collagen, respectively. For new scoring system, y = 0.91x + 0.94, r = 0.971 and y = 1.00x−0.08, r = 0.994 for APAL and CPAL, respectively.

Conclusions: Our results demonstrated that the CN-6000 is a robust automated coagulation analyzer for platelet aggregation testing including the new APAL and CPAL scoring system. The CN-6000 also provides high analytical performance as a high throughput analyzer for routine and specific coagulation parameters and is suitable for total laboratory automation.

PB0544 | Basic Evaluation of Novel Automated Coagulation Analyzer CN-6000

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Background: A fully-automated coagulation analyzer is a key component of a high-throughput laboratory. The CN-6000 (SYSMEX, Kobe, Japan) is a new haemostasis analyser with blood coagulation, amidolytic, immunoturbidometric, and light transmission aggregometry capabilities. The CN-6000 has a smaller footprint yet a higher throughput than the CS-5100 and maintains the ability for cap piercing, detection at multiple wavelengths and HIL check function; the same as CS-5100.
Aims: In this study, we have evaluated basic performance of CN-6000 and throughput compared with CS-5100.
Methods: An Interfering substances study based on CLSI guideline EP07A2 was conducted to evaluate the effects of lipaemia, hemoglobin and bilirubin. A precision study based on CLSI guideline EP05A3 was conducted to evaluate; repeatability and within-device performance. In both studies, results from the coagulation, chromogenic and immunoturbidimetric assay methods were analyzed with Analyze-it software as recommended by CLSI guidelines. Throughput of the CN-6000 and CS-5100 were compared for the following test parameters; PT, APTT, Fbg, AT, FDP and D-Dimer.
Results: The interference study showed better performance with the CN-6000 compared to the CS-5100. This was achieved by the new function "gain change function of light source". In the precision study, the Coefficient of Variations (CV) in 'repeatability' and 'within-device' were found to be less than 5% and 6.5%, respectively. The CN-6000 achieved greater throughput in comparison to the CS-5100 for all assay parameter sets. For the multi-assay parameter set; PT, APTT, Fbg, AT, FDP and D-Dimer, throughput of the CN-6000 and CS-5100 was 235 and 141 tests per hour, respectively.
Conclusions: Our results showed that the CN-6000 can provide high analytical performance with reduced impact from interfering substances from a high throughput analyzer whilst maintaining accuracy and precision. The CN-6000 showed great suitability for total laboratory automation.

PB0545 | Sample Stability for a Broad Panel of Coagulation Assays on the Cobas t 711 Analyser Using Plasma Stored under Different Conditions

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Background: Preanalytical procedures impact the accuracy of coagulation assay results and thus patient diagnosis and monitoring of anticoagulant therapy. Sample stability under different storage conditions is a key parameter and specific temperature and time criteria need to be defined for individual assays.
Aims: To evaluate the influence of plasma storage conditions on test results using routine coagulation assays on the fully automated cobas t 711 analyser (Roche Diagnostics).
Methods: Experiments were performed at a routine laboratory in Germany using anonymised residual platelet-poor-plasma samples. Eligible samples had to be processed within 2 hours of collection and were selected based on initial measurements using the laboratory's routine assay. Fresh samples were tested in triplicate at time zero (t_0) and measurements repeated after storage at predefined conditions. Test storage temperatures/durations, concentration

TABLE 1 Storage conditions tested, acceptance criteria and results (all time points and concentrations

Assay	Storage temperature	Time points	Concentration range	Acceptance criteria	Mean deviation from t_0, %
DD2	18-25°C	0, 6, 8, 12-18 hrs	0.2-9 µg FEU/mL	0.2-0.5 µg FEU/mL: ±0.05 µg FEU/mL; >0.5 µg FEU/mL: ±10%*	−2.7 to 0.9
	2-8°C	0, 6, 12, 24 hrs, 2, 4, 5 days			−6.3 to 0.9
	−20°C; freeze/thaw	0, 1, 2, 4, 5 wks; 2x 48 hrs			−6.5 to 1.3; −4.8 to −2.2
PT Rec	18-25°C	0, 10, 12, 13, 16, 17 hrs	0.8-5 INR	±10%	−4.5 to 10.6
TT	18-25°C	0, 1, 2, 4, 5 hrs	14-120 secs	±10%; ±20% for heparin samples	−18.7 to 1.5
	−20°C; freeze/thaw	0, 3, 7, 14, 21, 28 days, 6, 8 wks; 3x 48 hrs			−32.5 to 1.6; −48.8 to 3.3
aPTT	18-25°C	0, 1, 2, 4, 5 hrs	25-160 secs	<100 secs: ±10%; >100 secs: ±20%	−0.5 to 16.6
FIB	18-25°C	0, 1, 2, 4, 5 hrs	60-900 mg/dL	±10%	−1.7 to 3.0

*Results for high concentration (>0.5 µg FEU/mL) samples presented. FEU, fibrinogen equivalent unit

groups (covering the relevant measuring range) and acceptance criteria (% deviation of mean/median measurements from t_0) for each assay are summarised in Table 1. Tina-quant® D-Dimer Gen 2 (DD2), Prothrombin Time (PT Rec), Thrombin Time (TT), activated partial thromboplastin time (aPTT) and fibrinogen (FIB) assays (Roche Diagnostics) were evaluated.

Results: Ranges of mean deviation from t_0 are summarised by assay and storage condition in Table 1. Sample stability criteria were defined as follows: DD2 assay, 8 hours at 18-25°C, 4 days at 2-8°C and 28 days at −20°C, one freeze-thaw cycle; PT Rec assay, 12 hours at 18-25°C; TT assay, 4 hours at 18-25°C (2 hours in samples from heparin-treated patients), no storage at −20°C or freeze/thaw cycles; aPTT assay, 2 hours at 18-25°C; FIB assay, 4 hours at 18-25°C.

Conclusions: Sample stability criteria are demonstrated for the DD2, PT Rec, TT, aPTT, and FIB assays on the cobas t 711 analyser, for application in routine laboratory practice.

PB0546 | Comparison of a Novel Viscoelastic Device with Standard Laboratory Tests for its Clinical Validation in Peri-Operative Cardiac Patients

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Background: The Rotem Sigma is a fully automated cartridge- based viscoelastic device which differs technologically from its predecessor, the Rotem Delta.

Aims: This study aimed to clinically validate the Rotem Sigma for use in patients undergoing cardiac surgery at the Royal Papworth Hospital, to verify published reference ranges and to develop a population specific transfusion algorithm.

Methods: In this prospective observational study, blood samples were obtained pre, intra and post cardiopulmonary bypass (CPB). Testing included the Rotem Sigma Complete + Hep cartridge and Standard Laboratory Tests (SLTs) inclusive of the prothrombin time,

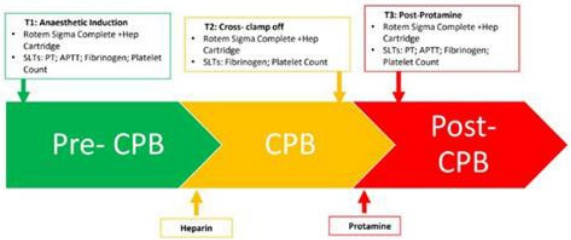

Figure 1: Flow diagram of study procedures. *Blood samples were obtained at three time-points: T1: anaesthetic induction (baseline); T2: cross- clamp removal (full heparinisation) and T3: post- protamine thus providing samples before, during and post by- pass. Testing at T1 and T3 included the Rotem Sigma Complete + Hep cartridge and SLTs including PT, APTT, Clauss fibrinogen and platelet count. Testing at T2 included the Rotem Sigma Complete + Hep cartridge and standard laboratory tests including Clauss fibrinogen and platelet count. Assessment of the PT and APTT was not possible at T2; the effects of heparin prevent fibrin clot formation leading uninterpretable results. T1: Pre-bypass; T2: On bypass; T3: Post-bypass; CPB: cardiopulmonary bypass; PT: Prothrombin time; APTT: Activated partial thromboplastin time.*

FIGURE 1 Flow diagram of study procedures

activated partial thromboplastin time, Clauss fibrinogen and platelet count (Figure 1). Median & 2.5th-97.5th percentiles were used to derive reference ranges. Spearman's rho assessed correlation between Rotem-derived parameters and SLT results. Receiver operating characteristic curve (ROC) analysis assessed the sensitivity and specificity of Rotem derived parameters.

Results: Samples were obtained from 42 CPB patients. The Fibtem A5 demonstrated strong correlation with the Clauss Fibrinogen pre, intra and post CPB (r_s.874; r_s.908; r_s.952). Good correlation pre and post CPB was identified between the Heptem CT and APTT (r_s.636; r_s.708) and the Extem CT and PT (r_s .607; r_s.794). The Extem A5 showed good correlation with platelet count pre and intra CPB (r_s.698; r_s .632) and moderate correlation post- CPB (r_s.508). By choosing optimum classification by ROC which were further adjusted based on reference ranges we determined that an Extem A5 < 50mm with platelet count < 100 identified requirement for platelets; an Extem A5 < 50mm with a Fibtem A5 < 9mm identified need for fibrinogen replacement; an Extem CT>90s with Fibtem A5 >9mm or Heptem CT >280s indicated requirement for factor replacement (Figure 2).

Conclusions: Our validation allows a rapid transfusion guidance strategy based on the earliest available Rotem Sigma parameters with best possible compromise of sensitivity and specificity, including during CPB.

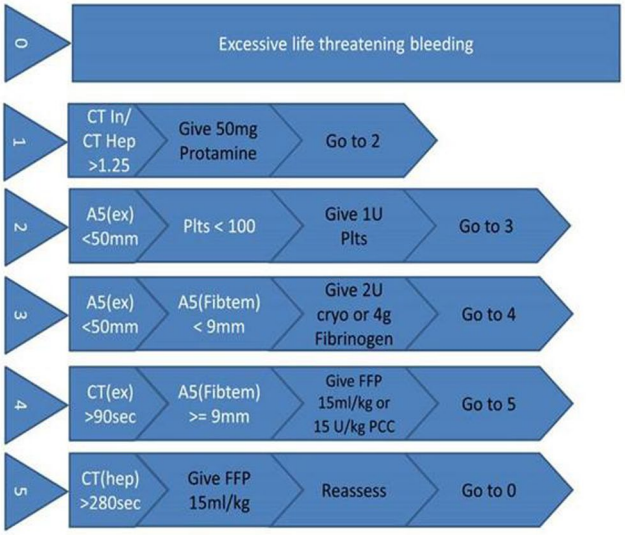

Figure 2: Royal Papworth Transfusion Algorithm. *This simplistic transfusion algorithm is designed specifically for use during and post cardiac surgery in the bleeding patient. The algorithm is optimised for the fastest possible time to transfusion decision and may serve as a template for other similar centres. CT: Clotting time; A5: amplitude 5 minutes post clot initiation; in: intem; hep: heptem; ex: extem; FFP: fresh frozen plasma; cryo: cryoprecipitate; PCC: prothrombin complex concentrate.*

FIGURE 2 Royal Papworth Transfusion Algorithm

PB0547 | The PFA Results Are Affected by Low Number of Platelets, Low Hematocrit and High Proportion of Immature Platelets

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Background: The PFA-200 analyzer is used for screening of impaired primary haemostasis and for evaluation of antiplatelet drugs therapeutic effectiveness. The results are reported as closure time (CT) (the time to occlusion of the aperture). The blood volume (BV) (the volume of blood flowed through the aperture until its obstruction) is reported using the research mode. Three cartridges are available: COL/EPI with collagen and epinephrine as inductors, COL/ADP with collagen and ADP, and P2Y with ADP, prostaglandin E1 and calcium chloride. The results are affected by thrombocytopenia and anemia using COL/EPI and COL/ADP cartridges; this has not yet been reported with P2Y cartridge.

Aims: To evaluate the correlation of CT and BV results with platelet count (PLT), plateletcrit (PCT), immature platelet fraction (IPF), mean platelet volume (MPV), hematocrit (HCT) and product of PCT and HCT (PCTXHCT).

TABLE 1 The results of selected blood count values

	PLT (*10^9/L)	MPV (fL)	PCT (%)	IPF (%)	HCT
min.	18.00	8.00	0.02	2.30	0.26
max.	98.00	13.30	0.11	19.80	0.48
mean	62.91	9.74	0.06	15.03	0.38
median	67.00	9.70	0.06	17.60	0.38
st. derivation	21.22	1.34	0.02	5.75	0.06

TABLE 2 The correlations of PFA results and selected blood count values. r correlation coefficient p statistical significance

	COL/ADP CT	COL/ADP BV	COL/ EPI CT	COL/ EPI BV	P2Y CT	P2Y BV
PLT	r=−0.3713 p=0.011	r=−0.4976 p=0.000	r=−0.3279 p=0.026	r=−0.3977 p=0.006	r=−0.1883 p=0.210	r=−0.1879 p=0.211
PCT	r=−0.2950 p=0.047	r=−0.4436 p=0.002	r=−0.3521 p=0.016	r=−0.3696 p=0.011	r=−0.1096 p=0.468	r=−0.1094 p=0.469
IPF	r=−0.3301 p=0.025	r=−0.2852 p=0.055	r=−0.0909 p=0.548	r=−0.2777 p=0.062	r=−0.5939 p=0.000	r=−0.6523 p=0.000
HCT	r=−0.4500 p=0.002	r=−0.5416 p=0.000	r=−0.3413 p=0.020	r=−0.5959 p=0.000	r=−0.4623 p=0.001	r=−0.5703 p=0.000
HCTx PCT	r=−0.4556 p=0.001	r=−0.5840 p=0.000	r=−0.4438 p=0.002	r=−0.5443 p=0.000	r=−0.2738 p=0.066	r=−0.3086 p=0.037

Methods: Patients with thrombocytopenia (PLT< 100x10^9/L) and no medication affecting platelet function were investigated using all three PFA cartridges. The correlation analysis was used for statistical analysis using the STATISTICA CZ software.

Results: 54 patients (30 males, 24 females, mean age 65 (range 19 to 93)) were investigated. The results of selected blood count values are summarized in table No 1.

No correlation of MPV with PFA results was found. The correlations of PFA results with others investigated blood count values are summarized in table No 2.

Conclusions: 1. The results of CT and BV are significantly affected not only by blood platelets count and PCT, but also by HCT; the role of HCT is at least as strong as the role of PLT and PCT. 2. Using the P2Y cartridge, the correlations of CT and BV with HCT are stronger than the correlations with PLT and PCT. 3. Using the P2Y cartridge, the correlation of CT and BV with IPF is significant.

PB0548 | Hemostatic and Inflammatory Markers in Patients with Alzheimer's Disease or Fronto-Temporal Dementia

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Background: Inflammatory and hemostatic factors may be directly involved in Alzheimer's disease (AD). Correlation between

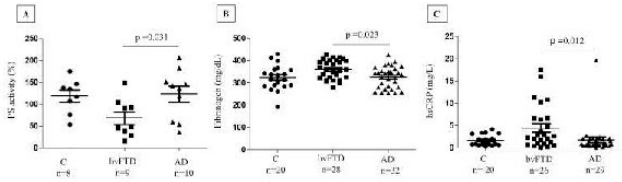

FIGURE 1 Medians and interquartile ranges for PS activity (A), and Fibrinogen levels (B), and usCRP (C) in AD, FTD and controls

an increased procoagulant state and decline cognitive has been reported. However, such factors have not yet been investigated in patients with behavioral variant frontotemporal dementia (bvFTD).

Aims: To compare hemostatic and inflammatory parameters in patients with AD or bvFTD.

Methods: A total of 90 patients participated in the study (AD, n= 32 and bvFTD, n= 29) recruited from the Hospital School at Federal University of Minas Gerais, Brazil, in addition to 29 individuals with no cognitive decline (controls). Plasma levels of fibrinogen and protein S (PS) were determined by coagulometric methods and ultrasensitive C-reactive protein (usCRP) by turbidimetry. Data were shown as median and interquartile range. Statistical analyzes were performed by Kruskal Wallis and Mann Whitney and $p < 0.05$ as significant. The study received approval from the local Ethics Committee.

Results: Regarding the hemostatic profile, Fig. 1 shows an decrease in the percentage of PS activity in the bvFTD group (48.9; Q1: 33.7 - Q3: 98.3) compared to the AD group (138.9; 58.6-158.3) (Figure 1A; p = 0.031) and a increase for fibrinogen levels in the bvFTD group (359.2; Q1: 321.5 - Q3: 400.5) compared to the AD group (339.5; 280.3-360.6) (Figure 1B; p = 0.023), were observed. With regard to usCRP, higher levels were observed in the FTD group (2.44; Q1: 0.83 - Q3: 6.62) compared to the AD group (0.88; 0.61-1.93) (Figure 1C; p = 0.012). There were no differences in these biomarkers in relation to controls.

Conclusions: The data taken together revealed more significant hemostatic and inflammatory changes in bvFTD compared to AD patients, suggesting impairment of natural anticoagulation in the former. Further studies are needed to confirm these preliminary findings.

Support: CAPES, FAPEMIG (APQ 00512-14) and CNPq (426933/2016-8).

PB0549 | Effect of Antiepileptic Therapy on Platelet Aggregation

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Background: It has already been shown that some antiepileptic drugs, especially valproic acid (VA) and its derivatives, alter platelet function. However, little is known about effect of dual antiepileptic therapy and/or combination of antiepileptic drugs with antiplatelet therapy such as acetylsalicylic acid (ASA).

Aims: To investigate an effect of mono and dual antiepileptic therapy and in combination with ASA applied as an antiplatelet drug.

Methods: The study included 102 patients using antiepileptic therapy. Testing was done on Sysmex CS2500 analyzer (method: light transmission aggregometry (LTA)) using ADP (2 μmol/L), epinephrine (5 μmol/L) and collagen (2 μg/mL) as agonists (all HYPHEN BioMed, France). Platelet rich plasma and platelet poor plasma were obtained from 3.2% citrate plasma centrifuged at 500 xg/10 minutes and 2000 xg/10 minutes, respectively. The differences between subgroups were tested using Kruskal-Wallis test.

Results: LTA results were: ADP 50% (44-74); epinephrine 54% (41-78) and collagen 71% (43-79). One third (31.3%) of patients were using combination of drugs (other antiepileptic or ASA) and 65 (63.7%) were taking VA. There was no difference between ADP (P=0.144), epinephrine (P=0.246) and collagen (P=0.431) results depending on antiepileptic used in all patients. Results differed between patients using monotherapy *vs* dual antiepileptic therapy for epinephrine 65% (44-79) *vs* 44% (37-51); P=0.001] and collagen [73% (47-79)] *vs* 43% (29-70); P=0.005] (Table 1).

Difference is obtained in patients using only VA *vs* VA combined with ASA for epinephrine [73% (44-80) *vs* 42% (23-74); P=0.020] and in patients using only VA *vs* dual antiepileptic therapy [65% (41-78) *vs* 84% (53-85); P=0.020] (Table 2).

Conclusions: Dual antiepileptic drug therapy inhibit platelet aggregation induced by epinephrine and collagen. Platelet aggregation is inhibited if induced by epinephrine in patients treated with valproic acid as monotherapy and if it is combined with ASA.

TABLE 1 Differences of ADP, EPI and COL values between patient subgroups (mono vs all on dual therapy; mono vs dual antiepileptic and mono vs ASA

	Monotherapy	Dual therapy					
	Antiepileptic N=70	AllN=32	P(mono *vs* all)	Dual antiepilepticN=22	P(mono *vs* dual antiepileptic)	Combination with ASAN=9	P(mono *vs* ASA)
ADP (%)	52 (44-74)	48 (42-55)	0.170	48 (44-51)	0.082	46 (44-51)	0.938
Epinephrine (%)	65 (44-79)	45 (38-74)	0.055	44 (37-51)	0.001	84 (41-85)	0.132
Collagen (%)	73 (47-79)	45 (41-79)	0.076	43 (29-70)	0.005	80 (48-84)	0.184

TABLE 2 Differences between ADP, EPI and COL in patients using VA (VA vs dual antiepileptic and VA vs combination with ASA

	Valproic acid usersN=65					
	Mono antiepilepticN=58	Dual antiepilepticN=7	P(mono *vs* dual antiepileptic)	Mono antiepilepticN=54	Combination with ASAN=11	P(mono *vs* ASA)
ADP (%)	50 (44-74)	79 (48-81)	0.192	51 (44-74)	47 (39-66)	0.189
Epinephrine (%)	65 (41-78)	84 (53-85)	0.020	73 (44-80)	42 (23-74)	0.020
Collagen (%)	72 (43-79)	83 (53-84)	0.092	74 (47-82)	41 (25-77)	0.062

PB0550 | Analysis of Megathrombocytes as a New, Fast, and Low Cost Tool to Indicate Thrombosis and Predict Prognosis in Sickle Cell Disease

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Background: Sickle cell disease (SCD) is a neglected genetic disease associated with high occurrence of thrombotic vaso-occlusive (VOC) crisis. In spite of exhaustive evidence of platelet's hyperactivation in SCD, there is a lack of knowledge about the correlation between Megathrombocytes and the occurrence of VOC in SCD.
Aims: We aim to evaluate the correlation between platelet's counts and the thrombotic state of patients using a new morphology-based evaluation method, which allows the determination of clinical indicators to predict VOC in a fast, easy and low-cost way.
Methods: Thirty-five patients diagnosed with SCD and receiving medical assistance at the University Hospital (UERJ, Brazil) participated in the study (ethics committee/ CEP-UERJ 455.394). D-dimer was quantified by ELISA to indicate the occurrence of thrombosis in patients. Platelets total count was obtained using the automatic counter. Blood smears were used for manually counting of platelets and megathrombocytes using a validated method developed by our group, called Modified Fonio's method 2 (FM2), which allows the reading of 20 spots/ sample. All statistical analyses were performed by GraphPad Prism 5
Results: SCD patients treated and non-treated with Hydroxyurea (HU) have shown higher median platelets counts (PC) (>300x10^3/ mm^3, $p< 0.01$), D-dimer values (>300ng/ml, $p< 0.05$) and equal megathrombocytes counts (MC) (6.9%, $p>0.05$) when compared to healthy individuals. Platelets abnormalities such as higher granulations and megathrombocytes counts were found in non-treated group. FM2 analysis has shown a strong correlation between D-dimer levels and MC in patients without HU treatment ($p< 0.05$ and $r=-0.45$), different to FM method by Lima and co-works (2001).
Conclusions: Finally, our results demonstrated that FM2 analysis of megathrombocytes should be used as sign of occurrence of thrombotic VOC in SCD. As an easy, fast, and low-cost method, the FM2 can be a valuable tool to help prognosis assessment and treatment follow-up of SCD patients, especially in underdeveloped countries.

PB0551 | Does Normal Diluted Russell Viper Venom Time Test Result Can Exclude Concentrations of Direct Oral Anticoagulants below 30 ng/mL?

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Background: Direct oral anticoagulants (DOACs), i.e. dabigatran, rivaroxaban and apixaban, have strong effect on dilute Russell viper venom screen (dRVVTs) and confirm (dRVVTc) test results. Therefore, it is possible to assume potential application of these assays in exclusion of DOACs presence in circulation.
Aims: To investigate whether normal dRVVT test result expressed as calculated lupus anticoagulant (LA) ratio i.e., dRVVTs/dRVVTc, can exclude clinically relevant concentration (>30 ng/mL) of DOACs.
Methods: DOACs concentrations were measured using commercial assays on BCS XP analyzer (Siemens Healthineers, Germany): Innovance heparin (Siemens Healthineers, Germany) calibrated with specific calibrators (Hyphen BioMed, France) for rivaroxaban and apixaban; Innovance DTI assay (Siemens Healthineers, Germany) for dabigatran. LA ratio was calculated from measured dRVVTs and dRVVTc tests with commercial LA1 Screening and LA2 Confirmation reagents using original protocols (Siemens Healthineers, Germany). Differences between groups were tested using Kruskal-Wallis test.

TABLE 1 Receiver operating characteristics (ROC) analysis for excluding direct oral anticoagulants at cut-off of 30 ng/mL

DOAC drug	Drug conc. in false positive LA results Median (IQR)	Drug conc. in neg. LA results Median (IQR)	LA ratio in false positive LA results Median (IQR)	LA ratio in negative LA results Median (IQR)	AUC (95% CI)	Sensitivity % (95% CI) at drug. conc. > 30 ng/mL	Specificity % (95% CI) at drug. conc. > 30 ng/mL
Dabigatran n = 59 False positive LA = 37 Neg. LA = 22	148 (53-250)	101 (19-168)	1.61 (1.41-1.77)	1.24 (1.19-1.29)	0.625 (0.489-0.747)	86.5 (71.2-95.5)	31.8 (13.9-54.9)
P	0.111		<0.001		0.106		
Apixaban n = 28 False positive LA = 10 Negative LA = 18	171.5 (82-202)	91 (56-208)	1.59 (1.54-1.80)	1.14 (1.01-1.24)	0.625 (0.423-0.799)	90.0 (55.5. -99.7)	11.1 (1.4-34.7)
	0.280		<0.001		0.277		
Rivaroxaban n = 70 False positive LA = 55 Negative LA = 15	194 (122-286)	14.3 (8-22)	1.80 (1.50-1.99)	1.20 (1.10-1.28)	0.967 (0.894-0.995)	92.7 (82-98)	80.0 (51.9-95.7)
	<0.001		<0.001		<0.001		

Receiver operating characteristics (ROC) analysis was performed for diagnostic accuracy estimation at cut-off value of 30 ng/mL for each DOACs. The study was funded by the Croatian Science Foundation as part of the research project IP- 2016-06-8208.

Results: LA ratios showed significant differences between false positive (FP) and true negative (TN) LA results (P< 0.001) for all three DOACs (Table 1). Among patients with FP and TN results of LA, concentrations of DOACs were significantly different for rivaroxaban only (P< 0.001). ROC analysis confirmed that normal LA ratio result can't exclude concentration below 30 ng/mL for dabigatran and apixaban (area under curve (AUC=0.625, P>0.05), whereas normal LA ratio could exclude rivaroxaban concentration below 30 ng/mL, AUC=0.967, P< 0.001 (Table 1).

Conclusions: Based on the normal dRVVT result (LA ratio), it is not possible to exclude low concentration of dabigatran and apixaban. On the contrary, normal dRVVT (LA ratio) could be potentially useful for excluding low concentration of rivaroxaban.

PB0553 | Method Comparison of Eight Coagulation Assays on the Cobas t 411 versus Cobas t 511/t 711 Analysers

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Background: The cobas t 411 (100 tests/hour), cobas t 511 (195 tests/hour) and cobas t 711 (390 tests/hour) analysers (Roche Diagnostics) can perform screening and specialised coagulation tests. Various systems may be used simultaneously (e.g. in routine/STAT laboratories) but differences in reagent and endpoint detection methods or different analyser reagent combinations can lead to discrepancies e.g. different thromboplastins used on cobas t 411 vs cobas t 511/t 711 analysers.

Aims: We investigated comparability between cobas t 411 and cobas t 511/t 711 platforms.

Methods: The study was performed at Roche Diagnostics, Penzberg (October-November 2018). Method comparison testing was performed using the following assays on each analyser: antithrombin, three different activated partial thromboplastin time (aPTT) reagents (based on lupus/heparin sensitivity), D-Dimer Gen 2, fibrinogen, prothrombin (PT), and thrombin time (TT). Passing-Bablok regression and Pearson's correlation were calculated. Plasma samples were obtained from donors at the in-house clinic and pathological donors (all stored at −80°C).

Results: Good/high correlation was shown between cobas t 411 and cobas t 711 analysers for seven coagulation assays (Pearson's r, 0.969-0.996; Table); moderate correlation was shown for fibrinogen (Pearson's r, 0.947). For PT, good correlation was shown for INRs < 4.5 (calibration limit) but greater differences were observed for higher INRs, particularly INRs > 6. Post-hoc analysis was performed using 13 samples with differences of > 0.5 INR between recombinant and human thromboplastin reagents (i.e. affecting a dosing change decision). Levels of factor II, V, VII and X activity were measured; 10 samples had FVII activity < 10% and 7 samples had FX activity < 10%.

Conclusions: Good/excellent comparability was demonstrated between cobas t 411 and cobas t 511/t 711 analysers. Our findings suggest the cobas t 411 analyser could be used as an appropriate satellite/back-up system for the larger cobas t 511/t 711 systems.

TABLE 1 Correlation between cobas t 411 and cobas t 711 analysers for eight coagulation assays

Assay	Number of samples	Measured range	Pearson's r
Antithrombin	112	20.8-118.6%	0.978
aPTT HighS	106	20.9-160.6sec	0.989
aPTT MedS	101	23.0-171.8sec	0.995
aPTT LowS	104	20.3-169.8sec	0.973
D-Dimer Gen 2	111; 97	0.2-9.0µg/mL; 0.25-5.5µg/mL	0.971; 0.980
Fibrinogen	102	65-880mg/dL	0.947
PT	130; 130; 49	7.8-56.7sec; 6.3-142.0%; 0.96-4.38 INR	0.990; 0.983; 0.996
TT	100	16.4-131sec	0.969

PB0554 | Effect of Activated Charcoal in Removing Interference in Thrombophilia Assays: Resistance to Activated Protein C, Activity of Coagulation Factor VIII and Antithrombin Activity

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Background: Treatment with direct oral anticoagulants (DOACs) has significant interfering effect on results of many specialized coagulation tests, such as thrombophilia assays, due to possible false negative (FN) or false positive (FP) results.

Aims: To investigate the impact of DOACs (dabigatran, rivaroxaban and apixaban) on thrombophilia assays: resistance to activated protein C (APCR), coagulation factor VIII (FVIII) and antithrombin (AT) activities and to evaluate the efficiency of our own optimized method using activated charcoal (AC) in removing interference.

Methods: DOACs concentrations and thrombophilia assays were firstly determined in native patient plasma samples (total n=65). Secondly, 100 mg of medical AC powder (Jadran Galenski laboratorij, Rijeka, Croatia) was added to 500 µL plasma allowing DOACs adsorption for 10 minutes. Treated samples were centrifuged 20 minutes at 1800xg and all measurements were repeated in supernatant. All assays were performed using commercial methods on BCSXP analyzer (Siemens Healthineers, Germany): Innovance anti-FXa assay with drug specific calibrators for rivaroxaban and apixabann (Hyphen BioMed, France); Innovance DTI assay for dabigatran; ProC Global for APCR; one-stage coagulation assay for FVIII and Innovance Antithrombin for AT activities. Wilcoxon test was used to test differences between pairs of samples. The study was funded by the Croatian Science Foundation as part of the research project IP- 2016-06-8208.

Results: All three DOACs have shown significant and different interfering effect on particular thrombophilia assays evaluated in this study (Table 1), indicating that most of these assays could not be performed in patients on DOACs, and suggesting the effective solution in removing interference by application of procedure with AC (Figure 1). In AC treated samples, concentrations of DOACs were below limit of detection.

Conclusions: AC has been found an effective in vitro agent to overcome effect of dabigatran, rivaroxaban and apixaban on particular thrombophilia assays, confirming potential of AC application before testing in patients treated with DOACs.

DOAC drug	Resistance to activated protein C (APCR)	FVIII activity	Antithrombin (AT) activity
Dabigatran	Dabigatran has strong interfering effect on APCR testing in terms of false positive result. Testing for APCR is not possible in patients treated with dabigatran. Medical AC is an effective in vitro agent in removing interference on APCR testing in patients treated with dabigatran.	Dabigatran has interfering effect on FVIII testing in terms of false negative result (falsely lower FVIII activity)* Testing for FVIII activity is not possible in patients treated with dabigatran. Medical AC is an effective in vitro agent in removing interference on FVIII testing in patients treated with dabigatran	Evaluation not performed, since dabigatran does not interfere in AT determination based on FX principle Testing for AT activity based on FXa principle can be performed in patients treated with dabigatran
Rivaroxaban	Rivaroxaban has strong interfering effect on APCR in terms of false positive result. Medical AC is an effective in vitro agent in removing interference on APCR testing in patients treated with rivaroxaban.	Rivaroxaban has no impact on FVIII measurement. Testing for FVIII activity can be performed in patients treated with rivaroxaban	Rivaroxaban has a slight interfering effect on AT testing in terms of false negative result (falsely higher AT activity) showing statistically significant difference, although without clinically significant difference. Medical AC is an effective in vitro agent in removing interference on AT testing in patients treated with rivaroxaban
Apixaban	Apixaban could have an interfering effect on APCR testing in some patients in terms of false positive test result. Medical AC is an effective in vitro agent in removing interference on APCR testing in patients treated with apixaban	Apixaban has no impact on FVIII measurement. Testing for FVIII activity can be performed in patients treated with apixaban.	Apixaban has a slight interfering effect on AT testing in terms of false negative result (falsely higher AT activity) showing statistically significant difference, although without clinically significant difference. Medical AC is an effective in vitro agent in removing interference on AT testing in patients treated with apixaban.

*FVIII activity >150% represents an independent thrombophilia risk factor

FIGURE 1 Interpretation and recommendation of thrombophilia assays (APCR, AT, FVIII) in patients on DOACs and with activated charcoal application

TABLE 1 Results of thrombophilia assays (APCR, FVIII, AT) before and after addition of medical activated charcoal (AC) in patients treated with DOACs

Direct oral anticoagulants (DOACs)	DOAC conc. before AC addition Median (95% CI), IQR	DOAC conc. after AC addition Median (95% CI), IQR	APCR normalized ratio Positives < 0.86 (n, ratio)		AT activity (%) Median (95% CI), IQR		FVIII activity (%) Median (95% CI), IQR	
			Before AC	After AC	Before AC	After AC	Before AC	After AC
Dabigatran n = 24	120 (86-186) 70-203	<2.8	23 (0.96)	0 (0)	NA	NA	106 (84-121) 76-129	161 (150-171) 149-174
P			<0.001		NA		<0.001	
Rivaroxaban n = 13	168 (33-238) 39-237	<5.9	11 (0.85)	2 (0.15) both true positives	106 (101-123) 101-122	95 (85-98) 86-98	130 (115-150) 117-150	135 (121-152) 121-152
P			<0.001		0.001		0.787	
Apixaban n = 28	151 (127-182) 108-203	<4.1	11 (0.73)	0 (0)	116 (109 125) 107-129	94 (90-104) 89-106	202 (176-206) 167-206	199 (168-206) 159-206
P			<0.001		<0.001		0.599	

PB0555 | Modified Anti-Xa Assay for Measuring Betrixaban (Bevyxxa®) Activity in Plasma on STA-R Max

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Background: Portola Pharmaceuticals has developed Bevyxxa® a direct factor-Xa inhibitor approved in the US for the venous thromboembolism prophylaxis in medically ill patients. Bevyxxa® is mainly excreted via the bile and has a low renal clearance. The plasma concentration of this drug has been estimated to be approximately ten times less than other factor-Xa inhibitors. An inhibitor-specific Anti-Xa assay may be used to assess Bevyxxa® Anti-Xa activity.

Aims: Proof of concept for the determination of Bevyxxa® concentration in plasma with specific calibrators and controls using STA-Liquid anti-Xa on STA-R Max.

Methods: The assay was optimized using commercial STA-Liquid anti-Xa and two Stago analysers. Assay calibration and quality control (QC) recovery were performed with specific freeze dried calibrator and controls respectively. Normal pooled plasma spiked with seventeen levels of Bevyxxa® were tested. Six *ex-vivo* samples from Bevyxxa® study were also tested. The following parameters were determined.

Limit of blank (LoB) using five normal pooled plasma samples.

Limit of detection (LoD) and linearity limit using Bevyxxa®-spiked plasma (n=8).

Day-to-day precision (at least 5 series) using two levels of QC and two other levels of Bevyxxa®-spiked plasma.

Accuracy measurement according to LC-MS reference method using Bevyxxa®-spiked plasma and *ex-vivo* samples.

Long term stability of freeze dried calibrator and QC.

Results: Results are summarized in the table I.

Conclusions: This new Bevyxxa® Anti-Xa activity assay automated on STA-R Max is accurate in the 8-110ng/mL range and reproducible with STA-Liquid Anti-Xa using new specific freeze-dried calibrator and controls. This is the very first study with Bevyxxa® samples and Stago commercial system. The results with *ex-vivo* samples showed good agreement with LC-MS reference method.

TABLE 1 Summary of Stago assay performances

ITEM	Stago Bevyxxa® Anti-Xa Assay
Limit of blank (LoB)	5.5 ng/mL
Limit of detection (LoD)	8.0 ng/mL
Linearity	Relative deviation from linearity (DLi) is less than 15% within the 15-110 ng/mL Bevyxxa® range. Absolute DLi is less than 2.5 ng/mL in the low range (< 15 ng/mL)
Day-to-day precision	Maximum CV of 10% with 10-20-30-60 ng/mL Bevyxxa® samples
Accuracy	Good agreement between Stago modified assay and LC-MS reference method on spiked and ex-vivo samples: Mean relative difference with LC-MS is below 16% within the 15-110 ng/mL Bevyxxa® range. Mean absolute difference with LC-MS is below 3 ng/mL in the low range (< 15 ng/mL)
Long term stability	Freeze-dried Bevyxxa® calibrators and controls stable up to 33 months at 2-8°C.

PB0556 | An Improved Algorithm for the Detection of Lipemia as Interfering Substances on a Fully Automated Coagulation Analyzer

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Background: The approach commonly employed to model optical interference through lipemia is to use an artificial substance (e.g., INTRALIPID emulsion). Because turbidity caused by lipemia is very heterogeneous, as the native lipoproteins' VLDL and chylomicrons vary in size and optical density, the current technique for measuring such turbidity is limited. A new evaluation algorithm** was developed on the Atellica® COAG 360 System to improve the detection of native lipids as interfering substances.

Aims: Improvement of the detection of native triglycerides as interfering substances compared to clinical chemistry systems.

Methods: The HIL module on the Atellica COAG 360 System consists of a capillary measurement cell with a photometric unit (LEDs at wavelengths l = 365, 415, 470, and 645 nm) attached.

The module reports the HIL assessments in index levels from 1 (low) to 9 (high).

Results from a previous study on the functionality of the HIL module were used to prove the new DeltaE evaluation algorithm. All results were compared to visual inspection and to the results from the clinical chemistry analyzer. Sensitivity and specificity were calculated and compared to the results from the clinical chemistry system.

Results: INTRALIPID curves compared to real native lipid spectra showed a different deflection, which causes the heterogenous behavior related to turbidity. The new evaluation algorithm DeltaE compared to the previous check algorithm for lipemic samples showed a better correlation to visual inspection and the clinical chemistry analyzer. Sensitivity for lipemic samples improved compared to the results from the clinical chemistry analyzer.

Conclusions: The new evaluation algorithm on the Atellica COAG 360 System enables much better differentiation of lipemic samples, with very good correlation to the real triglyceride content in native patient samples.

*Product not available for sale in the U.S. Product availability varies by country: **This feature is not commercially available. Its future availability cannot be ensured.

PB0557 | Fibrinogen Prothrombin Time Derived Method Is Not Useful in Anticoagulated Patients with Apixaban

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Background: We have showed that fibrinogen (FIB) prothrombin time derived method (FibPT-d), an easily available and economic method, is not useful when LMWH or rivaroxaban are present in the blood samples.

Aims: To compare FIB results obtained by Clauss method (FIB-C) and FibPT-d with two thromboplastins in anticoagulated patients with apixaban.

Methods: Population: 40 consecutive patients taking apixaban 5 mg twice a day (with normal PT and APTT before initiating anticoagulation) and 40 healthy controls (NC).

Methods: Fib C by HemosiL Fibrinogen C (FIBC) Fib PT-d with rabbit brain (HemosiL PT Fibrinogen HS plus, FibPT HS) and human recombinant (HemosiL Recombiplastin 2 G, FibPT RP). Apixaban levels by Liq Anti Xa with specific calibrators (HemosiL Apixaban calibrators). FIB assays were calibrated with Calibration Plasma and performed on ACL TOP platform.

Data Analysis: BIAS % between FibC and FIBPT-d with each thromboplastin were calculated. Comparison between methods was performed by Bland Altman plots.

Results: The table shows fibrinogen level by Clauss and the BIAS % between FIBC and Fib PT-d with each thromboplastin. Bland Altman analysis shows that there is a positive bias (p<0.001) for FibPT-d in either NC or anticoagulated samples. With FIB PT HS the bias was even bigger, in a concentration dependent manner, compared to NC. The results show a greater bias when rabbit brain compared to human recombinant thromboplastin was used.

Conclusions: Although the FibPT-d is an economic and rapid method, it should not be used in anticoagulated patients with apixaban because results could be overestimated. This behavior is like that previously shown by fibrinogen prothrombin time derived assay in plasmas containing rivaroxaban. As the origin of thromboplastin affects the results, these findings should not be extrapolated to other reagents / coagulometers systems.

TABLE 1 Bias Fib PT-d vs Fib C in samples from patients anticoagulated with apixaban

	FIB-C g/L (mean and range)	% BIAS Fib PTHS	% BIAS Fib PTRP
Normal controls n=40	3.10 (2.03-5.10)	13 CI 95%: 11.6-15.9	19.0 CI 95%:17.1-20.8
Apixaban anticoagulated patients n=40 (10-571 ng/mL)	3.90 (2.78-7.25)	21.7 CI 95%:14.6-28.9 P<0.0001	15.7 CI 95: % 8.7-22.64 P<.0001

PB0558 | Platelet Aggregation Testing by Light Transmission Aggregometry with Drug Specific Agonists in Patients on Mono and Dual Antiplatelet Therapy

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Background: Recently, platelet function testing is increasingly included in assessment of the effectiveness of antiplatelet therapy in research and clinical settings. Literature data suggest high residual platelet reactivity measured by light transmission aggregometry (LTA) as >20% when induced with arachidonic acid (AA) and >70% when induced with adenosine diphosphate (ADP).
Aims: To evaluate platelet reactivity by LTA using drug specific agonists in patients on long-term mono and dual antiplatelet therapy.
Methods: Study included 191 consecutive patients on dual antiplatelet therapy, 158 patients on monotherapy with acetylsalicylic acid (ASA) and 37 on clopidogrel referred to our laboratory for platelet aggregation testing. Platelet rich plasma was obtained from 3.2% citrate plasma centrifuged at 500xg/10 minutes. LTA was done on Sysmex CS2500 (Siemens Healthineers, Germany) using AA (1 mmol/L) and ADP (2 μmol/L) as agonists (Hyphen Biomed, France). Parametric and nonparametric analysis of variance was used to test the differences between studied groups.
Results: Among 191 patients with median age 64 yrs (range 26-90) on dual therapy, 170 were using clopidogrel (75 mg) and ASA (100 mg) whereas 21 were using ticagrelor (90 mg) and ASA (100 mg). Results for AA were 7% (interquartile range (IQR) 3-10)) and for ADP 35%±17%, respectively. Group of patients using ticagrelor and ASA has significantly lower ADP results than those using clopidogrel and ASA (median 20%; IQR 16-27) *vs* median 37%; IQR 23-49; P< 0.001). Patients on ASA monotherapy (N=158) had AA 9% (IQR 4-16) while in those on clopidogrel therapy (N=37), aggregation with ADP agonist was 68%±10%. Only 8 (4%) patients on dual therapy did not reach suggested cut-off for AA in contrary to 20 (13%) patients on monotherapy with ASA (Table 1).
Conclusions: Our study has shown that a higher proportion of patients on long term ASA monotherapy has high residual platelet reactivity compared to patients on dual antiplatelet therapy.

PB0559 | Multicentre Performance Evaluation and Reference Ranges for a New Assay for Quantitative Determination of von Willebrand Factor (vWF) Activity

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Background: vWF is important for platelet adhesion at wound sites and regulates the half-life of FVIII. Deficiency and/or functionally abnormal vWF results in delayed clot formation and bleeding characteristic of von Willebrand disease. ABO blood group also influences vWF levels; blood group O is associated with a significantly lower reference range.
Aims: To determine the analytical performance and establish reference ranges for the new quantitative vWF Act assay (Roche Diagnostics) on the cobas t 711 analyser.
Methods: Anonymised residual or commercially purchased 3.2% citrated plasma samples were evaluated at three sites. Five human

TABLE 1 Results of platelet aggregation testing in patients on mono and dual antiplatelet therapy

	Dual therapy			P	Monotherapy (all n = 196)		P
	All (n = 191)	Clopidigrel + acetylsalicylic acid n = 170	Ticagrelor +acetylsalicylic acid n = 21		Acetylsalicylic acid n =158	Clopidogrel n =37	
Age median (min-max)	64 (26-90)	64 (26-90)	61 (29-87)	0.219	69 (30-93)	69 (46-90)	0.712
Sex, female (n, %)	107 (54%)	97 (57%)	10 (0.48%)	0.412	69 (44%)	13 (0.35)	0.300
ADP (%) mean±SD	35±17	36±17	20 (16-26)	<0.001	/	38±17	/
AA (%) median, IQR	7 (3-10)	7 (3-10)	6 (4-11)	0.918	9 (4-16)	/	/
ADP > 70% (n, %)	2 (0.01%)	0 (0%)	0 (0%)	1.000	/	1 (0.03)	/
AA > 20% (n, %)	8 (4%)	7 (4%)	1 (0.05%)	1.000	20 (13%)	/	0.009 (all vs mono ASA)
AA < 20% median, IQR	30 (26-44)	/	/	/	26 (24-29)	/	0.069 (all vs mono ASA)

plasma pools and two controls were tested to determine within-run precision (n=21 determinations/sample; one run; three reagent lots), total reproducibility and intermediate precision (five aliquots/sample); coefficients of variation (CV) were calculated. Lot-to-lot comparisons (three reagent lots) were performed using three sample sets (n≥120) covering the measuring range and evaluated by Passing-Bablok regression. Method comparison versus the INNOVANCE® VWF Ac assay (Siemens; Sysmex CS-5100 analyser) was performed using the same samples; Deming regression and Pearson's r were calculated. Reference ranges were determined in three cohorts (blood type O, non-O and ABO) using ≥120 fresh samples from healthy donors aged ≥18 years, not receiving anticoagulant medication.

Results: Excellent within-run precision was demonstrated across sites, with CVs 0.5-5.9% (mean vWF activity, 4.77-132 IU/dL). Total reproducibility and intermediate precision were high, with CVs 1.6-7.2% and 0.8-7.3% (mean vWF activity, 4.91-132 IU/dL), respectively. The vWF Act assay showed low lot-to-lot variability (Pearson's r, 0.997-0.998; Table 1), and high correlation with the comparator assay (Pearson's r, 0.973-0.996; Table 2). Reference ranges (2.5-97.5th percentiles) according to blood type: O, 46.2-135 IU/dL (n=164); non-O, 68.4-186 IU/dL (n=210); ABO, 50.4-180 IU/dL (n=374).

Conclusions: The vWF Act assay (cobas t 711 analyser) demonstrated robust analytical performance, supporting introduction into routine laboratory practice.

TABLE 1 Lot-to-lot comparison for the vWF Act assay (cobas t 711 analyser)

Parameter	Freiburg	Sheffield	Vienna
Lot	A vs B	C vs A	B vs C
Sample, n	137	128	133
Slope (Passing-Bablok)	0.988	1.006	1.044
Intercept	0.317	−1.03	−1.51
Pearson's r	0.998	0.998	0.997
Relative bias at 30 IU/dL, %	−0.110	−2.85	−0.637

TABLE 2 Method comparison of the Roche vWF Act assay versus the INNOVANCE VWF Ac assay

Parameter	Freiburg	Vienna	Sheffield
Lot	A	B	C
Sample, n	139	133	126
Slope (Deming)	0.933	0.948	0.916
Intercept	−0.260	0.0827	−1.25
Pearson's r	0.992	0.996	0.973
Relative bias at 30 IU/dL, %	−7.52	−4.97	−12.5

PB0560 | Comparison of Two Quantitative Methods for Determination of Dabigatran Concentration: Diluted Thrombin Time Test and Innovance Dabigatran Test in Real Life Patients Treated with Dabigatran

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Background: Clinical application of dabigatran does not require routine therapeutic monitoring. However, there are special clinical situations in which measurement of dabigatran concentration in circulation should be performed using specific quantitative methods.

Aims: The aim of this study was to compare two quantitative methods for dabigatran concentration i.e. commercial chromogenic Innovance Dabigatran assay (DTI) and in house optimized coagulometric diluted thrombin time (dTT) test.

Methods: Dabigatran concentrations were determined in 38 patients using two methods on BCS XP coagulation analyzer (Siemens Healthineers, Germany): Innovance Dabigatran assay (Siemens Healthineers, Germany) with drug specific calibrators (Siemens Healthineers, Germany) and dTT test as an in house applied protocol with thrombin time reagent (BC Thrombin, Siemens Healthineers, Germany) calibrated with commercial dabigatran calibrators (Diagnostica Stago, France). Passing-Bablok regression and Bland-Altman methods were used for method comparison and Wilcoxon test was used to test differences between pairs of samples. The study was funded by the Croatian Science Foundation as part of the research project IP- 2016-06-8208.

Results: Passing and Bablok regression analysis (y=−7.653 (−15.085 to −2.782) +0.986x (0.939 to1.033) revealed constant difference between two methods (Figure 1). Bland-Altman analysis showed statistically significant mean difference of 7.7% (P< 0.001) between dTT assay compared with DTI. Further, comparison of the results for dabigatran concentrations using DTI and dTT assays showed statistically significant difference (P=0.0002), but without clinically significant difference (Table 1).

Conclusions: Although two evaluated methods for quantitative determination of dabigatran concentration showed significant constant difference, that difference is not clinically significant, so both

TABLE 1 Results of dabigatran concentrations measured with two methods in real life patients treated with dabigatran (n= 38)

Method	Dabigatran concentration (ng/mL) Median (95% CI); IQR
Diluted thrombin time (dTT) test	163.5 (95-228) 68-252
Innovance Dabigatran (DTI) test	171 (114-240) 70-259
P	0.0002

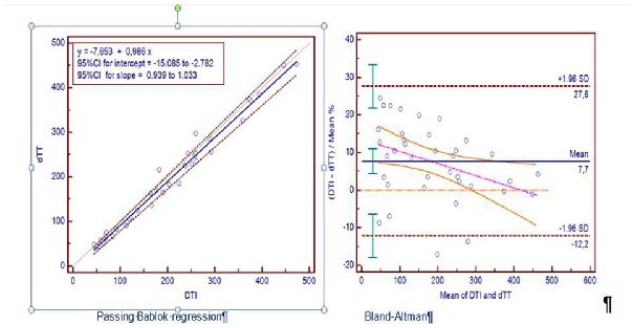

FIGURE 1 Passing-Bablok regression and Bland-Altman methods for comparison of two methods (dTT and DTI) for dabigatran concentration measurement

can be used interchangeably. However, commercial DTI assay could be recommended as a method of choice for quantitative measurement of dabigatran concentration, since it has much wider measurement range without sample dilution (up to 500 ng/mL) compared to dTT assay (up to 256 ng/mL), uses chromogenic principle and time of testing is much shorter.

PB0561 | Pre - Analytical Variables in Haemostasis: Results of the UK National External Quality Assessment Scheme for Blood Coagulation Haemolysis Supplementary Exercise

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Background: Haemolysis is considered one of the major contributors of non-conformities and sample rejection in coagulation testing.

Aims: To investigate effects of haemolysis on methodology and results in a multicentre exercise, and gather information about laboratory approaches to dealing with haemolysed samples.

Methods: In November 2018 UK National External Quality Assessment Scheme for Blood Coagulation (UK NEQAS BC) conducted an exercise where two different lyophilised plasma samples were distributed to 800 participants comprising of centres registered for prothrombin time (PT), activated partial thromboplastin time (APTT) and either Clauss fibrinogen or thrombin time (TT) in the UK NEQAS BC programme. The same pooled normal plasma was used to prepare 2 samples S18:19 and S18:20. A haemolysate consisting of freeze/thaw red blood cells was added to sample S18:20 in order to create haemolysis at 3g/l concentration. Participants were asked to provide answers to a questionnaire about their laboratory approach to dealing with haemolysed samples including strategies used to deal with different levels of haemolysis.

Results: There was variability in the number of received results for samples S18:19 and S18:20 which depended on the test registered by participants. Results are shown in the table. Variability in practice was observed, out of 501 responses 226 centres indicated they reject haemolysed samples solely on visual checks, with 163 using initial visual checks with further sample rejection evaluation by analyser flags. 333 centres indicated that the level of haemolysis affects sample rejection decisions while 132 stated it does not.

Conclusions: In this exercise, results for performed tests did not show great variability between two samples. This may be explained by artificial construction of the haemolysed sample in this exercise. Variability of responses for dealing with haemolysed samples reflects a lack of clear guidelines in the pre analytical area of sample processing.

PB0562 | Loop-Mediated Isothermal Amplification without DNA Extraction for Simultaneous Detection of Factor V Leiden and Prothrombin-G20210A Genetic Variants

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Background: Genotyping Factor V Leiden (FVL) and Prothrombin-G20210A (PT) genetic variants is not recommended in all cases diagnosed with Venous Thromboembolism (VTE). However, they are among the most requested clinical tests included in work-up for VTE, perhaps because homozygotes or certain special situations (such as pregnancy) need particular medical considerations. Most of available diagnostic techniques need prior genomic DNA (*g*DNA) extraction and are time-consuming. Thus, promising tests potentially able to save time have come out on the market. We show preliminary results obtained with a new *in vitro* diagnostic method, based on a loop-mediated isothermal amplification (LAMP) (CE-IVD LaCAR MDx; Liège, Belgium), which simultaneously detects FVL and PT on whole blood samples.

TABLE 1 A summary of received results for samples S18:19 and S18:20

Test	N results	Median ratio S18:19 (range)	Median ratio S18:20 (range)	Median %difference
PT	501	1.05 (0.86-1.7)	1.1 (0.85-9.23)	4.8
APTT	503	0.95 (0.76-1.47)	0.95 (0.8-1.60)	0
Clauss Fibrinogen	504	2.46 (1.67-8.3)	2.56 (1.62-8.0)	4.1
Thrombin time	338	1.05 (0.67-1.37)	1.01 (0.71-1.42)	−3.8

Aims: To compare the genotyping of FVL and PT variants with LAMP assay to the routine diagnostic method performed in our Centre.

Methods: From Dec 1st 2019 until Jan 31st 2020, 50 patients (median age 40 years [8-82], M/F: 11/39) were tested for FVL and PT. The LAMP method simultaneously detects both the variants, using a different fluorescent colour for each of them. We identified FVL and PT variants with the TaqMan methodology (Applied Biosystems).

Results: Overall, we identified 47 wild-type and 3 FVL heterozygotes, whereas 48 wild-type and 2 heterozygotes for the PT, with a 100% agreement with the current method. We calculated the turnaround time for both tests. Using the new method, we recorded a median turnaround time of 55 min (45-70 min), whereas the median turnaround time calculated for the routine method was 11 days (range 8-13 days).

Conclusions: Our results show that the LAMP method can detect FVL and PT variants with an accuracy of 100%. This new rapid diagnostic method could help improving efficiency of care.

PB0563 | Comparative Evaluation of the Aggregation Studies in a Sysmex CS2500 Coagulometer with a Standard Turbidimetric Technique

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Background: The evaluation of platelet function by aggregometry techniques is complex, time consuming, with a high manual component that requires dedicated personel and expert teams. The Sysmex CS-2500 (Siemens) is a high performance automated coagulation analyzer designed for aggregometry studies.

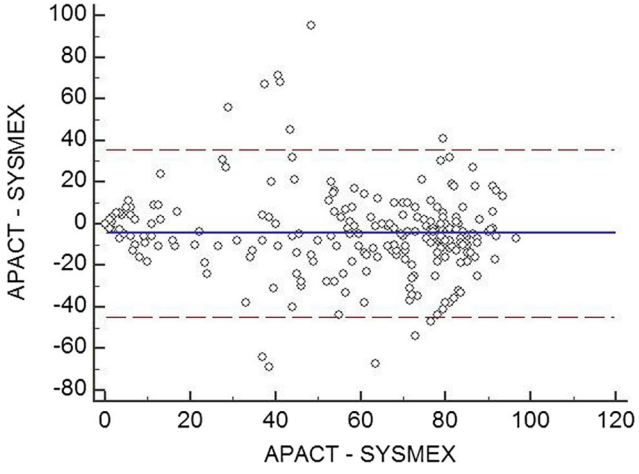

FIGURE 1 Bland-Altman plot

Aims: To assess the efficacy, reliability and ability to detect alterations of platelet function in the Sysmex CS-2500 in a comparative study with conventional aggregometry (APACT, Helena), both using light transmission aggregometry (LTA).

Methods: We obtained platelet rich plasma from healthy individuals, from patients with suspected platelet functional disorders and from blood samples exposed to antiplatelet therapy. We evaluated the aggregation response against arachidonic acid (1.4 mM), ADP (4 μM), collagen (2.5 g / ml), epinephrine (10 μM) and ristocetin (1 mg / ml) in 48 samples. We analyzed the correlation between the results with both techniques and used the Bland-Altman test to calculate the confidence intervals of the differences.

Results: The results obtained with the two techniques showed a good correlation ($r = 0.79$; $p < 0.001$). The degree of coincidence between the results in the two aggregometers was very high, with 95% of the results included within the standard deviations of the mean (image). The individual results of the different aggregating agents was similar in the two types of aggregometer. The Sysmex CS-2500 detected the presence of anomalies n a similar way to the APACT used as a reference. No technical difficulties were observed in the management of the automated equipment.

Conclusions: LTA is still considered the "gold standard" technique for the evaluation of platelet function. The CS-2500 offers diagnostic possibilities similar to conventional aggregometry in a high performance desktop coagulometer. This automated equipment may offer alternatives in the future allowing laboratories to assess and diagnose the different alterations of primary and secondary hemostasis.

PB0564 | Multicentre Performance Evaluation and Reference Ranges for the Quantitative Determination of Factors II, V and VII by One-Stage Factor Assay on the Cobas t 711 Analyser

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Background: Deficiency of coagulation factors FII, FV and FVII causes prolonged prothrombin time (PT) and bleeding disorders of variable severity depending on factor level activity. We performed a multicentre evaluation of three PT one-stage factor assays for quantitative determination of FII, FV and FVII activity (Roche Diagnostics).

Aims: Evaluate the analytical performances of FII, FV and FVII factor assays on the cobas t 711 analyser.

Methods: Experiments were performed at three laboratories using 3.2% citrated, anonymised, ethically approved, residual/commercially purchased plasma samples. Five human plasma pools (HPPs)

TABLE 1 Lot-to-lot comparisons for the Roche FII, FV and FVII factor assays

Assay	FII			FV			FVII		
Site	Freiburg	Sheffield	Vienna	Freiburg	Sheffield	Vienna	Freiburg	Sheffield	Vienna
Lot	A vs B	C vs A	B vs C	A vs B	C vs A	B vs C	A vs B	C vs A	B vs C
Sample, n	128	140	133	126	126	133	125	124	124
Slope (Passing-Bablok)	0.998	0.980	0.993	0.992	1.000	1.022	1.031	1.031	1.003
Intercept	0.198	0.264	0.611	0.162	−0.900	−0.053	−0.70	−1.52	0.75
Pearson's r	0.999	0.999	0.999	0.999	0.998	0.999	0.999	0.999	1.000

TABLE 2 Method comparisons between the Roche and Siemens FII, FV and FVII factor assays

Assay	FII			FV			FVII		
Site	Freiburg	Vienna	Sheffield	Freiburg	Vienna	Sheffield	Freiburg	Vienna	Sheffield
Lot	A	B	C	A	B	C	A	B	C
Sample, n	124	128	132	124	129	124	125	124	123
Slope (Deming)	0.952	0.935	0.925	0.929	0.997	0.915	0.896	0.967	0.907
Intercept	−0.439	−0.821	−0.705	2.95	2.02	1.27	−0.68	0.14	−1.58
Pearson's r	0.997	0.997	0.995	0.989	0.996	0.992	0.997	0.997	0.996

and two controls were used for determination of assay repeatability (1 run/site; n=21 replicates per sample), intermediate precision and total reproducibility (5 aliquots/sample per day over 5 days); coefficients of variation (CV) were calculated. Lot-to-lot variability (3 reagent lots) and method comparison (versus Siemens Coagulation FII/FV/FVII Deficient Plasma [with Dade Innovin] on Sysmex CS-5100 analyser), using three sets of ≥120 samples, were evaluated by Passing-Bablok and Deming regression, respectively, and Pearson's r calculated. Reference ranges were determined (one site) using fresh plasma samples from healthy individuals aged ≥18 years, not receiving anticoagulant medication.

Results: Across sites, CV ranges for repeatability for each assay were: FII, 0.6-3.0%; FV, 0.8-2.5%; FVII, 1.0-1.7%. High intermediate precision (CV: FII, 1.0-2.0%; FV, 1.1-7.0%; FVII, 1.3-3.5%), and good reproducibility (CV: FII, 1.7-2.6%; FV, 2.7-6.1%; FVII, 1.9-3.3%) were shown for each assay. All assays demonstrated excellent lot-to-lot variability (Pearson's r, 0.998-1.000; Table 1) and high agreement with comparator assays (Pearson's r, 0.989-0.997; Table 2). Among healthy individuals (n=200), 2.5th and 97.5th percentiles for coagulation factor activity were: FII, 78.4-124 IU/dL; FV, 64.0-143 IU/dL; FVII, 56.1-158 IU/dL.

Conclusions: FII, FV and FVII factor assays demonstrated robust analytical performance on the cobas t 711 analyser, supporting use in routine laboratory practice.

PB0565 | Low Molecular Weight Heparin Calibrated Anti-Xa Assays can Identify Patients with Low Apixaban or Rivaroxaban Drug Levels

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Background: Patients on apixaban or rivaroxaban may not benefit from anticoagulant reversal strategies when their drug levels are below 30-50 ng/mL (ISTH, 2016). It is unknown if anti-Xa low molecular weight heparin (LMWH) assays can reliably detect drug levels in this range.

Aims: To establish if LMWH anti-Xa assays can identify patients with apixaban or rivaroxaban levels < 50 ng/mL.

Methods: We selected 100 clinical samples from Hamilton Regional Laboratory Medicine Program's Special Coagulation Laboratory with known apixaban or rivaroxaban drug levels up to 100 ng/mL (determined using Diagnostica Stago's STAr/Evolution analyzer with rivaroxaban and apixaban drug-specific calibrators). Three LMWH calibrated anti-Xa assays were performed for each sample using: Instrument Laboratories ACL TOP 300 (IL), Siemens BCS XP (Siemens) and Diagnostica Stago STAr/Evolution (Stago) as per manufacturer protocols, reagents and calibrators. We used linear regression and Pearson correlation coefficients to compare LMWH anti-Xa activities with drug levels. We determined the LMWH anti-Xa

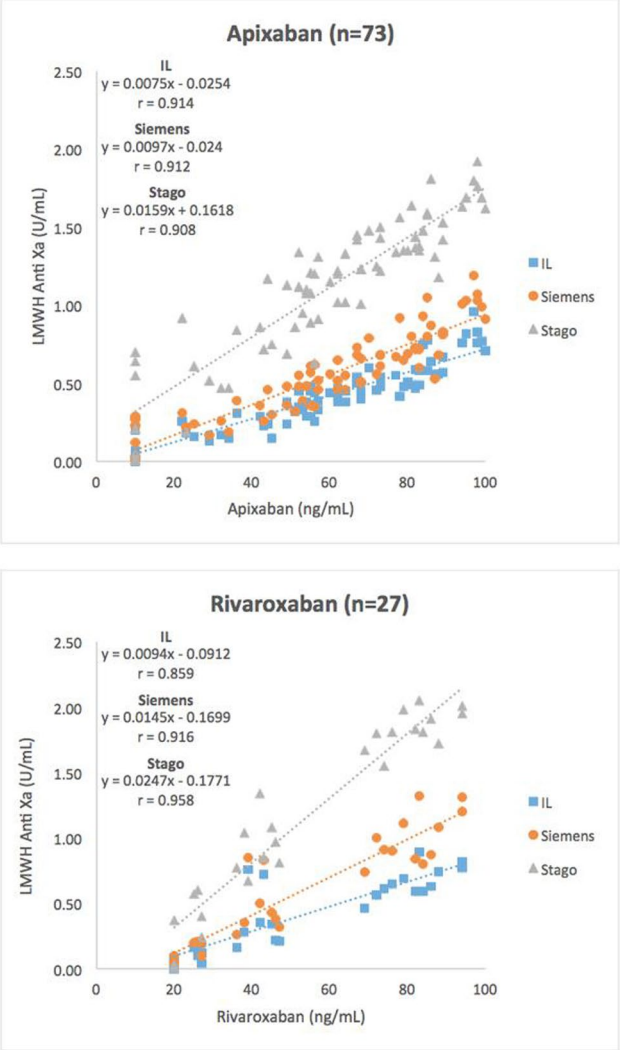

FIGURE 1 LMWH anti-Xa activities corresponding to known drug levels of apixaban and rivaroxaban (n=100)

activity that corresponded to 95% negative predictive value (NPV) for an apixaban or rivaroxaban drug level greater than 50 ng/mL. The Hamilton Integrated Research Ethics Board reviewed this study.

Results: In 73 apixaban and 27 rivaroxaban samples, all 3 LMWH anti-Xa assays correlated linearly with drug levels (r=0.91 for apixaban, r=0.86-0.96 for rivaroxaban) (Figure 1). A LMWH anti-Xa activity below 0.26 U/mL (IL), 0.31 U/mL (Siemens), or 0.84 U/mL (Stago) had 95% NPV for an apixaban drug level > 50 ng/mL. A LMWH anti-Xa activity below 0.35 U/mL (IL), 0.50 U/mL (Siemens), or 1.34 U/mL (Stago) had 95% NPV for a rivaroxaban drug level > 50 ng/mL.

Conclusions: LMWH anti-Xa assays can identify patients who are unlikely to benefit from anticoagulant reversal. The predictive threshold varies widely between instrument/reagent combinations.

PB0566 | Bethesda Assay for the Detection and Titration of an Anti-VIII Inhibitor: Interest of the Chromogenic Assay

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Background: Screening and titration of factor VIII inhibitors allows monitoring of hemophilia A (HA) treatment and the diagnosis of acquired hemophilia. The classic Bethesda assay is based on the FVIII one stage clotting assay (BOSA) which can be impacted by the presence of lupus anticoagulants or interfering treatments.

Aims: The aims of this study was to compare analytical performances of the modified Bethesda assay using FVIII chromogenic assay (BCH) and BOSA.

Methods: The intra and inter assay precisions of the 2 techniques were calculated on a weak positive (1UB) control (Cryopep®). For 180 samples (20 patients with suspected acquired HA, 79 HA patients with or without inhibitors, 11 HA patients treated with Hemlibra®), inhibitor titers were determined by BCH (COAMATIC, Chromogenix, bovine reagents) and BOSA (Synthasil, Hemosil, deficient FVIII, Werfen). Calculated residual FVIII levels and inhibitor titers were then statistically compared (Mann and Whitney test and calculation of the kappa cohen coefficient).

Results: The intra assay CV on the positive control was 3% and 10% respectively for BCH and BOSA. The inter assay CV determined on the positive control was 10.7% for BCH and 17.5% for BOSA. The coefficients of correlation of residual FVIII and titers obtained on the 180 samples were 0.75 and 0.94 respectively (Figure 1). The titers obtained for the 53 positive plasmas were not significantly different between the two assays (P=0.859) except titers of HA patients treated with emicizumab significantly higher (+15%) with BCH. The approval rate between both techniques were 98.3% (kappa :0.96) with only 3 discrepancies:

1. plasma with LA weakly positive (0.8 UB) with BOSA and negative with BCH
2. plasmas weakly positive (0.6 and 0.8 BU) only with BCH (suspected Acquired HA and severe HA with a history of inhibitors).

Conclusions: Bethesda technique using chromogenic assay improves performance and appears to improve specificity.

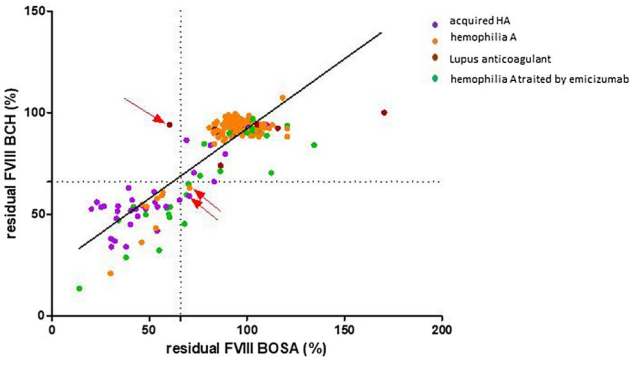

FIGURE 1 Correlation of residual FVIII: BCH vs BOSA

PB0567 | Thrombin Generation Analysis in Patients with Venous Thromboembolism and Atrial Fibrillation on Anticoagulant Tehrapy: Comparison between Direct Oral Anticoagulants and Antivitamin K Antagonists

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Background: Thrombin generation (TG) is a global coagulation analysis that evaluate the individual haemostatic balance between pro and anticoagulant activity. Direct oral anticoagulants (DOAC) have shown large inter-individual variability, both in atrial fibrillation (AF) and in venous thromboembolism (VTE), as measured with different coagulation test.
Aims: To evaluate TG analysis in patients with VTE and AF treated with the four available DOAC (apixaban, dabigatran, edoxaban and rivaroxaban) and anti vitamin K antagonists (AVK).
Methods: 300 patients (150 AF and 150 VTE), 60 for each drug (30 AF, 30 VTE), were enrolled.
Plasma samples were collected at C-trough and C-peak in DOAC patients, while in AVK nearly 16 hours after the last dose intake, 3 months after starting anticoagulant treatment. Prothrombin time (PT INR) for warfarin, chromogenic assays specific calibrated for each DOAC and TG were performed on all plasma samples. ST-Genesia (STG, Stago, Asnières-sur. Seine, France), a new system fully automatized, was used.
TG parameters measured were: normalized lag time (min), endogenous thrombin potential (ETP nM*min) and peak height (nM).
Results: DOAC high inter-individual variability was confirmed through specific measurements and TG analysis. All patients on warfarin showed PT INR within 2.0-3.0 and mean ETP nM*min=467, while DOAC patients showed a significant higher ETP mean values (>1000 nM*min), both at C-peak and C-trough, without differences among the four DOAC. Significant differences (p< 0.0001) were found in ETP and lag-time between DOAC and warfarin patients.
Conclusions: Our data confirmed the high DOAC inter-individual variability as measured both with specific coagulation test than with ST-Genesia. TG comparison between DOAC and AVK showed significant differences, suggesting a more stable anticoagulant effect in AVK treatment and the importance of improving methods for assessing global individual anticoagulation status, particularly in high risk patients. ST-Genesia TG could be a useful method to better tailor DOAC dosage in the single patient.

PB0568 | Validation of a Warfarin Dose Prediction Model: A Single Center Retrospective Study

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Background: Warfarin dose initiation is often a trial and error challenging process which is often time consuming. Dose prediction nomograms, pharmacokinetic formulas and computer softwares have all been tried to facilitate and speed the process achieving a stable steady state INR. In a recent study (JTH 2010; 8: 90) a simple one week warfarin loading nomogram was used successfully for such patients.
Aims: To investigate the validity on the warfarin loading model in a cohort of warfarin first time users.
Methods: Consecutive patients starting warfarin treatment were routinely dosed with the use of the nomogram at our center starting from 2015. All patients were followed at our outpatient clinic and their steady state warfarin maintenance dose (dose resulting in 2 successive INRs in the desired therapeutic range) was compared to the predicted dose. We compared the difference between predicted to actual doses to the null hypothesis of zero difference and used reliability testing to study the correlation between the predicted and actual daily doses.
Results: Thirty five patients (23 females, median age 47.5 IQR 21.5) were studied. Warfarin treatment indication was mainly venous thromboembolism (31/35) except for four patients treated for atrial fibrillation or arterial thrombosis. Steady state (median ± IQR) predicted daily warfarin dose was 5.51±2.28 mg compared to 5.53±1.97 mg actual daily dose and not statistically different (P=0.98). The median difference between predicted and actual warfarin steady state dose was 0.015 mg (IQR 0.61) and was not different from zero (P=0.332). The intra-class correlation coefficient was 0.970 (0.938-0.985).

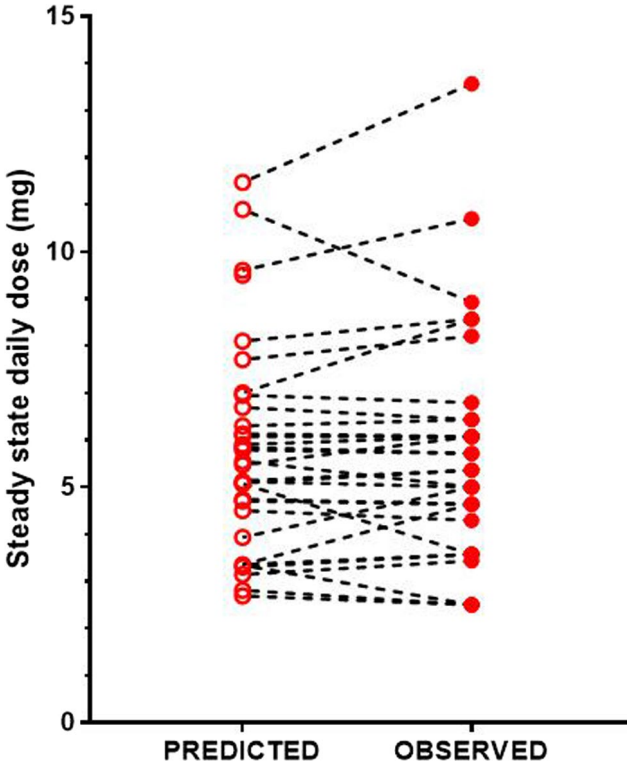

FIGURE 1 Predicted vs. actual daily steady state warfarin doses

Conclusions: The warfarin steady state dose prediction tool was able to precisely predict warfarin steady state maintenance dose with good accuracy within a week after starting warfarin treatment and may be a useful tool to shorten the time period to reach safe warfarin anticoagulation.

PB0569 | Biosimilar Enoxaparin Sodium Inhixa® Compared with Clexane®: An in vitro Study

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Background: Recently three biosimilar/generic molecules of enoxaparin (Inhixa®, Gmemaxan® and Thorinane®) have been authorized by the European Medicines Agency (EMA), declared as identical to the reference product Clexane®, showing a good comparability in term of biological activity and chemical properties.

Aims: To compare in vitro anti-FXa activity of Inhixa® and Clexane® on normal plasma samples added with different amount of the two molecules.

Methods: Increasing amount of both LMWH were added to normal plasma pool to reach expected final concentration, ranging from 0 UI/ml up to 3.0 UI/ml, for a total of 8 different dilutions (0 UI/ml, 0.125 UI/mL, 0.25 UI/mL, 0.5 UI/mL, 1.0 UI/mL, 1.5 UI/mL, 2.0 UI/mL, 3.0 UI/mL). Activated partial thromboplastin time (aPTT) and anti-FXa chromogenic assay were performed in duplicate, within 1 hour after sample preparation. Mean±standard deviation (SD), coefficient of variation (CV%) and linear regression analysis were calculated. Bland Altman method was used to evaluate bias between the two molecules.

Results: Clexane® and Inhixa® anti-Xa and aPTT ratio mean values were 0.84 UI/mL and 0.79 UI/mL and 1,81R and 1,76 R, respectively. Anti-FXa mean CV% between the two molecules was 5.45%, showing a costant small, but not significant, underestimation of Inhixa®. Bias between Clexane® and Inhixa® for anti-FXa assay and aPTT were 7.7% and 2.3% respectively. Coefficient of correlation (r) was > 0.999, both in the comparison of the two molecules and the expected values.

Conclusions: In vitro anti-FXa activity of Clexane® and Inhixa® showed very good correlations with a small underestimation of Inhixa®, lower than anti-FXa intra/inter assay variability. Limits are represented by the "in vitro" evaluation that cannot give any information respect "in vivo" pharmacokinetic, pharmakodynamic, clinical efficacy, safety and collateral effect as immunogenic reactivity. A strict pharmacovigilance should be recommended and anti-FXa monitoring, at least in particular clinical settings, should be advised.

TABLE 1 Comparison between anti-FXa activity, aPTT and expected results, using the two molecules

aFXa UI/ml	aFXa UI/ml	aFXa UI/ml	aPTT (ratio)	aPTT (ratio)
Expected final concentration	Clexane®	Inhixa®	Clexane®	Inhixa®
3	2*	2*	3.14	2.95
2	1.83	1.71	2.24	2.21
1.5	1.41	1.34	1.99	1.93
1	0.98	0.94	1.68	1.65
0.5	0.49	0.44	1.36	1.35
0.25	0.24	0.23	1.19	1.17
0.125	0.14	0.12	1.07	1.06

*Upper limit of detection

PB0570 | Discrepant Interpretation of HIT Screening Results on the Same Sample - Data from a UK NEQAS for Blood Coagulation Exercise

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Background: Heparin Induced Thrombocytopenia (HIT) is an immune complication of treatment with heparin, in some cases resulting in life-threatening thrombosis. An accurate diagnosis is required to ensure appropriate clinical decisions are made.

Aims: We describe here a further EQA exercise in which laboratories employing different HIT assays were asked to test and interpret results for a sample from a different donor thought to have HIT based on clinical and laboratory findings.

Methods: Plasma was obtained with informed consent from a patient identified as having HIT antibodies by the Werfen Acustar assay. Plasma was buffered and lyophilised in aliquots and sent to participants in the UK NEQAS for Blood Coagulation (UK NEQAS BC) HIT programme.

Median, coefficient of variation (CV) and range of results were determined for each method and reagent group. Participants were also asked to make an interpretation (positive, equivocal or negative) for the sample.

Results: Results were received from 66 centres. 62% of centres reported a positive screen on the test sample, with 35% reporting a negative screen, and 3% equivocal results. 15/16 centres using ELISA-based assays reported a positive HIT screen, the 12 of which used the Lifecodes/Immucor PF4 IgG kit (median OD 0.72). 13/13 centres using the Werfen Acustar assay reported a positive HIT screen (median 4.09u/ml), compared to just 5/12 using the HemosIL HIT-ab PF4-H kit. 8/14 centres using the Biorad Diamed (ID-PaGIA heparin/PF4 antibody) test and 12/15 centres using the Stago STic Expert kit reported a negative screen. 1 centre reported a positive functional HIPA screen on the sample, and two centres reported heparin neutralisation or inhibition of their assay.

Conclusions: This exercise demonstrated further variability in interpretations for a sample with HIT antibodies, and the need for laboratories and clinicians to understand the clinical implications of reported HIT assay results.

PB0571 | Antifactor Xa Levels vs. Activated Partial Thromboplastin Time for Monitoring Unfractionated Heparin

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Background: The antifactor Xa heparin assay (anti-Xa) and the activated partial thromboplastin time (aPTT) are commonly used to monitor the anticoagulant effects of unfractionated heparin (UFH).

Aims: The purpose of this study is to examine the relationship between the values of anti-Xa and aPTT, simultaneously collected, to monitor UFH infusion.

Methods: This single-center, retrospective and observational *study uses a*nti-Xa and aPTT values from patients in a Brazilian University Hospital gathered from 2018 to 2019. We analyzed 506 paired values from 52 patients. Therapeutic range for anti-Xa was 0.3-0.7 IU/mL and values > 0.7 IU/mL were considered supratherapeutic. The aPTT based heparin therapeutic range was calculated on laboratory using regression analysis: Therapeutic range for aPTT was defined as 60-80 s and values > 80s were considered supratherapeutic. Results with decreased level of antithrombin (AT) III were excluded.

Results: From 506, we evaluated 441 values, where 65 (13%) values met the exclusion criteria were decreased level of ATIII (36 ±8 mg/dL). For the 194 antiXa values in the therapeutic range, aPTT values were in subtherapeutic range (< 60 s) for 67 samples (35%) and in supratherapeutic range (>80 s) for 30 samples (15%). Only 97 samples (50%) were in aPTT therapeutic range (60-80 s). For 246 anti-Xa values in subtherapeutic range, 187 (76%) resulted in aPTT values < 60 s, for 41 (17%) aPTT values were between 60-80 s and for 18 (7%) aPTT values were >80s. There was one supratherapeutic anti-Xa levels (0.8 IU/mL), in agreement with an aPTT>80s. This patient had no bleeding episodes on the medical records.

Conclusions: Anti-Xa therapeutic range and APTT values are discordant in 97 samples which represent 50% of total samples, presenting to 67 samples aPTT values lower and for 30 samples higher than expected considering the anti-Xa values.

PB0572 | Effects of Rivaroxaban on Thrombophilia Screening Tests - Results from a UK NEQAS (Blood Coagulation) Exercise

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Background: Current recommendations suggest investigation of heritable thrombophilia should not be carried out on patients receiving anticoagulation. However, information about the effects of direct oral anticoagulants (DOACs) on thrombophilia screening tests is useful, as clinical information is not always available when samples are received in the laboratory.

Aims: To investigate effects of rivaroxaban on a wide range of methods used in thrombophilia screening.

Methods: A sample from a patient with protein S deficiency and taking rivaroxaban (level 93ng/ml) was sent to UK NEQAS laboratories for thrombophilia testing, without relevant clinical information. Participants were asked to perform (Protein C (PC), Protein S (PS), Antithrombin (AT) and activated protein C resistance (APCR)) on the sample and interpret their results.

Results: 347 centres returned results. Medians were calculated for all methods employed for each test. Clot-based PC activity assay results were significantly higher than chromogenic-based assay results

TABLE 1 Number of samples of Antifactor Xa and activated partial thromboplastin time on the therapeutic range

Total (n=441)	Terapeuthic range (Total: 194), n (%)	Subtherapeutic range (Total=246), n (%)	Supratherapeutc range (Total=1), n (%)
aPTT <60s	67 (35%)	187 (76%)	-
aPTT : 60 to 80s	97 (50%)	41 (17%)	-
aPTT >80s	30 (15%)	18 (7%)	1
activated partial thromboplastin time (aPTT)			

(122.7u/dl vs 101.7u/dl, p< 0.001). Thrombin-based antithrombin assays gave lower results than Xa-based methods (96.0u/dl vs 107.0u/dl, p< 0.001). PS activity assay results were higher than free PS antigen results (24.4u/dl vs 11u/dl, p< 0.001). These differences were seen across the range of methods employed by participants, confirming the consistent effect of direct Xa inhibitors on these assays. Despite this, all centres performing a full thrombophilia screen obtained the correct interpretation of PS deficiency, although 3 additionally reported APC resistance.

Conclusions: Anticoagulation with DOACs is not reported to affect chromogenic PC assays or free PS assays, so false negative results are unlikely if these tests are employed in a thrombophilia screen, as they are by the large majority of UK NEQAS participants. However, AT assays may be affected by either thrombin- or Xa-based inhibitors dependent on the substrate used, and false positive results may arise in patients with mild AT deficiency - our data show both these methods are widely used.

PB0573 | Analytical Performance Validation of the T-TAS 01 PL Assay

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[1]*Hikari Dx, Inc., San Diego, United States;* [2]*Fujimori Kogyo Co., Ltd, Tokyo, Japan*

Background: The T-TAS01 PL assay is a novel in vitro diagnostic system that passes whole blood through a collagen-coated microcapillary bed at arterial shear stress to measure the platelet thrombus formation process. The test is used to assess overall primary hemostatic function. Results are reported as the area under the pressure-time curve (AUC).

Aims: To validate the analytical performance of the T-TAS01 PL assay by evaluating precision, reproducibility, limit of quantification (LoQ), and analytical specificity.

Methods: Blood samples were collected from apparently healthy volunteers with and without taking antiplatelet therapy to produce a wide range of results, and measured with the T-TAS01 PL assay. Reproducibility testing was performed at three locations; all other testing was performed at one location.

Results: Precision and reproducibility testing demonstrated that the repeatability and reproducibility has less than 15% CV or less than 39 SD across the analytical measurement range, which was determined to be 10.4-467.7 AUC. The primary sources of error from highest to lowest were within-run > between-lot > between-operator > between-instrument. The PL assay is not significantly influenced by common potentially interfering substances and is not affected by dabigatran or rivaroxaban, which specifically cause secondary hemostasis defects. A dose-response effect was observed for cilostazol, tirofiban, ibuprofen, and dipyridamole, which are known to affect primary hemostatic function. Taken together, the results indicate that the test is highly specific for primary hemostatic function and is not significantly influenced by secondary hemostatic function.

Conclusions: The T-TAS01 PL assay is highly specific for the measurement of primary hemostatic function, with acceptable precision and reproducibility and a sufficiently wide analytical measurement range. T-TAS01 PL assay measurements may be useful for the assessment of overall primary hemostatic function in patients with bleeding tendencies, defects in primary hemostatic function, or prior to clinical procedures involving bleeding risk.

PB0574 | Clinical Validation of the T-TAS 01 PL Assay in Patients with von Willebrand Disease and Glanzmann's Thrombasthenia

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Background: The T-TAS01 PL assay is a novel in vitro diagnostic system that passes whole blood through a collagen-coated microcapillary bed at arterial shear stress to measure the platelet thrombus formation process. The test is used to assess overall primary hemostatic function. Results are reported as the area under the pressure-time curve (AUC).

Aims: To validate the diagnostic performance of the T-TAS01 PL assay using a pre-specified AUC< 260 cutoff in subjects with confirmed normal primary hemostatic function and in subjects with von Willebrand disease (vWD) and Glanzmann's thrombasthenia (GT).

Methods: Healthy subjects and subjects with confirmed vWD or GT were enrolled according to an IRB-approved protocol at five investigational sites. Blood samples were collected into vacuum tubes containing benzylsulfonyl-D-Arg-Pro-4-amidinobenzylamide (BAPA), a thrombin and factor Xa inhibitor. Healthy subjects were screened for possible primary hemostasis defects and confirmed to have normal primary hemostatic function.

Results: 170 subjects were enrolled. The T-TAS01 PL AUC< 260 cutoff was 96% specific, and 72% and 100% sensitive for abnormal primary hemostatic function in vWD and GT, respectively. AUC results were highly correlated with vWF antigen, vWF activity, and factor VIII activity levels (Spearman's rho = 0.55, 0.83, and 0.71, respectively, all p < 0.01). vWD patients with AUC>260 had either normal PFA-100 results, or vWF antigen, vWF activity, and FVIII:C results that were above levels typically associated with vWD, suggesting that these subjects had mild vWD and did not have significantly impaired primary hemostatic function.

Conclusions: The T-TAS01 PL assay is highly sensitive and specific for impaired primary hemostatic function, and AUC results decrease in proportion to the severity of impairment. T-TAS01 PL assay

TABLE 1 Summary Data

Group	N	Mean Age	% Female	Mean AUC	% < 260 AUC
Healthy Controls	142	38	67.6%	381.5	4.2%
vWD Type 1	12	42	83.3%	247.9	50.0%
vWD Type 2	10	49	80.0%	65.5	90.0%
vWD Type 3	3	59	33.3%	28.1	100.0%
GT	3	25	100.0%	7.1	100.0%

measurements may be useful for the assessment of overall primary hemostatic function in patients with bleeding tendencies or defects in primary hemostatic function.

PB0575 | Clinical Validation of the T-TAS 01 PL Assay in Patients Taking Antiplatelet Therapy

J. Dahlen[1]; P. Gurbel[2]; A.H.B. Wu[3]; K. Bliden[2]; C.-M. Ong[3]; U. Tantry[2]; K. Hosokawa[4]

[1]Hikari Dx, Inc., San Diego, United States; [2]Sinai Hospital, Baltimore, United States; [3]University of California, San Francisco, United States; [4]Fujimori Kogyo Co., Ltd, Tokyo, Japan

Background: The T-TAS01 PL assay is a novel in vitro diagnostic system that passes whole blood through a collagen-coated microcapillary bed at arterial shear stress to measure the platelet thrombus formation process. The test is used to assess overall primary hemostatic function. Results are reported as the area under the pressure-time curve (AUC).

Aims: To validate the diagnostic performance of the T-TAS01 PL assay using a pre-specified AUC< 260 cutoff in subjects taking antiplatelet therapy.

Methods: Healthy subjects and subjects confirmed to be taking 81-325 mg daily aspirin monotherapy (ASA) or dual antiplatelet therapy (DAPT) were enrolled according to an IRB-approved protocol at three investigational sites. Blood samples were collected into vacuum tubes containing benzylsulfonyl-D-Arg-Pro-4-amidinobenzylamide (BAPA), a thrombin and factor Xa inhibitor. Healthy subjects were screened for possible primary hemostasis defects and confirmed to have normal primary hemostatic function.

Results: 259 subjects were enrolled. The T-TAS01 PL AUC< 260 cutoff was 96% specific, and 71% and 100% sensitive for abnormal primary hemostatic function in ASA and DAPT patients, respectively. The AUC results correlated with the potency of antiplatelet therapy, as well as vWF antigen and activity levels. The AUC>260 results in aspirin patients reflect the established variability in response to aspirin and existence of poor response or "aspirin resistance". Subjects taking ticagrelor DAPT had lower AUC results compared to subjects taking clopidogrel DAPT (mean AUC 46.2 vs 20.7) and had significantly less variability in AUC results ($p < 0.01$).

Conclusions: The T-TAS01 PL assay is highly sensitive and specific for impaired primary hemostatic function caused by antiplatelet therapy, and AUC results decrease in proportion to the potency of antiplatelet therapy. T-TAS01 PL assay measurements may be useful for the assessment of overall primary hemostatic function in patients taking antiplatelet therapy either while on therapy or following cessation prior to procedures involving bleeding risk.

PB0576 | Comparison of the T-TAS 01 PL Assay with PFA-100 for Assessment of Primary Hemostatic Function

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Background: The T-TAS01 PL assay is a novel in vitro diagnostic system that passes whole blood through a collagen-coated microcapillary bed at arterial shear stress to measure the platelet thrombus formation process. The test is used to assess overall primary hemostatic function. Results are reported as the area under the pressure-time curve (AUC). The PFA-100 assay uses collagen-epinephrine (CEPI) and collagen-ADP (CADP) coated apertures for the detection of platelet dysfunction. CADP is interpreted with CEPI is abnormal.

Aims: To compare the diagnostic performance of the T-TAS01 PL assay to PFA-100 in subjects with normal primary hemostatic

TABLE 1 Summary Data

Group	N	Mean Age	% Female	Mean AUC	% < 260 AUC
Healthy Controls	142	38	67.6%	381.5	4.2%
ASA	82	67	34.1%	221.0	70.7%
DAPT	35	63	25.7%	33.8	100.0%

TABLE 1 Summary Data

				T-TAS 01 PL Assay		PFA-100 CEPI		
Group	N	Mean Age	% Female	Mean AUC	Sensitivity/ Specificity	Mean CT (s)	Sensitivity/ Specificity	Agreement
Healthy Controls	142	38	67.6%	381.5	95.8% (specificity)	127	89.4% (specificity)	88.0% (125/142)
ASA	82	67	34.1%	221.0	70.7% (sensitivity)	196	51.2% (sensitivity)	63.4% (52/82)
vWD	25	46	78.0%	149.3	72.0% (sensitivity)	261	80.0% (sensitivity)	92% (23/25)
GT	3	25	100.0%	7.1	100.0% (sensitivity)	300	100.0% (sensitivity)	100.0% (3/3)

function, subjects taking aspirin (ASA), subjects with von Willebrand disease (vWD), and subjects with Glanzmann's thrombasthenia (GT).

Methods: Healthy subjects, subjects taking ASA, and subjects with confirmed vWD or GT were enrolled according to an IRB-approved protocol at five investigational sites. Blood sample collection and testing were performed according to the manufacturer's instructions. Healthy subjects were screened for possible primary hemostasis defects and confirmed to have normal primary hemostatic function.

Results: 252 subjects were enrolled. The T-TAS01 PL assay had significantly higher specificity (p=0.029) than PFA-100 for identifying conditions associated with impaired primary hemostasis.

The T-TAS01 PL assay had significantly higher sensitivity for aspirin-induced platelet dysfunction (p=0.004) and comparable sensitivity for vWD and GT (p>0.05). The overall percent agreement between the T-TAS01 PL and PFA-100 assays was 80.5% (203/252), primarily influenced by the 30 discordant results due to the higher sensitivity of the PL assay in ASA subjects.

Conclusions: The T-TAS01 PL assay is highly sensitive and specific for impaired primary hemostatic function, with equivalent or better performance compared to PFA-100 for assessing primary hemostatic function. T-TAS01 PL measurements may be useful for the assessment of overall primary hemostatic function in patients with bleeding tendencies or defects in primary hemostatic function, or prior to procedures involving bleeding risk.

PB0577 | Evaluation of a New Benzylsulfonyl-D-Arg-Pro-4-Amidinobenzylamide (BAPA) Blood Collection Tube for Platelet Function Studies

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Background: Benzylsulfonyl-D-Arg-Pro-4-amidinobenzylamide (BAPA) is a potent inhibitor of thrombin and factor Xa. BAPA has previously been described as an alternative to citrate or hirudin anticoagulants for the collection of blood samples to be used for platelet function studies.

Aims: Using the BAPA tube for T-TAS01, to 1) compare the performance of BAPA anticoagulated blood samples to citrate and hirudin anticoagulated blood samples using light transmittance aggregometry and T-TAS PL measurements, respectively, and 2) to determine the stability of BAPA anticoagulated whole blood samples.

Methods: Blood samples were collected from healthy volunteers with or without aspirin into vacuum tubes containing the anticoagulant to be evaluated. With the exception of the sample stability determination, all measurements were performed within 3 hours after sample collection using the manufacturer's recommended procedures. LTA measurements were performed using 5 μM ADP, 2 mM arachidonic acid, 2 μg/mL collagen and 4 μg/mL collagen.

Results: Results from the comparison between BAPA and citrate using N = 22 matched samples yielded average percent differences of 3%, 4%, 1%, and 3% for ADP, arachidonic acid, 2 μg/mL collagen and 4 μg/mL collagen, respectively. Both tubes also correctly identified two donors that were taking medications that affect AA and collagen-induced aggregation. Using the T-TAS PL assay, results from N = 9 matched BAPA and hirudin samples were highly correlated, with r^2 = 0.95, and a Deming regression slope = 1.00 and intercept = −19.3 (all not significantly different from y = x). Using T-TAS PL assay measurements of N = 4 samples, BAPA blood samples exhibited no significant decay within 8 hours after sample collection.

Conclusions: The highly comparable performance and superior sample stability suggest that BAPA is a suitable alternative to citrate and hirudin for anticoagulation of blood samples to be used for platelet function studies.

PB0578 | Correlation between Thrombin Generation and Prothrombin Fragment F1+2 Plasma Levels

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Background: The thrombin generation assay (TGA) is a global coagulation procedure evaluating the balance between thrombin generation and decay. It has been developed to overcome the poor potential of the basic coagulation tests to represent the coagulation balance as it occurs in-vivo. However, TGA itself is an ex-vivo procedure, estimating the potential of the test plasma to produce thrombin, and not representing the real amount of thrombin in the test plasma. Markers of coagulation activation in-vivo do exist and one of these is the prothrombin fragment F1+2, which is a degradation peptide produced when prothrombin is converted to thrombin.

Aims: The study aimed to correlate TGA parameters versus F1+2 plasma levels in a population of healthy volunteers and patients on vitamin K antagonists (VKA), known to be at normal or reduced levels of TGA/F1+2, respectively.

Methods: We used a homemade TGA according to Hemker et al. and performed the test in platelet-poor plasma with or without triggers [tissue factor (1pM) and synthetic phospholipids (1μM)], without or with thrombomodulin (2nM). F1+2 was measured with an immunoenzymatic assay (Siemens).

Results: Fifty-seven healthy subjects and 28 patients on VKAs were enrolled. As expected, F1+2 and TGA parameters were significantly different ($p < 0.001$) between patients on VKA and healthy subjects. The comparison of results showed a significant linear correlation (Spearman's Rho=0.5, $p < 0.01$) between F1+2 levels and four of the TGA parameters [LagTime, Peak, Time to Peak and Endogenous Thrombin Potential (ETP)], when analyzed with triggers with or without thrombomodulin.

Conclusions: The study showed a linear correlation between F1+2 plasma levels and TGA parameters, indicating that subjects with high potential to generate thrombin in-vitro, have also an ongoing thrombin generation in-vivo.

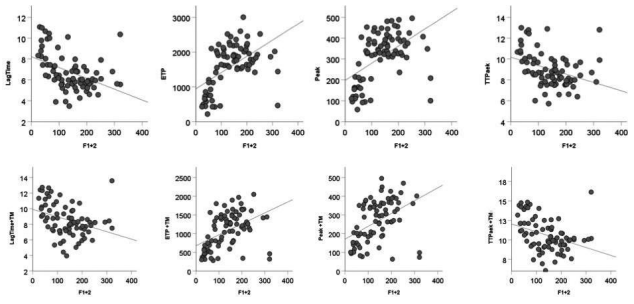

FIGURE 1 Correlation between F1+2 plasma levels and TGA parameters without (upper panel) and with (bottom panel) thrombomodulin

PB0579 | Global Thromboelastometry in Patients with Acute Bleedings on Direct Oral Anticoagulant (DOA) Treatment: The RO-DOA Bleed Study

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Background: Point-of-care tests to measure the anticoagulant effect of direct oral anticoagulants (DOAs) might be useful in emergency situations such as bleedings (when quick assessment and treatment are required).

Aims: To assess the effects of DOAs on global thromboelastometry (ROTEM) in patients hospitalized for bleeding.

Methods: Coagulation parameters were assessed at baseline, at 24 and at 72 hours in patients receiving apixaban, dabigatran or rivaroxaban, and hospitalized for an acute bleeding, and compared to those of healthy volunteers (controls). Citrated blood samples were tested by ROTEM using diluted EXTEM assay, with and without the addition of an anti-FXa catcher, and using ECATEM-B, with and without the addition of an anti-FIIa catcher.

Results: Overall, 10 patients hospitalized for major (8) or clinically-relevant-non-major (2) bleeding and 15 controls were included. Mean age of patients was 83±5 years (range 76-93), males were 70%. Three patients had intracranial hemorrhage (ICH) (post-traumatic in one), 5 had major gastrointestinal (GI) bleeding, 1 had clinically-relevant-non-major GI and 1 had muscular bleeding. DOAs were withdrawn in all patients, red blood cell transfusion administered in 5 and reversal agents used in those with ICH.

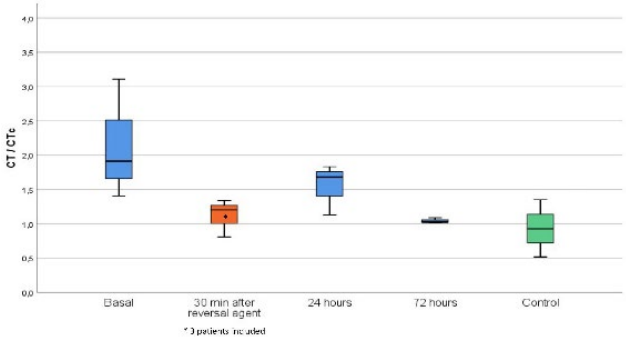

FIGURE 1 Clotting time / clotting time+catcher in patients according to assessment time and in controls

Mean clotting time (CT), CT/CT+catcher (CTc) and clot formation time CFT/CFT+catcher (CFTc) values of patients at admission were significantly higher than those of controls (p=0.01, p< 0.001 and p=0.04, respectively), patients at 72 hours (p=0.02, p=0.03 and p=0.01, respectively) and patients after reversal (p=0.01, p=0.02 and p=0.02, respectively).

Compared to controls, the CT was still significantly longer at 72 hours (p=0.04), while no significant differences were found for CT/CTc and CFT/CFTc at 74 hours and for any of the three parameters after reversal.

Conclusions: The use of ad-hoc designed reagents and catcher molecules at ROTEM was able to identify DOAs activity and the effect of reversal agents in patients with acute bleeding.

PB0580 | Laboratory Assessment of Efficacy and Safety of Direct Oral Anticoagulants in Real Clinical Practice

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Background: Traditional coagulation tests are not sensitive for DOAC, and unacceptable to assess the degree of induced hypocoagulation. It is estimated that using of a chromogenic analysis anti-Xa-activity can evaluate the concentration of rivaroxaban/apixaban in blood plasma.

Aims: To assess laboratory efficacy and safety of therapy rivaroxaban/apixaban.

Methods: The subjects were 50 patients (18 men, 32 women), aged 26 to 86 years (Me = 49), of whom 27 received rivaroxaban, 23 - apixaban. It was collected the following information: gender, age, serum creatinine, DOAC type, dose, indication, time of last dose, thrombin generation assay (TGA). The concentration of rivaroxaban/apixaban were measured using anti-factor-Xa chromogenic assay. Statistics were performed using R.

Results: Indications for DOAC were DVT (42%), PE (28%), atrial fibrillation (28%), splenic artery thromboembolism - 2%. None of the patients had hemorrhagic and/or thrombotic complications during therapy. The range of DOAC concentrations ranged from 0 to 432.7 ng/ml (Me=105.24). Zero concentration of the drug was detected in 10%, which indicates a complete absence of adherence. Statistically significant differences in concentration were obtained between men and women (W = 340, p-value = 0.009707) and between working and non-working patients (W = 405, p-value = 0.03841). The median glomerular filtration rate (GFR) was 68.5 ml/min (Q1 = 59.25; Q2 = 89). The inverse relationship of medium strength between GFR and drug concentration was found (r = −0.36, p-value = 0.03544). The inverse relationship of medium strength between thrombin peak and drug concentration was found (r = −0.45, p-value = 0.0009).

Conclusions: 10% of patients showed a lack of laboratory adherence to therapy. A relationship was found between the concentration of DOAC and the sex and employment of patients. There are the relationship between the peak of thrombin/GFR and the concentration of DOAC.

PB0581 | Effect of Vitamin K Antagonist Therapy on the Dynamics of Hypercoagulation Syndrome in Patients with Deep Vein Thrombosis

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Background: To date, anticoagulant therapy with vitamin K antagonists requires the determination of known standard indicators of the hemostasis system, which is often insufficient, due to the development of bleeding, retrombosis, even in the presence of laboratory monitoring.

Aims: of the study is to analyze the effect of warfarin therapy on the dynamics of hypercoagulation syndrome in patients with DVT.

Methods: A retrospective study was carried out on the basis of the anticoagulant clinic. Methodologically the study included 3 points: 1- 1st day of heparin therapy and warfarin; 2-7 day of warfarin therapy; 3 - the 15th day of warfarin therapy. At 68 patients with deep vein thrombosis were examined in the AC for changes and comparability of parameters global and standard tests of the hemostasis system with the assessment of dynamics by ultrasound dopplerography.

Results: On the 1st and 7th day of warfarin administration, the formation of spontaneous plasma clots without an activator was observed in 11 and 5 patients respectively at the 1st minute. The initial growth rate of the clot on the 1st day of the study, which characterizes the coagulation initiation phase, was 31.0 [17.0-43.0] mcm/min (reference value 38.0-56.0 mcm/min), which reflected the hypocoagulation during treatment with heparins. The standard levels of indicators of hemostasis of the AVK to 15 days of the effectiveness of therapy was achieved in terms of international normalized ratio and D-dimer, data was recorded on doppler ultrasound in the form of recanalization of blood flow by 15 days.

Conclusions: The expediency of local and global methods of hemostasis system at the stage of initiation of warfarin dose together with ultrasound control of blood flow in patients with deep vein thrombosis is shown.

PB0582 | An International Study on the Interpretation of Test Results of the APTT Mixing Test

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Background: The APTT mixing test is a useful tool to gain insight in the potential cause of a prolonged APTT. However, it has been demonstrated that, using case scenarios, it can be problematic to correctly interpret APTT mixing test results (Ajzner *et al*, Clin Chem Lab Med, 2015: 1593-603). Therefore a follow-up study was conducted to investigate the interpretation of APTT mixing test results on the basis of real test results.
Aims: A follow-up study was conducted to investigate the interpretation of APTT mixing test results on the basis of real test results.
Methods: Two lyophilized citrated plasma samples together with case histories were distributed to 340 European laboratories. Laboratories were asked to perform an APTT and mixing test according to their own in-house procedures. The participants were also asked to interpret the obtained test results in the perspective of the provided case history and classify the sample according to the following categories: normal, coagulation factor deficiency, presence of an inhibitor, presence of anticoagulant or unknown. Multiple selections were allowed.

TABLE 1 The results of the classification of case 1 and 2

Classification	Case 1 (heperin contamination)	Case 2 (factor VIII deficiency)
Normal	0.0%	1.%
Coagulation Factor Deficiency	4.5%	85.5%
Inhibitor (specific or non-specific)	35.1%	3.4%
Presence of anticoagulant	14.9%	0.0%
Normal / Coagulation Factor Deficiency	0.0%	0.4%
Factor Deficiency / Inhibitor	4.1%	1.9%
Factor Deficiency / Anticoagulant	3.0%	3.8%
Inhibitor / Anticoagulant	33.2%	0.0%
Factor Deficiency / Inhibitor / Anticoagulant	3.4%	2.3%

Results: Results of 270 laboratories were evaluated. For case 1 (sample contaminated with heparin), 100% found a prolonged APTT and about 90% a prolonged initial and incubated mixing test result. For case 2 (factor VIII deficiency) almost all participants reported a prolonged APTT result and 78% found a correction to normal in the mixing test. The results of the classification of both samples is given in the table below.
Conclusions: This study demonstrates again that for particular scenarios the interpretation of APTT mixing test results can be problematic.

PB0583 | Challenges in Anticoagulation Management at an Academic Medical Center, 20 Years of Clinical Observations

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Background: Best practices for monitoring of both old and newer anticoagulants rely on a combination of published research, pharmaceutical companies' drug approval applications and package inserts, expert opinion, and clinical experience. Clinical laboratories now face a constantly changing environment that challenges the ability of centers to provide timely, accurate, and appropriate guidance for clinical care.
Aims: To summarize major challenges in anticoagulation management at an academic medical center during a two decade period.
Methods: Review of changes in laboratory testing, including in reagents, in the context of anticoagulation management, through examination of the changes in test procedures and recommendations for dose adjustment during the study period. Comparison of these procedures and recommendations with conclusions of published research, pharmaceutical company recommendations, and the guidance of expert organizations.
Results: Major challenges in laboratory management of anticoagulation experienced at an academic medical center have included making recommendations on
(I) monitoring of intravenous therapeutic heparin anticoagulation, including challenges related to:
(a) changes in activated partial thromboplastins,
(b) changes in the ability to offer aPTT and anti-Factor Xa activity assays,:
(c) ambiguity regarding target values for anti-Factor Xa using reagents with or without exogenous antithrombin, and
(d) limited information on appropriate anticoagulation in pediatrics (including patients receiving extracorporeal membrane oxygenation;
(II) monitoring intravenous antithrombin medications in a manner that would not heighten the likelihood of adverse events;
(III) anticoagulation monitoring in the setting of lupus anticoagulant; and
(IV) monitoring of direct acting oral anticoagulants when such monitoring is not recommended by the pharmaceutical manufacturers.

Conclusions: A multidisciplinary team approach of the coagulation laboratory, clinical hemaotologists (both adult and pediatric), hospital pharmacists, and transfusion medicine specialists has enabled challenges in anticoagulation monitoring to be addressed, even when randomized controlled trials for the described challenges may not have been available to guide such decisions.

PB0584 | Influence of Phospholipids on the Measurement of Extended Half-Life Factor VIII Activity in One-Stage Clotting Assay

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Background: Accurate measurement of factor VIII:C (FVIII) activity is a major concern in patients with hemophilia A. Extended half-life (EHL) FVIII products have been introduced for treatment of these patients, and it is important to understand how EHL-FVIII concentrates behave in the different one-stage coagulation assay (OSA), especially the different contents of phospholipids in APTT reagents.
Aims: To evaluate the influence of phospholipids on EHL-FVIII activity in OSA.
Methods: FVIII deficient plasma was spiked with 0.05–1.0 IU/mL of standard full-length (FL) FVIII: (FL-FVIII) and three EHL-FVIII concentrates (FL-FVIII-PEG, BDD-FVIII-Fc, and BDD single-chain [BDD-FVIII-SC]). APTT reagents were prepared at 50–300 mg/L of total phospholipids with ellagic acid as the activator. In addition, reagents containing variable composition of phospholipids were prepared by mixing of phosphatidylserine (PS; 0–70 mg/L) and phosphatidylcholine (PC; 70–0 mg/L) with constant concentration of phosphatidylethanolamine (PE; 30 mg/L) as PE is crucial for the assay. FVIII activity was calculated by each calibration curve using standard plasma on an automated blood coagulation analyzer.
Results: PS was essential to establish calibration curve for FVIII activity, and slope of the curve at low levels was steepening with increase of PC/PS ratio. Variations in the phospholipid concentration did not affect the activity of FL-FVIII, BDD-FVIII-Fc and BDD-FVIII-SC. In contrast, dilution of the phospholipids had a substantial influence on the activity of FL-FVIII-PEG at high levels where the apparent activity decreased. Composition of phospholipids with variable concentrations of PS and PC did not influence on activities of any FVIII products, when 10 mg/L of PS was present in the reagents.
Conclusions: Our data show that concentration of phospholipids influences on activity of FL-FVIII-PEG, and that compositions of PS and PC do not affect any EHL-FVIII products in OSA.

PB0585 | Application of EP15A3 and EP15A2 Protocols to Verify the Performance of a Coagulometer in the Coagulation Laboratory of the National Institute of Respiratory Diseases in Mexico City

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Background: The culture of quality among clinical laboratories has increased the need to evaluate the performance of the analytical methods used for the tests offered to the patient.
Aims: To verify the performance of a coagulometer under the conditions of the INER clinical laboratory based on the verification protocol EP15-A3 *User Verification of Performance for Precision and Trueness*, proposed by CLSI (*Clinical and Laboratory Standards Institute*).
Methods: The coagulometer with viscosimetric (mechanical) detection principle: accurately evaluates weak instrument clots of the STA® range.
20 samples of blood bank donors are used; Global coagulation tests were determined (PT, PTT TT, Fibrinogen and D-dimer). With the results obtained, precision, veracity, method comparison, verification of reference ranges 6, linearity 7, total error 8 and Sigma metric were calculated. A statistical analysis was performed where the following programs were used: GMigliarino SC version 4.8; EP Evaluator version 8.0.0.172.
Results: It was observed that the coefficients of variation obtained are lower than estimated by the manufacturer. The verification of the instrument is acceptable. Perform a verification of methods in an analyte measurement laboratory, it is very important since the data provided by the manufacturer are compared in their own conditions, allowing the results generated by the instruments to be useful in the diagnosis, prognosis and treatment of a large number of diseases.
Conclusions: The coagulometer at the height of Mexico City (2250 meters above sea level), is able of work above the minimum acceptance specifications established by the manufacturer, complying with the CLSI international verification recommendations.

TABLE 1 Precision results for level 1 and level 2 control. Where: Sr (Deviated standard in repeatability conditions); CVr (Coefficient of variation)

Analyte Level 1 control	PT	PTT	Fibrinogen	D-dimer	Analyte Level 2 control	PT	PTT	Fibrinogen	D-dimer
Sr	0.17	0.22	6.71	0.0502	Sr	0.152	0.259	5.22	0.0554
CVr	1.2	0.6	2.5	4.7	CVr	0.8	0.5	3.3	9.1
CVr-Fab	1.5	1.5	4.0	17.5	CVr-Fab	2.0	2.0	5.0	15.2
UVL CVr Fab	2.0	2	5.2	22.9	UVL CVr Fab	2.6	2.6	6.6	19.9
Interpretation	ACCEPTED	ACCEPTED	ACCEPTED	ACCEPTED	Interpretation	ACCEPTED	ACCEPTED	ACCEPTED	ACCEPTED

TABLE 2 Intermediate precision results, where: Swl (Standard deviation in intermediate precision conditions); CVwl (Coefficient of variation in intermediate p)

Analyte level 1 control	PT	PTT	Fibrinogen	D-dimer	Analyte level 2 control	PT	PTT	Fibrinogen	D-dimer
Swl	0.205	0.782	8.79	0.0502	Swl	0.314	0.767	6.01	0.0581
CVwl	1.4	2.2	3.2	4.7	CVwl	1.7	1.6	3.9	9.5
CVwl-Fab	5.0	5.0	6.0	26.3	CVwl-Fab	5.0	5.0	6.0	30.3
UVL CVwl Fab	8.0	8.0	8.7	38.1	UVL CVwl Fab	8.0	8.0	8.1	47
Interpretation	Accepted	Accepted	Accepted	Accepted	Interpretation	Accepted	Accepted	Accepted	Accepted

PB0586 | Accuracy of Commercial Universal Calibrators for Measurement of Heparin and Low Molecular Weight Heparin

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Background: Plasma levels of heparin (UFH) and low molecular weight heparin (LMWH) can be monitored by anti-factor Xa assays relative to commercial calibrators. Unitage of these calibration plasmas are claimed to be traceable to the International Standard (IS). Universal heparin calibrators (unicalibrators) with same labelled potencies for UFH and LMWH are available from several manufacturers and are recommended for use to measure both UFH and LMWH.

Aims: The aim of this study was to determine the traceability and accuracy of these unicalibrators relative to the IS.

Methods: The individual calibrator plasma in unicalibrator sets from three different manufacturers were tested against the ISs for UFH and LMWH using an anti-Xa parallel line bioassay. The potency for each plasma calibrator was estimated and the recovery against quoted values were calculated.

Results: Each spiked calibrator plasma tested validly against both the UFH and the LMWH IS. In all cases the potency estimates against the UFH IS gave higher values than against the LMWH IS (figure 1). Recovery of spiked heparin in all calibration plasmas, irrespective of manufacturer, gave a higher value when assayed against the UFH IS, ranging from 120% (Manufacturer C, Cal 5) to 170% (Manufacturer B, Cal 2) of the labelled values (figure 2). Recovery against the LMWH was good for both manufacturer A, 99% and 100% and manufacturer C, 90 to 107% but was higher for manufacturer B, 103 to 133%.

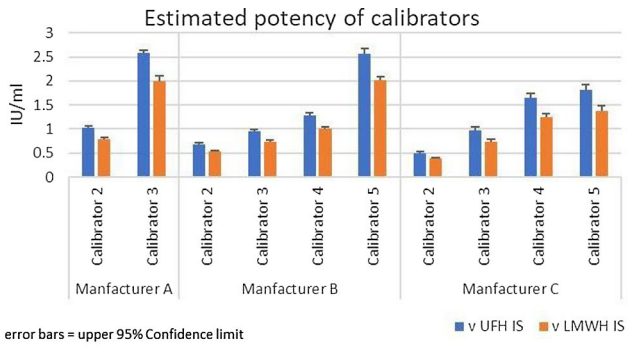

FIGURE 1 Potency by parallel line bioassay against either the IS for UFH or for LMWH of each calibrator in a manufacturers' calibrator set

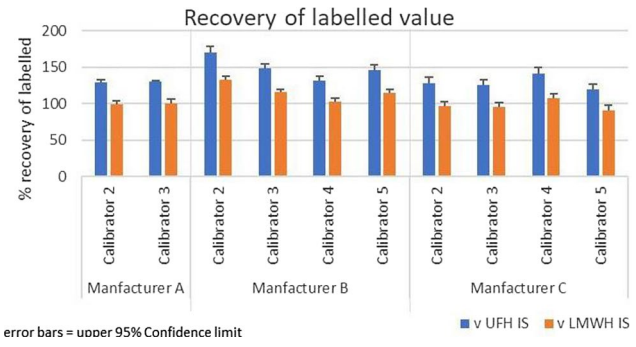

FIGURE 2 Recovery of the labelled value for each calibrator in a manufacturers' calibrator set against either the 6th IS for UFH or 3rd IS for LMWH

Conclusions: The traceability and accuracy of all calibration plasmas tested was found to be poor against the UFH IS, values were at least 20% higher than labelled unitage. Whilst there was good agreement against the LMWH IS for the spiked plasmas from manufacturers A and C, there was poor agreement in values for calibrators from manufacturer B. Current commercial unicalibrators are not suitable for estimation of UFH in patient plasmas.

PB0587 | The Effect of trough Dabigatran Plasma Concentration on Thrombin Generation

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Background: The goal of anticoagulation therapy is to reduce generation of thrombin and to prevent thromboembolic event. Thus, an assay that measures plasma potential to produce thrombin may be used for estimation of anticoagulant effect of dabigatran. Thrombin generation assay (TGA) is a global hemostasis assay. It evaluates generation of thrombin in plasma resulting from the action of procoagulant and anticoagulant factors.
Aims: Our aim was to assess the effect of trough dabigatran ex vivo plasma concentration on thrombin generation.
Methods: In 12 patients taking 150mg bd dabigatran for thromboprophylaxis at steady state, we measured trough dabigatran concentration in plasma. Informed consent was obtained and the medical ethics committee approved the study. Blood was collected into 3.2% buffered Na-citrate tubes, (BD Vacutainer). Plasma was stored at −20°C for maximum three weeks until analysis. Dabigatran levels were measured on Diagnostica Stago analyzer (STA-R Evolution) using Stago STA-ECA II assay. Endogen thrombin potential (ETP AUC), peak thrombin generation (Cmax), time to peak (tmax) and lag time (tlag) were measured on Siemens analyzer (BCSXP) using Siemens Innovance ETP reagent.
Results: Mean dabigatran concentration (ECA) was 65.3 ng/ml ± 37.9 SD. Spearman's coefficient of rank correlation (rho) was not statistically significant between dabigatran concentration and ETP AUC (rho=−0.287, p=0.3416), Cmax (rho=−0.245, p=0.4169) and tmax (rho=0.559, p=0.0635). There is a very strong, positive correlation between dabigatran concentration and tlag (rho=0.867, p=0.0040). Regression equation for ECA and tlag is y=0.1486x + 21.6082.
Conclusions: Estimation of dabigatran concentration in plasma could be made by measuring tlag in TGA. Beyond estimation of dabigatran concentration, TGA as a global hemostasis assay, may have additional clinical value in evaluating thrombotic or bleeding risk in patient receiving dabigatran. Therefore, TGA as well as dabigatran concentration should be evaluated in a large clinical trial to correlate them with clinical event.

PB0588 | Sigma Metrics Scores for Routine Haemostasis Assays in a Small Laboratory

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Background: Six sigma (6σ) concept has been put in statistical quality control (QC) radar to monitor the quality performance of analytical instruments. The 6σ depicts a standard deviation of process that should fit within the tolerance limits of the quality requirement. Generally, assay with high σ score (≥3.0) demonstrates better analytical performance and lower variability.
Aims: We aimed to measure our two-year sigma scores for our haemostasis assays using total allowable error (TEa) based on desirable biological variation (BV) data by the Analytical Quality Commission of the Spanish Society for Clinical Chemistry (SEQC, 2004).
Methods: Sigma scores for two routine analytes; Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) were measured by tabulating our two-year internal QC data at normal and pathological levels for two separate lots. Both assays were performed on STA-Compact Max (Stago, France) using Stago reagents (STA-Neoplastine and STA-PTT Automate).
Equation; σ=(TEa − bias)/CV (CV;Coefficient of variation) was used to measure sigma scores. Statistical indices i.e. CV and bias were obtained from internal QC data and external quality assurance programme that we had participated within the corresponding period.
Results: Based on TEa by desirable BV, we performed poorly for both assays at both levels in both years as our σ scores were between 1.0 to 2.3. However, we noticed that our σ scores were slightly better for normal level compared to pathological level. We also noticed both analytes demonstrating consistent σ scores in both years. In this study, we focused on 6σ determination using desirable BV data and demonstrated that applying 6σ concept on IQC data results in σ value below minimum acceptable value of 3.0.
Conclusions: In our laboratory, unsatisfactory performances (σ≤3.0) were observed in our routine haemostasis assays. We are able to have an overview of our quality performance by utilizing sigma metrics as a standard of excellence.

TABLE 1 Sigma scores for analytes using total allowable error (TEa) based on desirable biological variation (BV) data

Year	Analyte	Quality Control Level	Number of data (n)	Mean	Standard Deviation (SD)	Coefficient of Variation (CV)	Bias (obtained from external quality assurance data)	Total allowable error, desirable biological variation (TEa for BV)	Sigma score
2018	Prothrombin Time (PT)	Normal	240	12.9	0.3	2.2	0.3	5.3	2.3
		Pathological	239	22.9	0.8	3.3	0.3	5.3	1.5
	Activated Partial Thromboplastin Time (APTT)	Normal	241	34.9	0.7	2.0	1.7	4.5	1.4
		Pathological	242	59.5	1.5	2.5	1.7	4.5	1.1
2019	Prothrombin Time (PT)	Normal	182	13.4	0.3	2.3	0.4	5.3	2.1
		Pathological	182	23.7	0.8	3.2	0.4	5.3	1.5
	Activated Partial Thromboplastin Time (APTT)	Normal	181	35.4	0.7	1.9	2.0	4.5	1.3
		Pathological	181	66.1	1.6	2.4	2.0	4.5	1.0

PB0589 | Laboratory Analysis of Half-Life-Prolonged Coagulation Factors VIII Rurioctocog Alfa Pegol and IX, Nonacog Beta Pegol and of rVWF - Vonicog Alpha in Spiked Human Plasma Samples - First Results of a Multicentre Study by the Austrian Laboratory Societies ÖGLMKC and ÖQUASTA

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Background: Introduction of new coagulation factor concentrates may confront laboratories with technical problems in selecting suitable test batteries. The study involves 11 centres and examines the test behaviour of three new factor preparations (two half-life-prolonged factor concentrates rurioctocog alfa pegol (rPEGVIII; Adynovi™), nonacog beta pegol (rPEGFIX; Refixia™) and vonicog alpha (rVWF; Veyvondi™).

Aims: The influence of test methods available in the laboratories for factor recovery, APTT based one stage clotting assay (OSA), or by chromogenic assay (CSA), if available, and for rVWF, VWF:Ag and VWF:Akt assay (if established VWF:RCo and VWF: CB - collagen binding), respectively, should be determined.

Methods: Factor deficient human plasmas were mixed by the manufacturers of the products in different concentrations and sent to the participating laboratories with information on the spiked factor concentrations but without further information.

Results: rPEGFVIII: Using OSA all laboratories achieved comparable results to the target: (max. < 25%). Combinations of the CS-5100 analyzer with Pathromtin SL (Siemens) and ACL Top with SynthAsil (Werfen), respectively, showed the best results. With both CSA tests Biophen FVIIIc, (Hyphen) and FVIIIc chromogenic assay (Siemens)) an overestimation of the recovery (>20%) was seen.

rPEGFIX: Using OSA only with SynthaFAx (Werfen) a recovery with an overestimation of maximum 25% was seen; all other APTTs Actin FS, Pathromtin SL, SynthAsil, C.K.Prest® (Stago) showed significant underestimations as well as massive overestimations of the recovery. CSA by Biophen FIX (Hyphen) showed a good agreement with the spiked concentrations.

rVWF: VWF:Akt tended to be >20% above the spiked concentrations; only HemosIL VWF:Act (Werfen) showed good agreement. Both VWF:Ag and VWF:RCo values (Siemens and Werfen) were within an acceptable range of +/- 20%.

Conclusions: Selecting appropriate test batteries are crucial. Supply of spiked plasma samples by manufactueres facilitates evaluation of the assays. The final study results are expected for mid 2020.

PB0590 | Comparison of Visual versus Reader Results of the Point of Care Test of Direct Oral Anticoagulants from Urine Samples

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Background: Direct oral anticoagulants (DOAC) are increasingly used for anticoagulation. In emergency situations, a quick and safe method to detect or exclude to presence of DOACs may guide clinical decision-making. Because DOACs are excreted via the kidney, they can be detected in the urine with the point of care test DOAC Dipstick. DOAC concentrations are manifold higher in urine compared to plasma, which is important for normal ranges.

Aims: To identify concentrations of DOACs providing 100% negative (DOAC absent) or 100% positive (DOAC present) results for each of the two methods of determination (i.e. naked eye or photometric reader (DOASENSE Reader, CE-labelled).

Methods: Samples of artificial (n=324) urine were spiked with concentrations of DOACs ranging from 0 ng/ml to 325 ng/ml. Three examiners (visual method) or 4 photometric readers (reader method) independently identified in triplicate the colours of the pads of DOAC Dipstick for positive or negative for factor Xa inhibitors (DXI: apixaban, edoxaban, rivaroxaban) and thrombin inhibitor (DTI) dabigatran on the respective test pads of DOAC Dipstick. Results are expressed as negative or positive.

Results: One hundred percent of negative and positive results were found by visual and reader analysis at the levels of DXI and DTI given in the table, respectively. Intermediate concentrations of DOACs may result in negative as well as positive evaluations for DXI and DTI by visual and reader analysis (see table).

Conclusions: Visual and reader analysis are in agreement for urinary concentrations to allow to exclude or to identify the presence of DOACs in urine, respectively. Clinical studies are ongoing for further validation.

PB0591 | Monitorig of Direct Oral Anticoagulants in Routine Practice

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Background: Three direct oral anticoagulants (DOACs) are currently registrated for use in Slovenia: dabigatran, rivaroxaban and apixaban. These DOACs are administrated in fixed doses without routine anticoagulant monitoring. However, there are some clinical situations where laboratory measurement may be needed, such as a serious bleeding and thrombotic events, need for urgent surgery, renal or liver insufficiency.

Aims: The classical coagulation tests such as activated partial thromboplastin time (aPTT) or prothrombin time (PT) cannot be used to quantify activity of the drug precisely. In contrast, specific assays (dTT—diluted thrombin time for dabigatran, chromogenic factor Xa assays for the factor Xa inhibitors, correlate well with plasma concentrations. To ensure safe patient treatment in special clinical situations we decided to introduce special assays for DOACs.

Methods: Specific assay for Rivaroxaban was introduced in march 2013, for Dabigatran in february 2015 and Apixaban in may 2016. Assays were performed on BCS XP coagulation analyser and accessibility was 12 hours per day. We analysed number of specific assays from introduction till the end of 2019 and in which clinical settings testing was orded.

Results: The number of assays for Rivaroxaban rise from 13 in 2013 to 267 in 2019; for Dabigatran from 36 in 2015 to 197 in 2019; for Apixaban from 19 in 2016 to 187 in 2019. Approximately 60% of assays were orded for urgent trauma, neurological, orthopedic, abdominal surgery and medical ill patients.

Conclusions: Our analyses shows the growing demand for specific assays, esspecialy in urgent clinical settings. From april 2018 assays are available 24 hours per day and safer treatment of patients is guaranteed.

TABLE 1 Results of methods for detection of DXI and DTI in urine with DOAC Dipstick

Methods	Visual	Reader	Visual	Reader	Visual	Reader
Results	NEGATIVE		negative or positive		POSITIVE	
DXI (ng/ml)	<100	<100	100–200	100–275	>200	>275
DTI (ng/ml)	<50	<75	50–125	75–300	>125	>300

PB0592 | Performance of Thrombin Generation Test (TGT) versus Conventional Coagulometric Methods in Snakebites: Preliminary Results

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Background: Hemostatic abnormalities usually occur in snakebite envenoming and prescription of antivenom is based on clinical signs and laboratory tests, including PT/INR, aPTT and fibrinogen. However, none of the conventional methods is able to assess early hemostatic changes.

Aims: To evaluate the performance of TGT to investigate early hemostatic changes, compared to conventional coagulometric methods in victims of Bothrops snake envenoming, before and within 24 hours after starting antivenom therapy.

Methods: Blood samples were collected from 16 victims of *Bothrops* jararaca snake *envenomation* at times T0 (on admission to the Hospital) and T≤ 24 (up to 24 hours after starting antivenom therapy). TGT was performed using the CAT method, with high (HTF) and low (LTF) tissue factor concentration. Among the parameters of TGT, endogenous thrombin potential (ETP) was chosen to assess possible hemostatic changes and results were expressed in mean±SD and analysed by t Student test. PT/INR, aPTT and fibrinogen data were obtained from medical records and results were expressed in median and interquartile range and analysed by Mann Withney ($p< 0.05$). The study received approval from the local Ethics Committee.

Results: Significantly higher median values of ETP (Low tissue factor) for T≤ 24 were observed in relation to those observed in TO (Table 1). There were no significant differences for ETP performed with HTF, PT / INR, aPTT and fibrinogen between T0 and T≤ 24 (Table 2).

Conclusions: TGT (ETP) has apparently higher sensitivity than conventional coagulometric techniques to investigate hemostatic changes in envenoming by Bothrops jararaca. If confirmed, TGT can be a useful tool for optimization of antivenom therapy and stratification of bleeding risk.

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TABLE 1 Laboratory findings by TGT in victims of Bothrops jararaca snake envenomation

Variable	T0	T≤ 24	p value*
ETP (nM.min) Low Tissue Factor	1.059 ±656	1.479 ±412	0.0086
ETP (nM.min) High Tissue Factor	1.304 ±699	1.542 ±394	0.3056

TABLE 2 Laboratory findings by conventional coagulometric tests in victims of Bothrops jararaca snake envenomation

Variables	T0	T≤ 24	p value*
INR	1.3 (8.88)	1.3 (0.76)	0.6334
aTTP (s)	34.4 (93.95)	28.2 (7.1)	0.2208
Fibrinogen (mg/dL)	123 (167)	65.6 (87.5)	0.3035

PB0593 | The Basic Evaluation of Light Transmission Platelet Aggregation Method on an Automated Coagulation Analyzer CN-6000

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Background: Light transmission aggregometry (LTA) is known as a gold standard method for assessing platelet function. The fully automated coagulation analyzer CS-5100 is already enabled to measure platelet aggregation using LTA. Recently, a new automated coagulation analyzer called CN-6000 was launched.

Aims: In this report, we have evaluated performance of the CN-6000 of platelet aggregation function with 2.0 μM adenosine diphosphate (ADP), 1.0 mM arachidonic acid, 2.0 μg/mL of collagen, 5.0 μM epinephrine and 1.2 mg/mL ristocetin.

Methods: We evaluated within-run precision, on-board stability and comparative study. Platelet-rich plasma (PRP) samples from healthy volunteers were used as normal samples and 1.0 mM of aspirin-spiked PRP or 0.05 μM of cangrelor (P2Y12 inhibitor)-spiked PRP were used as abnormal samples. Within-run precision was measured using 30 replicates of analysis. On-board stability was evaluated at 0, 4, 8 and 10 hour intervals. Comparative study between CN-6000 and CS-5100 was performed using 130 PRP samples with different levels of aggregation by adding several concentration of aspirin or cangrelor.

Results: For within-run precision evaluation, the coefficient of variation (CV) with all agonists tested with normal and abnormal samples was less than 5% and 13%, respectively. For on-board stability evaluation, the changes between 0 and 10 hours were within 5% CV, with each agonist.

In comparative study, $y = 1.08x–1.34$, $r = 0.978$ for ADP, $y = 1.01x +1.62$, $r = 0.995$ for arachidonic acid, $y = 1.01x + 0.61$, $r = 0.989$ for Collagen, $y = 1.06x–1.61$, $r = 0.980$ for epinephrine, $y = 0.98x +5.28$, $r = 0.989$ for ristocetin.

Conclusions: Our results demonstrated that the CN-6000 is a robust automated coagulation analyzer for platelet aggregation testing. The CN-6000 also provides high analytical performance as a high throughput analyzer for routine and specific coagulation parameters and is suitable for total laboratory automation.

PB0594 | Comparison of the One-Stage Clot-Based and Chromogenic Factor VIII Activity Measurement

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Background: Factor VIII (FVIII) measurement is important in the current era of evolving FVIII gene therapy in which a number of studies have shown discrepancy between the one-stage and chromogenic FVIII assays. There is a paucity of studies comparing the two assays.

Aims: To compare the one-stage clot-based and chromogenic assays in the measurement of FVIII in blood sample from a diverse spectrum of hematological conditions in a single tertiary academic centre.

Methods: This is a single centre retrospective study conducted at Sultan Qaboos University Hospital (SQUH). FVIII measurement at the SQUH requested from March 2009 to March 2018 was performed with both assays. Paired FVIII results were compared using Bland-Altman analyses and mostly of small sample size.

Results: A total of 2113 samples had both assays performed. The median age of all patients was 26 years (IQR: 17–37). The commonest indications of testing were recurrent pregnancy loss (7.8%), sickle cell disease (4.7%), hemophilia A (2.8%). The remaining (84.7%) included venous thromboembolism, von Willebrand disease, thalassemia, myeloproliferative neoplasms and others. The median FVIII level by one-stage clot-based and chromogenic assays were 1.17 IU/mL (IQR: 0.8–1.6) and 1.2 IU/mL (IQR: 0.9–1.6) respectively. Bland-Altman analyses showed excellent agreement with a mean difference of 0.02 IU/mL. The upper and lower agreement boundaries were −0.53 IU/mL and 0.49 IU/mL respectively. Visually, the points of difference were closer to the mean difference when the mean FVIII level was low. High baseline FVIII level and the diagnosis predicted the disagreement between the two assays ($P < 0.001$). Positive ANA and FVIII inhibitor had no impact on the disagreement (*P of* 0.2 and 0.7 respectively).

Conclusions: In this cohort of diverse hematological conditions, one-stage clot-based and chromogenic FVIII assays showed excellent agreement for measuring FVIII activity level especially in patients with low baseline FVIII level.

PB0595 | A Strategy for the Prenatal Diagnosis for Fetus with Hemophilia A without Genetic Defects

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Background: No genetic defects are found in the coagulation factor VIII gene of approximately 2% of patients with hemophilia A by routine DNA analysis. It confers a big challenge in genetic counseling and prenatal diagnosis for fetus, whose mother gave birth to children with hemophilia A or might be carrier of hemophilia A based on linkage analysis with genetic markers.

Aims: We aim to make a better strategy for the prenatal diagnosis for fetuses, whose mothers were carriers without confirmed genetic defects.

Methods: From 2010 to 2019, 57 cases of hemophilia A carriers, without confirmed genetic defects by routine DNA analysis, were performed prenatal diagnosis for fetuses. All fetuses were detected by linkage analysis utilizing five microsatellite markers. Additionally, FVIII:C level in cord blood of three male fetuses diagnosed to be genetical hemophilia A patients were evaluated.

Results: 18 fetuses were diagnosed as genetical hemophilia A patients, and 8 fetuses were carriers of hemophilia A. 18 male fetuses and 14 female fetuses were genetical normal. In the FVIII clotting activity assay, the FVIII:C level was less than 1% in two of the there male fetuses. The FVIII:C level was 55% in the other one male fetus and for one year's follow up he was doing normal. In one year's follow up, the coagulation was normal in all of the 18 male fetuses diagnosed as genetical normal.

Conclusions: The combination of linkage analysis and evaluation of FVIII:C level in fetuses may be an efficient way to prevent the birth of children with hemophilia A, and makes an alternative strategy for those pregnant carriers with the urgent requirement of prenatal diagnosis for fetus but with no genetic defect found.

PB0596 | The Effects of EDTA as an Anticoagulant and Delayed Processing on the Measurement of Plasma VWF Antigen

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Background: Von Willebrand factor antigen (VWF:Ag) is typically measured in blood collected into citrate tubes. Guidelines recommend processing samples within 4 hours. Reference ranges are based on citrate VWF:Ag. However, plasma samples deposited in biobanks are often derived from blood collected into a limited range of anticoagulants (e.g. EDTA) and are sent to a central location, resulting in a time delay in sample processing.

Aims:

1) Determine the association between VWF:Ag measured in EDTA and citrate plasma;: 2) Assess whether VWF:Ag measurements made in EDTA plasma vary over time.

Methods: All VWF:Ag measurements were made in platelet-depleted plasma using published methods (Heubel-Moenen et al., 2017). Firstly, blood was simultaneously taken into EDTA and citrate tubes from 99 healthy volunteers and processed within 2 hours. Secondly, from 3 volunteers, serial VWF:Ag measurements were made on EDTA samples stored at room temperature for 72 hours post venesection. Correlation between variables was calculated using Pearson's method. Written consent was provided by all volunteers and the study was approved by the Maastricht University Medical Center+ local ethics committee.

Results: VWF:Ag measurements in citrate plasma varied from 63 to 233 IU/dL (Figure 1) and were positively correlated with corresponding measurements made in EDTA ($R = 1$, $P < 2.2e^{-16}$). Measurements in EDTA plasma were higher than corresponding measurements made in citrate. There was a positive correlation ($R = 0.79$, $P = 1.739e^{-08}$) between baseline-adjusted VWF:Ag measurements and time since venesection (Figure 2). The regression line (with the 95% confidence interval) and equation is shown on each figure.

Conclusions: We demonstrate a method for estimating the citrate VWF:Ag from a measurement made in EDTA, and the baseline VWF:Ag from a sample where there has been a delay in processing. To further the scope of our analysis, we are now analysing individuals with von Willebrand Disease using the same protocol.

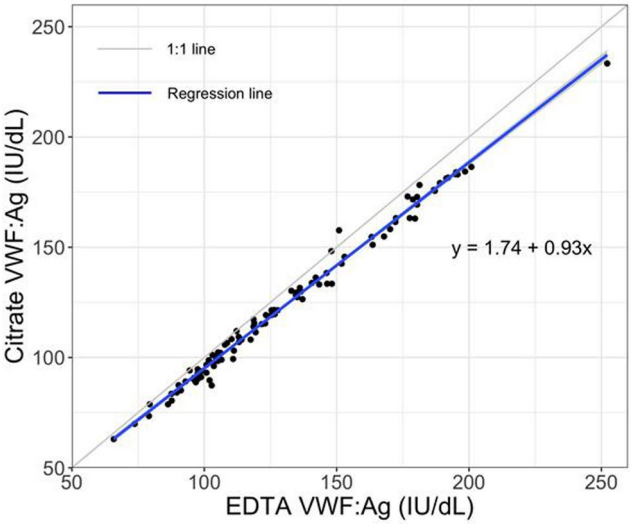

FIGURE 1

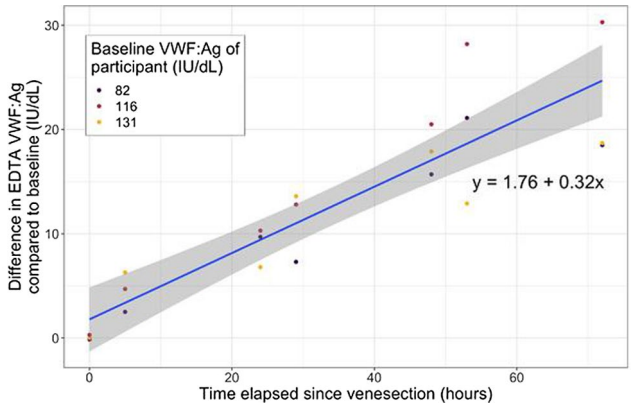

FIGURE 2

PB0597 | Evaluation of EQA Material on Lumira Dx Point of Care Platform: UK NEQAS for Blood Coagulation Study

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Background: The Lumira Dx is a point of care platform which uses cartridge based technology for INR testing in patients on VKA therapy.

Aims: To determine the suitability of UK National External Quality Assessment Scheme for Blood Coagulation (NEQAS BC) lyophilised plasma samples for use with Lumira Dx and to explore the possibility of establishing an external quality assessment (EQA) programme for this platform.

TABLE 1 Results of testing previously used samples in UK NEQAS POC INR programmes on Lumira Dx

Sample	POC EQA programme median INRs	Platform 1	Platform 2	Platform 3
XS19:04	1.6 (n = 3703)	1.8	2	1.9
XS19:05	1.9 (n =3754)	2.1	2.1	2.1
XS19:03	2.1 (n = 3698)	2.3	2.6	2.4
XS18:07	2.7 (n= 3899)	2.9	3.1	2.9
XS19:06	3 (n = 3635)	3.4	3.5	3.1
XS18:01	5.7 (n = 3762)	6.4	6.4	5.6

Methods: UK NEQAS BC conducted a study to determine suitability of NEQAS BC lyophilised plasma samples for Lumira Dx. After a series of testing using samples previously used in POC INR EQA programme a suitable diluent with an optimum time for sample reconstitution, calcium chloride concentration and volume was established. Precision was evaluated via repeated testing of an EQA sample previously used in POC INR programmes on three Lumira Dx platforms. This was followed by testing samples with increasing INR values previously used in POC INR programmes. Obtained results were compared to the POC INR sample specific medians established previously.

Results: A reasonable precision with CV% range 2.7–5.4 was observed across three platforms after repeated testing of a sample previously used in POC INR EQA programme (mean INR 3.0). The results of testing samples with increasing INR values previously used in POC INR EQA programmes are shown in the table. Obtained results on Lumira Dx were comparable to the sample specific medians established previously in POC INR programmes across three platforms.

Conclusions: In summary, Lumira Dx has demonstrated good precision (all < 10%) while testing NEQAS BC POC INR samples across three platforms. The study has demonstrated the suitability of NEQAS BC lyophilised plasma samples for testing on Lumira Dx. NEQAS BC can provide an EQA POC INR programme for this platform.

PB0598 | The Novel Prothrombin Time (MRX PT DOAC) Assay - Can Be Used to Detect Interfering DOACs in Thrombophilia Investigation

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Background: Direct oral anticoagulants (DOACs) have been shown to interfere with many coagulation assays. For thrombophilia investigations this might lead to falsely positive Lupus Anticoagulant (LA) results. A simple and rapid assay to rule out interfering DOACs is needed.

The MRX PT DOAC test is based on two simultaneously run prothrombin time (PT) assays, one DOAC-insensitive PT assay and one DOAC-sensitive PT assay. The INR (International Normalized Ratio) determined by the DOAC-insensitive PT assay and the DNR (DOAC Normalized Ratio) determined by the DOAC-sensitive assay will be the same for samples without DOACs, while for DOAC-containing samples there will be a difference. This difference is expressed as a clot time ratio (CTR). DOAC is present in samples with CTR greater than the reference interval.

Aims: To use MRX PT DOAC to detect interfering DOACs in samples during thrombophilia investigation.

Methods: Material leftover from 154 citrated blood samples that had undergone analysis for investigation of thrombophilia were analysed with MRX PT DOAC. LA results were obtained from the laboratory information system. MRX PT DOAC CTR reference interval was set using a reference material of 120 healthy volunteers. The study was performed according to Swedish law on biobanking and research ethics.

Results: LA was detected in 9 out of 154 patient samples. One sample was falsely LA positive, and a >CTR in the MRX PT DOAC test revealed the presence of DOAC in this sample, which was confirmed with an independent method. Seven samples were LA positive with < CTRs, *i.e.* MRX PT DOAC correctly cleared these samples from DOAC interference. One sample was LA positive (diluted Russel Viper Venom Time ratio, dRVVT ratio>3) with >CTR; MRX PT DOAC indicated DOAC in this sample.

Conclusions: MRX PT DOAC can detect the presence of interfering DOACs in patient samples during thrombophilia investigation.

PB0599 | Assessment of ExpertCor, a New Set of Frozen Plasmas for Assay Comparability Studies

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Background: Increasing accreditation requirements challenge the laboratory's time, resources and expertise. ExpertCor is a range of frozen plasma sets, spanning the full measuring range of the parameter of interest prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, D-Dimer, unfractionated heparin (UFH) or low molecular weight heparin (LMWH). The ExpertCor plasma sets can be used to supplement local patient samples, to ensure assay comparability as part of system to system comparisons, lab to lab comparisons or lot to lot conversions.

TABLE 1 Summary of linear correlations of Stago analyzers using the ExpertCor sets of plasma (equation and Pearson correlation coefficient (r))

	STA Compact Max vs STA R Max	STA Satellite vs STA Compact Max	STA Satellite vs STA R Max
PT (sec)	y = 1.0494x–0.9632 r = 0.998	y = 09376x + 0.7962 r = 0.999	y = 0.9859x – 0.1629 r = 0.999
APTT (sec)	y = 1.2254x – 3.3478 r = 0.998	y = 1.0488x – 2.1695 r = 0.998	y = 1.2885x – 5.8633 r = 0.998
Fibrinogen (g/L)	y = 1.1207x – 0.0754 r = 0.996	y = 0.9725x – 0.0336 r = 0.996	y = 1.0965x – 0.1208 r = 0.999
DDi (µg/mL)	y = 0.9788x + 0.077 r = 1.000	y = 0.96x + 0.0392 r = 0.994	y = 0.9406x + 0.107 r = 0.995
Anti-Xa (IU/mL)	y = 0.9965x – 0.0082 r = 0.997	y = 0.9675x + 0.0007 r = 0.993	y = 0.9703x – 0.0122 r = 0.996

Aims: We evaluated the ExpertCor plasma sets on various Stago analyzers to assess suitability for assay comparability purposes.

Methods: Each plasma set was assayed on the STA®-R Max, STA® Compact Max and the STA® Satellite.

Each sample of ExpertCor Routine was assayed for PT seconds, APTT and Fibrinogen using STA®-Neoplastin® CI Plus, STA®-PTT-Automate and STA®-Liquid Fib. 5 samples of the set were also assayed for PT in INR.

The ExpertCor DDi, ExpertCor UFH/LMWH sets were assayed for DDi and anti-Xa activity using the STA®-Liatest® DDi and STA®-Liquid Anti-Xa, respectively.

All analyzers and reagents are from Stago (Asnières-sur-Seine, France).

For each parameter, linear correlation was used to compare analyzers two by two.

Results: A summary of the computed linear regressions obtained is displayed in the table.

On the 5 INR samples, the delta in INR was lower than 0.6; the higher differences being observed on the sample with INR level around 7.

Conclusions: Our correlation results indicate that ExpertCor it is a convenient tool for use in verification studies and highlights the excellent system comparability within the Stago line of analyzers.

Moreover, while saving time to seek and collect a large number of plasma samples locally, ExpertCor can help laboratories gain operational efficiency.

PB0600 | Clinical Performance of the Tina-Quant D-Dimer Gen 2 Assay on the Cobas t 711 Analyser for Evaluation of Patients with Suspected Deep Vein Thrombosis (DVT) and/or Pulmonary Embolism (PE)

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Background: Fibrin degradation products, D-Dimer and X-oligomers, are biomarkers for activation of coagulation. A normal D-Dimer result (< 0.5 µg fibrinogen equivalent unit [FEU]/mL), in combination with low/intermediate clinical probability assessment, may identify patients at low risk of proximal DVT and PE.

Aims: Evaluate the clinical performance of the new Tina-quant® D-Dimer Gen 2 assay (cobas t 711 analyser; Roche Diagnostics) for identifying patients with low/intermediate pre-test probability (PTP), by Wells score, who are at low risk of proximal DVT/PE.

Methods: Prospective, observational, multi-centre study using samples collected from six European hospital emergency departments and specialist referral centres, or purchased from commercial vendors. Eligible patients: aged ≥ 18 years; presenting with DVT/PE symptoms; low/intermediate PTP for DVT/PE by Wells score. Main exclusion criteria: DVT/PE symptoms for > 7 days; previous DVT and/or PE. Written informed consent was obtained pre-enrolment. DVT and/or PE were diagnosed by physicians via local imaging protocols, and patients followed-up for ≥ 90 days. Patients were assigned to DVT and PE (with/without concomitant DVT) cohorts. Citrated plasma samples were analysed at a central site using the Tina-quant D-Dimer Gen 2 assay, considering a standard 0.5 µg FEU/mL cut-point; negative predictive value (NPV), positive predictive value (PPV), sensitivity and specificity were determined.

Results: The study population comprised 2516 evaluable patients, 1741 in the DVT cohort and 775 in the PE cohort. Overall NPV and sensitivity (95% CI) for ruling out proximal DVT and/or PE were high:

TABLE 1 Clinical performance of the Tina-quant D-Dimer Gen 2 assay by cohort and PTP classification

Parameter, % (95% CI)	DVT cohort			PE cohort		
	All (n=1741)	Low PTP (n=140)	Moderate PTP (n=1601)	All (n=775)	Low PTP (n=230)	Moderate PTP (n=545)
NPV	100.0 (99.7–100.0)	100.0 (97.0–100.0)	100.0 (99.7–100.0)	99.8 (98.8–100.0)	100.0 (98.0–100.0)	99.7 (98.1–100.0)
PPV	15.7 (12.2–19.8)	0.0 (0.0–17.7)	16.5 (12.8–20.8)	25.3 (20.6–30.6)	9.6 (3.2–21.0)	28.5 (23.1–34.5)
Sensitivity	100.0 (93.9–100.0)	0.0 (N/A)	100.0 (93.9–100.0)	98.7 (93.2–100.0)	100.0 (47.8–100.0)	98.7 (92.7–100.0)
Specificity	81.2 (79.2–83.0)	86.4 (79.6–91.6)	80.7 (78.6–82.6)	67.0 (63.3–70.4)	79.1 (73.2–84.2)	61.2 (56.6–65.6)

100.0% (99.7–100.0) and 99.3% (96.0–100.0), respectively. Overall PPV and specificity (95% CI) were 20.0% (17.1–23.2) and 77.0% (75.3–78.7), respectively. Table 1 presents results by cohort.

Conclusions: In our study population, a normal result (< 0.5 μg FEU/mL) on the Tina-quant D-Dimer Gen 2 assay (cobas t 711 analyser) excluded proximal DVT and PE with high sensitivity in patients with low/intermediate clinical probability.

PB0601 | Reference Values of 117 Healthy Donors on the ST Genesia with the STG-BleedScreen, STG-DrugScreen and STG-ThromboScreen Reagents

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Background: The ST Genesia is a benchtop, fully automated thrombin generation (TG) assay, related to the Calibrated Automated Thrombinography (CAT) assay. The ST Genesia is more standardized and ensures a stable and uniformly heat distribution throughout the measurement and the measuring cuvette.

Aims: To obtain reference values for the ST Genesia with all the reagents available. A comparison between men and women with/without oral contraceptives (OC) was evaluated.

Methods: Plasma samples from 117 healthy donors were measured on the ST Genesia with all three available reagents: STG-BleedScreen, STG-DrugScreen and STG-ThromboScreen. All reagents included at least two quality controls and a reference plasma. This reference plasma is used to normalize data and enables comparison between laboratories, between lots and eases multicenter studies. STG-ThromboScreen has a second trigger containing thrombomodulin (TM), to include the effect of the protein C pathway. One-way ANOVA was used to compare means of the three donor groups. Reference ranges limits were established with 2.5th–97th percentiles on both normalized and absolute TG parameters.

Results: Out of 117 donors, 49% were men, 38% women without OC and 13% women with OC, with an overall mean age (±SD) of 35 years (±12). Men and women without OC had, respectively, a mean peak height of 167nM and 164nM with STG-BleedScreeen, 335nM and 351nM with STG-DrugScreen and 192nM and 198nM with STG-ThromboScreen reagent. Women taking OC had a mean peak height of 263nM, 473nM and 312nM, respectively ($p < 0.05$). Interindividual variation for ETP was 23% for all reagents (similar to CAT). TM addition decreased ETP by 54% in men, 47% in women without OC and only 25% in women with OC ($p < 0.05$).

Conclusions: TG in men and women without OC was similar, however, significantly higher TG values were observed in women taking OC. TM addition decreased ETP significantly less in women taking OC.

PB0602 | The Severity of Hemophilia A Patients Varies among Different Types of Factor VIII Gene Mutation

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Background: Hemophilia A (HA) is a congenital X-linked recessive bleeding disorder, which is characterized by deficiency in factor VIII (FVIII) clotting activity.

Aims: In this study, we aimed at investigating the correlation between FVIII clotting activity and *FVIII* (*F8*) genotype.

Methods: FVIII clotting activity and DNA analysis of *F8* were evaluated in 223 HA patients from 219 unrelated families. Routine genetic testing for HA, including polymerase chain reaction for detecting intron 22 and 1 inversions (Inv22 and Inv1), sanger-sequencing of the entire *F8* coding sequence as well as the exon-intron junctions, was performed in all the patients. Large structural deletion in *F8* was determined by multiplex ligation-dependent probe amplification.

Results: We identified 197 cases with *F8* gene mutation in 223 patients (88.34%). In these 197 cases, 73 (37.06%) were caused by Inv22, 2 (1.01%) were Inv1, 109 (55.33%) were point mutations (41 cases of missense mutation, 29 cases of nonsense mutation, 28 cases of frameshift mutation, 11 cases of splicing mutation), 13 (6.60%) were large structural deletion, respectively. The FVIII clotting

activity was significantly different from these four gene mutations. The clinical manifestation of HA patients with point mutations, especially those with missense or splicing mutations, was milder than those with Inv22, Inv1 and large structural deletion in *F8*.

Conclusions: A combination of multiple methods can effectively improve the detection rate of *F8* mutations. The severity of HA patients with missense or splicing mutation seems to be milder. However, the unexplained 11.66% cases may be attributed to different genetic backgrounds and environments.

PB0603 | Measuring Thrombin Generation in the Presence of Haemoglobin on the ST Genesia

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Background: Haemolysis, i.e. the release of intracellular components of blood cells, is visible as a reddish colorization of the plasma after centrifugation. This phenomenon should not be ignored as it can interfere with laboratory measurements both because of optical disturbances (released haemoglobin absorbs light at specific wavelengths) and coagulation activation through membrane components exposure. Distinguishing both contributors can be of help in understanding observation of an impact of haemolysis on an assay.

Aims: To investigate whether the presence of haemoglobin in plasma samples can interfere with the measurement of thrombin generation (TG) with ST Genesia.

Methods: Plasma samples (N=3) were prepared by mixing a fixed volume of plasma (475 µl) with a fixed volume of buffer (25 µl) containing a varying amount of haemoglobin. In total, 7 samples were prepared with a haemoglobin concentration ranging from 0 mg/dl up to 190 mg/dl. An in-house prothrombin time assay on the STart was used to exclude the presence of heparin in the hemoglobin preparation and TG was measured with the ST Genesia using STG-BleedScreen reagent.

Results: The in-house prothrombin measurements revealed no presence of heparin in the samples. Normalized results (mean (±SD)) of lagtime, time-to-peak, peak height and endogenous thrombin potential were 99.6% (±3.7%), 101.0% (±3.7%), 99.5% (±6.2%) and 98.2% (±3.5), respectively. One-way ANOVA multiple comparison revealed no significant differences between the baseline sample without haemoglobin and all other samples containing haemoglobin ($p>0.05$).

Conclusions: No differences in TG could be observed in samples containing varying haemoglobin concentrations. The calibration method of the ST Genesia is able to correct for the colour of the plasma samples in the presence of haemoglobin. Any effect of haemolysis on TG would then be due to activation of coagulation with cell components and not to the colour of the released haemoglobin.

PB0604 | Variable Findings in One-Stage Clotting Assays in a Patient with Waldenstrom's Macroglobulinaemia

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Background: A patient with Waldenstrom's Macroglobulinaemia had samples sent to Laboratory-1 who found a raised prothrombin time (PT) ratio (3.7), activated partial thromboplastin time (APTT) ratio (1.3), factor (F)V≤1IU/dL, and FVII 18IU/dL. Samples were referred to Laboratory-2 for confirmation and to Laboratory-3 and Laboratory-4 in an inter-laboratory exchange.

Aims: Establish consensus between laboratories in diagnosis of rare inhibitors.

Methods: Laboratories performed screening, one-stage clotting assays (OSCA) and inhibitor screening by their routine methods.

Results: Laboratory-2 measured FVII≥59IU/dL, FII≥41IU/dL and FX≥23IU/dL (non-parallel curves), and FV≤1.0IU/dL at all dilutions. A Bethesda-principle inhibitor assay was consistent with a FV-inhibitor (18.1BU/mL). Lupus anticoagulant (LA) was detected by dilute Russell's viper venom time (DRVVT), dilute APTT and Taipan Snake Venom Time (TSVT) confirmed by Ecarin Clotting Time (ECT).
Laboratory-3 measured FV and FVII activity below 1IU/dL, a FV-inhibitor (16.6BU/mL), a FVII-inhibitor (not quantified) and LA detected by DRVVT, dilute APTT and TSVT/ECT.
Laboratory-4 found normal levels of FII, FV, FVII and FX using PT reagent, but reduced FX using an APTT-based OSCA, and a FX-inhibitor (16BU/mL). No LA was detected by DRVVT or Silica Clotting Time.
Further OSCA were performed using a variety of different reagents on new samples (see table 1). :
Using LA-sensitive reagents, PT and APTT were prolonged even when mixed with normal plasma, and OSCA showed non-parallel curves, indicating the presence of an inhibitor. Using LA-insensitive PT and APTT reagents showed mildly prolonged clotting times and normal factor assays.

Conclusions: This patient had a strong LA that interfered with all OSCA performed with LA-sensitive reagents, even at high dilutions. These findings highlight: that LA can affect PT *and/or* APTT; the importance of using LA-insensitive reagents for performing OSCA; the importance of checking parallelism in OSCA, even when factor levels appear to be normal; and that even expert laboratories find such scenarios challenging.

TABLE 1 Factor Assay Results

	Lab 1	Lab 2		Lab 3		Lab 4	
	Aug 19	Sept 19	Nov 19	Sept 19	Nov 19	Sept 19	Nov 19
Factor II [IU/dL]		40.5[Innovin]	69.0[Innovin] 92.3[ThromboreIS] 92.6[ActinFS]	>10.2[Innovin]	>36.9[Innovin]	52[Recombiplastin]	49[Recombiplastin]
Factor V [IU/dL]	<1[Innovin]	<1.0[Innovin]	<1.0[Innovin] 95.8[ThromboreIS] 162.8[ActinFS]	<1.0[Innovin] 70.7[Antigen]	>28.8[Innovin] 107.7[Antigen]	60[Recombiplastin]	79[Recombiplastin]
Factor VII [IU/dL]	18[Innovin]	>=59.2[Innovin]	>=73.1[Innovin] 77.4[ThromboreIS]	<1.0[Innovin]	>6.2[Innovin]	49[Recombiplastin]	46[Recombiplastin]
Factor X [IU/dL]		>=22.7[Innovin]	>=39.4[Innovin] 77.5[ThromboreIS] 81.1[ActinFS]	>11.7[Innovin] 58.6[Antigen]	>19.6[Innovin]	58[Recombiplastin] 17[Synthasil]	58[Recombiplastin] 14[Synthasil]
Factor VIII [IU/dL]		108.5[Chromogenic]	116.5[ActinFS] 60.8[ActinFSL] 82.9[PathromtinSL] 89.6[Chromogenic]				106[Synthasil]
Factor IX [IU/dL]		59.1[Actin FS]	77.2[ActinFS] 47.4[ActinFSL] 61.8[PathromtinSL]				20[Synthasil]
Factor XI [IU/dL]			61.5[ActinFS] 37.8[ActinFSL] 52.3[PathromtinSL]				25[Synthasil]
Factor XII [U/dL]			55.8[ActinFS] 49.0[ActinFSL] 55.8[PathromtinSL]				55[Synthasil]

PB0605 | Assessing the Effect of Freezing and Thawing of Samples for Anti-Factor Xa Testing for the Determination of Enoxaparin Activity

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Background: The validity of laboratory results is influenced by pre-analytical variables including the collection technique, transportation and storage. Laboratories often rely on a model of centralisation of specialised testing with onsite processing and subsequent transport to referral centres. Low molecular weight heparin (LMWH) offers improved predictability of anticoagulation effect but in certain clinical scenarios (e.g. extremes of age and renal dysfunction) anti-Factor Xa (aFXa)activity testing is indicated. This test is not always available in smaller laboratories and samples must be centrifuged, separated and frozen on-site. Freezing may influence test results and the impact should be assessed in line with the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Aims: Assess the effect of a freeze-thaw cycle on aFXa results of patients treated with enoxaparin.

Methods: Ethics approval for the study was obtained from the research committee. 25 random samples received within 4 hours of collection in citrate tubes at Johannesburg Academic Hospital, National Health Laboratory Service (NHLS) laboratory for aFXa testing were analysed on a STAGO-R MAX® coagulation analyser with STA-Liquid® aFXa reagent. The samples were then frozen (−80°C) and transported within 60 days to another NHLS laboratory at Chris Hani Baragwanath Academic Hospital and analysed on Sysmex CS-2500i® analysers with Siemens Innovance® reagent. The aFXa results were compared for agreement.

TABLE 1 Bias and limits of agreement of Siemens vs. STAGO anti Factor Xa (aFXa):

Bias	−0.0168
Standard deviation (SD) of Bias	0.02982
95% Limits of Agreement	
From:	−0.07525
To:	0.04165

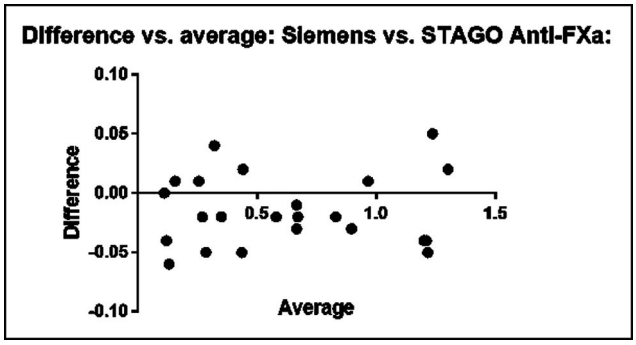

FIGURE 1 Bland-Altman difference plot Siemens vs. STAGO anti-Factor Xa (aFXa)

Results: The aFXa results on the 2 analytical platforms demonstrated acceptable agreement (Figure 1 and Table1):

Conclusions: The results demonstrated acceptable agreement of aFXa results after a freeze-thaw cycle in stored frozen samples analysed on 2 different analytical platforms.

PB0606 | Apixaban Plasma Concentration Estimation by Anti Xa Activity Assay Calibrated with Heparin Calibrators with 2 Different Reagent/Systems

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Background: Classical coagulation tests are not useful for Apixaban estimation in plasma samples. Its concentration can be measured by anti Xa assays specifically calibrated, but calibrators are not widely available in clinical laboratories.

Aims: To correlate heparin calibrated (HC) anti Xa activity, measured by 2 different reagent/systems, with apixaban plasma levels, and compare their performance

Methods: Samples: 32 from patients receiving Apixaban taken at through or peak (40–525 ng/mL) and 20 from patients receiving enoxaparin (0.01–1.33 U/mL) as a control group.

Plasma apixaban concentrations measurement: HemosIL Liquid anti Xa (HEM) ACL TOP Coagulometer. Calibrator and controls: HemosIL Apixaban Calibrators and Controls (Intrumentation Laboratory, IL).

HC anti Xa activity was measured in two different laboratories by HEM assay calibrated by HemosIL Heparin calibrators in ACL TOP coagulometer (IL) and Innovance Heparin Assay (INN) in a Sysmex CS-2500 coagulometer (Siemens Health Care).

Statistic: SPSS 23 software.

Results: Correlation between Apixaban concentration and HC anti Xa activity was very good for both reagent/systems. (table 1). Deming regression demonstrated not statistically significant differences between tests in either Apixaban or heparin samples. Bland Altman analysis demonstrated low and comparable Bias for both types of samples. Pared T test also demonstrated not differences.

Expected values of HC anti Xa activity calculated by regression equations with each test at 2 apixaban concentrations one safe for some procedures and the other in the upper limit at through are shown in table 2.

Conclusions: The good correlations of HC anti Xa results with apixaban concentrations and the comparable performance of HEM and INN in Apixaban samples, demonstrate that a good and similar estimation of apixaban concentration (evaluated range) is possible with both reagent/systems. More samples, particularly at low Apixaban

TABLE 1 Correlation and regression lines between HC anti Xa assays and apixaban levels

	HC Anti Xa activity HEM vs Apixaban (ng/mL)	HC Anti Xa activity INN vs Apixaban (ng/mL)	Deming regression HC Anti Xa activity HEM vs INN	Bland Altman HC anti Xa activity HEM vs INN
R2	0.9719	0.9533	-	-
Linear regression Slope (95% CI) (Apixaban samples)	0.0079 (0.0074 to 0.0083)	0.0082 (0.0075 to 0.0089)	-	-
Linear regression Y intercept (95% CI) (Apixaban samples)	−0.1199 (−0.2567 to 0.0168)	−0.2666 (−0.4694 to −0.0638)	-	-
Bland Altman BIAS (95% CI) Apixaban Samples	-	-	-	−0.03867 (−0.5079 to 0.4306)
Slope (95% CI) Apixaban Samples (n=30)	-	-	1.058 (0.8841 to 1.232)	-
Y intercept (95% CI) Apixaban Samples	-	-	−0.1506 (−0.4451 to 0.1439)	-
Slope (95% CI) LMWH samples	-	-	0.9018 (0.7676 to 1.036)	-
Y intercept (95% CI) LMWH samples	-	-	0.01039 (−0.1097 to 0.1305)	-
Bland Altman BIAS (95% CI) LMWH samples	-	-	-	−0.055 (−0.3415 to 0.2315)

TABLE 2 HC Anti Xa activity expected by linear regression equation

Apixaban concentration (ng/mL)	HEM (U/mL) value (95% CI)	INN (U/mL) value (95% CI)
50	0.27 (0.11 to 0.58)	0.14 (0 to 0.38)
200	1.45 (1.22 to 1.83)	1.38 (1.03 to 1.72)

levels, should be tested to confirm results. These results seem to be useful in clinical laboratories to manage emergency situations.

PB0608 | A Performance Evaluation of Chemiluminescent-Enzyme Immunoassays on a Routine Coagulation Analyser

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Background: The Sysmex CN-Series haemostasis analysers have integrated haemostasis immunoassay modules that perform chemiluminescent-enzyme immunoassays (CLEIA) in addition to coagulation, turbidimetric, chromogenic and platelet aggregation tests.
Aims: To evaluate the performance the CN-Series CLEIA with the predicate device (Sysmex HISCL-800) for thrombin-antithrombin (TAT), plasmin α2 plasmin inhibitor complex (PIC), tissue plasminogen activator/ plasminogen activator inhibitor 1 complex (t-PAI-C) and thrombomodulin (TM).
Methods: Imprecision was assessed by testing high and low quality control plasmas x10 on each of 5 separate days. Comparability was studied in 230 plasmas from normal donors, patients with suspected disseminated intravascular coagulation, sepsis or liver disease, lipaemic, haemolysed and icteric samples. Linearity, limit of detection and limit of quantitation was determined by testing doubling dilutions of a pooled normal plasma. Carryover was assessed by testing samples with normal plasma and plasmas with TAT and TPAI-C above 100 ng/mL.

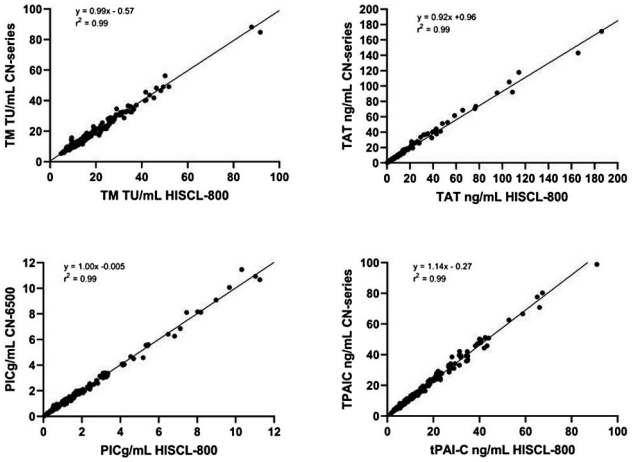

FIGURE 1

Results: The CN-Series performed 26 CLEIA tests per hour (time to first result was < 20 minutes), while simultaneous performing coagulation tests. Acceptable imprecision between-run was obtained for all four assays in high (%CV < 6%) and low (%CV < 7%) controls (Table 1). Excellent linearity was observed (slope 0.89–1.03; r^2 >0.99) across the measurement range. The lower limits of detection and quantitation were: TM < 0.3/0.6 TU/mL, TAT >0.1/< 0.2 ng/mL, PIC < 0.004/< 0.008 μg/mL and TPAI-C < 0.01/< 0.1 ng/mL respectively. All four assays showed excellent correlation between instruments (Figure 1) and were unaffected by haemolysis, icterus or lipaemia. No carryover was observed in the TAT or TPAI-C assays.
Conclusions: Our data demonstrate that the performance of the CLEIA module of the CN-Series is comparable to that of a stand-alone immunoassay analyser.

TABLE 1

High QC	Mean HISCL-800	SD HISCL-800	%CV HISCL-800	Mean CN-series	SD CN-series	%CV CN-series
TAT ng/mL	36.89	1.39	3.77%	38.00	1.59	4.17%
TM TU/mL	113.0	2.5	2.46%	115.0	3.8	3.29%
PIC μg/mL	7.257	0.254	3.49%	6.938	0.280	4.04%
TPAI-C ng/mL	15.59	0.62	3.98%	17.67	0.99	5.62%
Low QC	Mean HISCL-800	SD HISCL-800	%CV HISCL-800	Mean CN-series	SD CN-series	%CV CN-series
TAT ng/mL	9.56	0.32	3.36%	9.69	0.39	4.01%
TM TU/mL	27.4	0.7	2.48%	27.7	0.8	2.93%
PIC μg/mL	1.773	0.056	3.22%	1.620	0.046	2.84%
TPAI-C ng/mL	4.14	0.15	3.75%	4.67	0.31	6.74%

PB0609 | Assessment of the CN-6000; a Novel, High-Speed Coagulation Analyser, Utilising Coagulation, Chromogenic, Immuno-Turdibimetric and Light Transmission Aggregation Technologies

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Background: We evaluated a newly released coagulation analyser (CN-6000, Sysmex Corporation, Japan) capable of clotting, chromogenic, Immuno-turbidimetric and platelet aggregation assays.
This new high-throughput analyser has a small footprint, improved sample volume usage and enhanced software allowing improved end point detection and abnormal clot waveform interpretation.
Aims: Compare the performance of CN-6000 against CS-5100.
Methods: Performance was evaluated against the CS-5100 (Sysmex Corporation, Japan) for clotting (prothrombin time, activated partial thromboplastin time, Clauss Fibrinogen, Thrombin time, Factor VIII), chromogenic (Antithrombin, Protein C) and immuno-turbidimetric (D-Dimer, VWF antigen, VWF activity) assays. All reagents supplied by Siemens Diagnostic Healthcare (Marburg, Germany).
For each assay more than 120 samples were tested from both patients and normal subjects in order to assess correlation over a wide measuring range.
Calibration curves were tested by running 3 calibration curves over two days on both instruments. Commercial controls were tested 20 times over 8 days to obtain imprecision data.
Throughput was measured by testing PT only and PT/APTT in samples representative of a typical laboratory workload.
Results: The CN-6000 showed good linearity with reproducible calibration curves, along with low inter-assay imprecision using commercial normal (CV = 0.6–4.7%) and abnormal (CV = 0.8–6.2%) control plasmas.
Results showed excellent correlation between the CN-6000 and CS-5100 for each assay (r^2= 0.988–0.999) with no significant misclassification, and data points scattered close to the line of identity.
There was a 9% increase in sample throughput for PT with the CN-6000 compared to the CS-5100 (430 versus 390). More impressively, there was a 33% increase in throughput for samples requested for both PT and APTT (456 versus 344).
Conclusions: Our results show that using the CN-6000 analyser, routine coagulation and specialist assays can be performed with acceptable levels of imprecision and excellent comparability with the CS-5100.

TABLE 1 Test/Method Comparion (r2 value)

PT	0.999
APTT	0.988
Fibrinogen	0.990
Thrombin Time	0.997
Factor VIII	0.996
Antithrombin	0.991
Protein C	0.998
D-Dimer	0.998
VWF Antigen	0.994
VWF Activity	0.996

TABLE 2 %CV with Commercial Control Plasma

PT	Normal Control 0.8%	Abnormal Control 1.2%
APTT	0.6%	0.8%
Fibrinogen	4.7%	6.2%
Thrombin Time	2.3%	-
Factor VIII	3.2%	4.2%
Antithrombin	3.0%	4.7%
Protein C	1.2%	2.2%
D-Dimer	3.4%	2.0%
VWF Antigen	2.0%	2.8%
VWF Activity	1.5%	1.7%

PB0610 | Can Bone Marrow Cells Be Differentiated and Counted Using a Novel Hematology Analyzer?

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Background: Bone marrow (BM) cell populations have traditionally been assessed visually by light microscopy because most automated analyzers cannot adequately capture immature cells; however, advances in modern hematology analyzers have improved detection of immature cells. The rapid availability of complete blood count (CBC) results can be advantageous for patients and clinicians, but comprehensive CBC data using the new analyzer methods for BM samples are lacking.
Aims: The aim was to *i*) identify and count BM cells, *ii*) generate a gating algorithm for cells isolated from BM aspirates using a novel hematology analyzer.
Methods: Two hundred and sixteen BM aspirates were collected from 186 patients with oncohematologic diseases (leukemia *n*:68, multiple myeloma *n*:55, lymphoma *n*:22, myelodysplastic syndrome *n*:17, myelofibrosis *n*:15, aplastic anemia *n*:9) and 30 healthy donors for allogeneic transplantation. All samples were analyzed on a modified Sysmex XN20 Module® (Sysmex, Kobe, Japan), and were also blindly assessed by a hematology expert under microscopy. Data from the Sysmex XN20 Module® were saved as .fcs files and then

manually gated using the Software version 2.5.1 (Perttu Terho, Turku, Finland). Total nucleated cell counts were recorded and used to determine cellularity, myeloid/erythroid (M/E) ratio, and granulocyte/erythrocyte (G/E) ratio. Results were analyzed statistically.

Results: Compared with the healthy donors' findings, degree of cellularity was significantly higher for the patients with leukemia and lymphoma, respectively, and was significantly lower for patients with aplastic anemia ($p< 0.05$ for all). As well, mean M/E ratio was significantly higher for patients with leukemia and lymphoma, respectively, and mean G/E ratio was significantly higher for patients with leukemia ($p< 0.05$ for all). Three parameters were significantly correlated between the Sysmex XN20 Module®-analyzed and microscopy-evaluated BM specimens: cellularity r=0.18, p=0.02; M/E ratio r=0.76, p=0.01; G/E ratio r=0.78, p=0.001.

Conclusions: Our preliminary findings warrant further investigation to identify clinically useful novel analyzer parameters for BM cells.

PB0611 | Excluding Anticoagulants and Antiplatelet Drugs with Rotational Thromboelastometry

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Background: Rotational thromboelastometry (ROTEM) is a viscoelastic point-of-care device that measures clot formation in whole blood. It is currently used for transfusion management during cardiac surgery, liver transplantation and post-trauma care.

Aims: We aimed to evaluate whether ROTEM has additional value in excluding presence of anticoagulants and antiplatelet drugs.

Methods: In this prospective cohort 442 vulnerable patients that received PCI at MUMC+ between May 2014 and January 2019 were included. Vulnerable patients are defined as having ≥3 risk factors for either ischemic or bleeding events. Patients' 1 month post-PCI treatment consisted of a combination of a P2Y12 inhibitor with either aspirin (dual antiplatelet therapy; DAPT, n=324), a vitamin K antagonist (VKA; n=70) or a direct oral anticoagulant (DOAC; n=48). Four ROTEM assays (INTEM, EXTEM, tPA and FIBTEM) were performed and compared with a control group of 95 patients undergoing preoperative screening and not using antithrombotic medication.

Results: EXTEM CT was out of reference range (36–60 s) for all patients on anticoagulants, with respectively a median and range of 122s (74–203) and 132s (62–256) for VKA+P2Y12 and DOAC+P2Y12. Neither of the 4 ROTEM assays detected the use of DAPT when CT was compared to the control patients. Remarkably, both patients on DAPT and VKA/DOAC + P2Y12 showed significantly higher MCF and lower CFT for the EXTEM, INTEM and FIBTEM assays when compared to the control group.

Conclusions: An Extem CT within reference range can exclude the presence of anticoagulants: (in combination with a P2Y12 inhibitor) in this cohort. ROTEM CT cannot detect the use of DAPT, but surprisingly, an effect on CFT and MCF can be seen for DAPT. Mechanisms underlying this phenomenon remain to be investigated.

PB0612 | Rivaroxaban Plasma Concentration Values and Risk Factors for Adverse Events in Patients Experiencing Bleeding: Results from a Prospective Study

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Background: Adverse drug events from anticoagulants including direct oral anticoagulants (DOACs) are the major cause of emergency department admissions in the United States (1). Bleeding remains the main adverse event during DOAC use. Routine laboratory monitoring of DOACs plasma level is not recommended, but may be useful among certain patient groups, especially in emergency situations. In 2019 study examining relationship between chromogenic anti-FXa assay and LC-MS/MS for detecting rivaroxaban (RXN) plasma concentration (P-RXN) was conducted in North Estonia Medical Centre (2). During this, data about 43 patients: demographics, vital signs, utilisation of medicines, adverse events and laboratory results at 4 visits (days 0, 7, 30 and 180), was collected.

TABLE 1 Baseline characteristics of patients

	With bleeding	Without bleeding
Patients (n)	11	32
Male sex (n; %)	7 (64)	22 (69)
Age (years; mean; min- max)	61.4 (40–79)	57.9 (24–81)
Weight (kg; median; min- max)	83.9 (72.5–143.3)	88.4 (51.5–169.8)
eGFR (mL/min; mean; min- max)	88.3 (68–107)	82.9 (40–117)
Hemoglobin (g/L; mean; min- max)	133.9 (110–162)	138.9 (106–155)
RXN indication (VTE/AF)	8/ 3	25/ 7

Aims: The aim of this analysis was to describe differences between patient groups with and without bleeding complications in this dataset.

Methods: 43 patients were divided into two groups- patients with and without bleeding complications. Baseline characteristics of all patients at first visit (day 0) are presented in Table 1. P-RXN values used were detected by LC-MS/MS. Bleeding complications were classified by International Society on Thrombosis and Haemostasis (ISTH) definitions (3). We also conducted drug interaction review in Inxbase drug-drug interaction database.

Results: There were 11 patients with bleeding complications including 2 patients with 2 visits with bleeding (in total we recorded 12 minor and 1 major bleeding). P-RXN trough levels ranged from < 2 to 440 ng/ml (median 51 ng/ml; SD 108.7) in patients with bleeding and from 2.2 to 240 ng/ml (median 33 ng/ml; SD 48.9) in patients without bleeding. 2 patients with minor bleeding complications used non-steroidal anti-inflammatory drugs.

Conclusions: P-RXN levels in both groups had a very high variability, but tended to be higher in patients with bleeding. Bleeding events were mostly minor in patients using RXN.

References:

(1). JAMA 2016;316(20):2115–2125
(2). Eur J Clin Pharmacol (2019) 75(Suppl 1): 1. https://doi.org/10.1007/s00228-019-02685-2
(3). J Thromb Haemost 2015;13: 2119–26

PB0613 | Clinical Exomes for the Diagnosis of Inherited Bleeding, Thrombotic and Platelet Disorders: The Belgian Experience

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Background: The targeted high-throughput sequencing gene panel test ThromboGenomics was recently validated as potential diagnostic test for screening bleeding, thrombotic and platelet disorders.[1] Pathogenic variants and Variants of Unknown clinical Significance (VUS) have been detected in 49% for thrombotic, coagulation, platelet count, and function disorder patients while this diagnostic rate dropped to 3.2% for patients with unexplained bleeding disorders with normal laboratory test results. About half the genetic variants have never been described before and therefore are VUS.

Aims: We aimed to implement the international ThromboGenomics test in a fully accredited genetic lab and improve the diagnostic rate by studying VUS findings in research environment.

Methods: Thrombosis and hemostasis centers in Belgium recruited 69 patients. Clinical exomes have been used with the option for one of three panels: platelet genes, (anti)coagulation factor genes or vascular genes. Pathogenic variants have been reported to clinicians while VUS findings have been recorded for further research studies that involve family recall studies and additional laboratory tests.

Results: At least one pathogenic variant was identified in 23% of the exomes analyzed so far. A single VUS was detected in 13% of the patients while polygenic variants (including at least one VUS) were detected in another 13% of the patients with mostly thrombotic problems. These VUS cannot be used for clinical management and have been included in a research study. We have noticed that assays in routine haemostasis laboratories are often able to detect the quantitative defects but not all qualitative defects of plasma factors. A few examples will be discussed where co-segregation analysis and (improved) laboratory tests have been able to re-classify such VUS into pathogenic variants.

Conclusions: Our data confirms the findings of the ThromboGenomics study and emphasize the frequent occurrence of VUS, of which the functional and clinical relevance needs to be studied.

1Downes K, et al. Blood.2019;134:2082:

PB0614 | An Evaluation of the Assay Performance of the Antifactor Xa Assay for Rivaroxaban

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Background: Direct Acting Oral Anticoagulants (DOACs) such as Rivaroxaban, are direct inhibitors of factor Xa. With a more stable pharmacokinetic and pharmacodynamics profile, the use of them have been steadily increasing. In Khoo Teck Puat Hospital (KTPH), a 700-bed hospital in the northern area of Singapore, we have seen a similar increase in their use. KTPH laboratory performs routine prothrombin time testing, which may not be suitable for Rivaroxaban measurement.

Aims: The aim of the evaluation is to assess the assay performance of the reagents for the Antifactor Xa assay for Rivaroxaban, to be used in the laboratory.

Methods: The test protocol for the reagent was set up on the CS-2500 automated coagulation analyser (Sysmex Asia Pacific Pte Ltd, Japan). Calibration curves were set for both standard range (12–525 ng/mL) and low range (11–143 ng/mL) analytical measurement. The intra-assay imprecision was evaluated using two levels of the BIOPHEN™ Rivaroxaban Control Plasma and Control Low. A trueness of 146 samples were assessed with results from a referral laboratory.

Results: Intra-assay precision of 8.0% and 2.3% was obtained for Control Plasma 1 and 2 respectively. Results for Control Low 1 and

2 was 6.7 and 2.6% respectively. All results were acceptable and within the manufacturer claim of 15% for control plasma and 20% for control low. Trueness of 146 samples generated a slope of 0.982 with a coefficient determination of 0.962, showing good agreement with the referral laboratory.

Conclusions: Prothrombin time can be helpful and is prolonged if patient has Rivaroxaban in their system. However, the chromogenic method gives a more accurate assessment of the anticoagulant effect of the medication. Although Rivaroxaban does not require routine monitoring or dose titration, it may still be important for laboratories to perform the tests in urgent clinical circumstances.

PB0615 | ISTH SSC Plasma Standard Lot #5 - Results in a UK National External Quality Assessment Scheme for Blood Coagulation (NEQAS BC) Exercise

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Background: The ISTH SSC plasma standard is a secondary coagulation standard developed to aid manufacturers in the labelling of coagulation calibrators. Values are assigned for 24 haemostasis parameters through assessment against relevant WHO International Standards in multi-centre international collaborative studies. However, it is useful to compare results obtained on this plasma with a wide range of reagents and methods in routine laboratory use to confirm that commercial calibrants are accurately labelled.

Aims: UK NEQAS BC has regularly distributed the SSC plasma standard as a blinded sample for assay in proficiency testing exercises. We report here the first comparison of proficiency testing results to the assigned values for the lot #5 of the SSC plasma standard.

Methods: The plasma was sent to 870 centres, with a request to perform fibrinogen assays, and 385 centres for FVIII, FIX and VWF assays. Results along with method details for each assay were returned. Median results were determined, and compared to the SSC plasma standard assigned values.

Results: Table 1 shows the median results for all methods for fibrinogen, FVIII, FIX and VWF:Ag assays. Method specific VWF activity assay results are also shown. Reagent-related differences were noted, for example for FVIII:c assays, the median results for users of one widely used reference plasma was 81.3u/dl (n=187), compared to 91u/dl for users of another reference plasma (n= 52, p< 0.001).

Conclusions: it is useful to compare values assigned to commercial calibrants against the SSC plasma standard in an exercise with results obtained by a large variety of methods in a proficiency testing exercise. We have demonstrated good agreement with the assigned values for fibrinogen, FVIII, FIX, and VWF assays, including the method-specific target values for VWF activity, introduced for the first time with this lot of plasma. However, some method-related discrepancies require further investigation.

TABLE 1 Median results compared to assigned values

Test	Assigned Value Lot #5	no. of users, UK NEQAS BC	Median value, UK NEQAS BC
Clauss fibrinogen	3.19g/L	836	3.10g/L
FVIII:C	82.0 IU/dL	361	81.0 IU/dL
FIX:C	109.0 IU/dL	319	110.0 IU/dL
VWF:Ag	114.0 IU/dL	235	117.0 IU/dL
VWF activity Aggregometry (VWF:RCo)	82.0 IU/dL	37	82.0 IU/dL
Chemiluminescence (VWF:GPIbR)	95.0 IU/dL	18	97.3 IU/dL
IL VWF:RCo (VWF:GPIbR)	95.0 IU/dL	71	101.0 IU/dL
Siemens (VWF:GPIbM)	80.0 IU/dL	42	83.6 IU/dL
VWF:Collagen binding	102.0 IU/dL	42	102.6 IU/dL

PB0616 | Analytical Verification of Automated Latex-Enhanced Particle Immunoturbidimetric D-Dimer Assays on Diferent Analytical Platforms and Comparability of Test Results

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Background: Automation and the possibility of D-dimer analysis along with other coagulation tests on the same analytical platform makes the latex-enhanced particle immunoturbidimetric assays (LPIA) attractive to laboratory professionals.

Aims: The aim of the study was the analytical verification of the following automated LPIAs: INNOVANCE D-dimer on Sysmex CS-5100 and Atellica COAG360 analyzers (Siemens Healthineers, Germany), and HemosIL D-dimer HS500 on ACL TOP 550 (Instrumentation Laboratory, Italy).

Methods: Verification included: assessment of within-run and between-run precisions by analyzing commercial controls with low and high D-dimer concentrations in triplicate for five consecutive days, bias and measurement uncertainty (MU) estimation, verification of the cut-off and method comparison between all assessed assays as well as with the reference commercial enzyme-linked immunofluorescent assay (ELFA) VIDAS D-Dimer Exclusion II (bioMérieux, France) on a miniVidas analyzer.

Results: Within-run coefficients of variations (CVs) ranged from 1.6% (Atellica COAG360) to 7.9% (ACL TOP 550), while between-run CVs ranged from 1.7% (Sysmex CS-5100) to 6.9% (Atellica COAG360). Spearman's rank correlation coefficients were >0.99 between LPIAs and ≥0.93 when comparing ELFA with LPIA. Passing-Bablok regression analysis yielded constant and proportional difference for comparison of ACL TOP 550 with both Sysmex CS-5100 [y=0.09 (0.02–0.17)+0.88 (0.82–0.94)x] and Atellica COAG360 [y=0.08 (0.03–0.16)+0.85 (0.79–0.89)x], as well as for miniVidas with Atellica COAG360 [y=−0.27 (−0.48-(−0.05)+1.57 (1.34–1.85) x]. Small proportional difference was found between miniVidas and both Sysmex CS-5100 [y=−0.33 (−0.52–0.01)+1.6 (1.27–1.82)x] and ACL TOP 550 [y=−0.16 (−0.33–0.15)+1.37 (1.05–1.6)x]. Calculated MUs using D-dimer HS 500 calibrator were 12.6% (Sysmex CS-5100) and 15.6% (Atellica COAG360), while with INNOVANCE D-dimer calibrator 12.0% (Sysmex CS-5100) and 10.0% (Atellica COAG360). MU for ACL TOP 550 using INNOVANCE D-dimer calibrator was 28.1%. Excellent agreement of results was obtained (weighted Cohen's kappa coefficient: 0.85–1.00). The cut-off (0.5 mg/L FEU) was confirmed.

Conclusions: Obtained results prove the analytical validity of LPIAs, their high comparability and almost equal discriminatory characteristics, suggesting them as a valid alternative to ELFA.

PB0617 | Correlation between INR Pocket Test and Standard Coagulation Test

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Background: Over the last years, a lot of point of care (POC) testing devices to measure INR have been introduced. The INR-POC system has been found to be as accurate and reliable as the traditional method, in the outside setting.

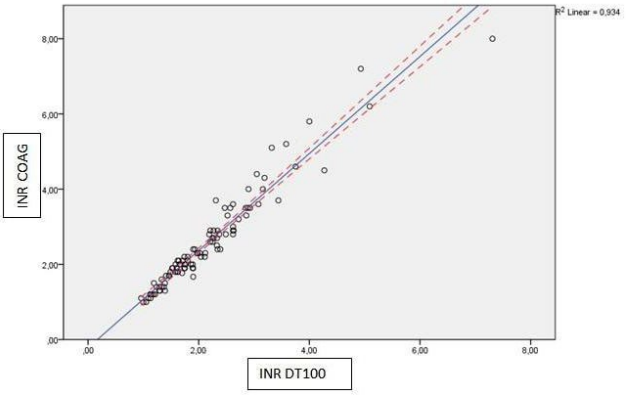

FIGURE 1 1 graph

Aims: We want to establish if there is a good correlation between standard coagulation test (SCT) and pocket INR test in the hospitalized patients.

Methods: We included 100 hospitalized patients under anticoagulation therapy with antivitamin K (AVK) 2 or 3 days before going home. All samples were taken from a peripheral vein, with syringe, first one drop was put on the pocket device Coaguchek ®, and then it was put in the citrate tube and processed with Triniclot PT HTF (Thromboplastin) in the Destiny DT 100 by Tcoag ®

Exclusion criteria: patients under bridging with LMWH.

Results: See Table 1 and Graph 1: The statistical correlation between SCT and Pocket test calculated with Pearson method was highly significant. (p>0.0001). The graph 1 shows the trend line (blue) and 95% CI (dotted red).

Conclusions: We observed a very good correlation between both methods, like described in the outside patients.

Our results could mean, it is safe for patient's treatment if we change between tests, adapting to patients necessities and settings.

TABLE 1 Correlation between methods

		INR COAGUCHEK	INR DT100
INR COAGUCHEK	Pearson Correlation	1	0.966
	Bil Sig		0
	N	100	100
INR DT100	Pearson Correlation	0.966	1
	Bil Sig	0	
	N	100	100

PB0618 | The SSC Working Party on Thrombin Generation: Inventory of Thrombin Generation Methods

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Background: Thrombin generation (TG) is a better determinant of the overall function of the haemostatic system than routinely used clotting time-based assays. Nowadays, more and more haemostasis groups integrated this assay to their range of tests. However, there is significant variability between laboratories regarding the pre-analytical phase, reagents, TG protocol, analysis interpretation and normalization.
Aims: In order to solve the problem related to variation between laboratories, we made an inventory of all the methods that are being used to measure TG.
Methods: The questionnaire about TG consisted of 51 questions regarding the different aspects of TG: participant, commercial methods, samples, in-house or adapted commercial methods, analysis and interpretation, normalization and quality control, clinical use and parameter of choice in specific conditions.
Results: Out of the 240 surveys that were started, 108 were completed (45%). However, not all questions were in scope for all laboratories. Half of the laboratories were research laboratories and half diagnostic laboratories. The most used TG assay was the Calibrated Automated Thrombinography (CAT)-based assay (56%). There was a significant amount of divergence regarding most aspects of the TG assay: type of needle, blood tubes, centrifugation steps, sample storage, storage temperature, freezing storage, thawing, commercial/in-house reagents, sample dilution, calibration, reference ranges, data normalization, …
Conclusions: There is still an important variation in the methods available for measuring TG. A standardized protocol and data normalization should be incorporated for comparing data from different laboratories and hospitals, especially for multicenter studies.

PB0619 | DOAC Intra-individual Variability in Patients with Non Valvular Atrial Fibrillation

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Background: Large inter-individual plasma level variability of the four direct oral anticoagulants (DOAC: apixaban, dabigatran, edoxaban and rivaroxaban), has been previously reported, showing a significant increase from phase II-III clinical trials up to post-marketing studies, both at standard and low dosages. At present poor data on intra-individual plasma level variability are available in real world.
Aims: To evaluate intra-individual variability, both at C-trough and C-peak, in patients with non valvular atrial fibrillation, treated with the four available DOAC, both at standard and low dosages.
Methods: After informed consent, 640 patients were enrolled and plasma samples were collected at C-trough and C-peak, after one month from starting DOAC (time 0) and each 3 months (time +3, +6). Three consecutive controls at C-trough and C-peak were considered for the analysis. Plasma DOAC concentrations were measured using Ecarin chromogenic assay for dabigatran and anti FXa activity, specifically calibrated for each anti-Xa drugs (Stago, France).
Results: From a total of 640 patients, 58 of them were excluded from the analysis: 40 had an incomplete collection of samples and 18 patients showed onset of new diseases or prescription of new potentially interfering drugs. 582 patients (73 dabigatran 150mgx2/die; 72 dabigatran 110mgx2/die; 76 rivaroxaban 20mg/die, 77 rivaroxaban 15mg/die; 75 apixaban 5mgx2/die, 78 apixaban 2.5mgx2/die, 68 edoxaban 60mg/die, 63 edoxaban 30mg/die) completed the study program and 3492 plasma samples were tested. DOAC mean CV% intra-individual variability at C-trough ranged from 23 to 31% while at C-peak from 22 to 30%. Details are showed in table 1.
Conclusions: The intra-individual variability was significantly lower in comparison with inter-individual variability, as shown in previous studies. Our data seems to suggest that DOAC levels are stable during the first six months of treatment. However, we can not exclude possible significant modifications of drug anticoagulant levels in case of new co-morbidities and/or changes in co-therapies.

PB0620 | Evaluation of INR and APTTs Using the Q Family of Analysers in Comparison to ACL TOP

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Background: The Q family of analysers (Grifols) are fully automated, random access, cap piercing analysers using clotting, chromogenic and immunoturbidometric endpoints. The QSmart, QNext and QXpert are suitable for low, medium and high throughput laboratories. Liquid PT/INR (recombinant human tissue factor), and APTT (ellagic acid/synthetic phospholipids) reagents are available for ease of use.
Aims: Compare INRs and APTTs determined using the Q family to ACL TOP.
Methods: We compared QNext, QSmart and ACL TOP in study 1, and then compared QXpert and QNext in study 2 using the following: PT/INR; liquid human recombinant thromboplastin (DG-PT RecombiLIQ)/Q series compared to Recombiplastin 2G/ACL TOP): APTT; liquid ellagic acid APTT (DG-APTT Synth)/Q series vs

TABLE 1 INR

	Study 1 n=	QSmart RLIQ	QNext RLIQ	IL TOP Rec 2G	Study 2 n =	QNext RLIQ	QXpert RLIQ
All	61	2.98	2.92	3.12	89	2.75	2.76
<4.0	49	2.51	2.47	2.57	77	2.42	2.45
>4.0	12	4.86	4.77	5.39	12	4.88	4.78

TABLE 2 APTT Ratio

	Study 1 n=	QSmart APTT ratio	QNext APTT ratio	IL TOP APTT ratio	Study 2 n=	QNext APTT ratio	QXpert APTT ratio
All	139	1.48	1.44	1.35	134	1.62	1.63
Single factor deficiency	16	1.58	1.54	1.46	26	1.87	1.85
UFH	15	3.90	3.69	2.79	16	2.73	2.73
Investigated for LAC	15	1.15	1.13	1.23	10	1.18	1.19
Liver disease	15	1.25	1.24	1.23	15	1.45	1.48
Critical care/ DIC	14	1.25	1.21	1.13	9	1.33	1.36
others	64	1.06	1.06	1.08	58	1.36	1.39

Synthasil/ACL TOP. The patient groups and numbers are shown in tables.

Results: INR Study 1 - Results with DG-PT RecombiLIQ were significantly lower than those with Recombiplastin 2G/ACL TOP (ANOVA $p< 0.05$) by on average < 5% for INRs < 4.0. There was excellent agreement between QNext and QSmart results. INR Study 2 - There was excellent agreement between QXpert and QNext INR results. The biggest difference was an average of < 3% for INRs >4.0.

APTT Study 1- APTT ratios with DG-APTT Synth were significantly higher than those with Synthasil/ACL TOP in subjects with isolated clotting factor deficiency, on critical care or during unfractionated heparin therapy. There were no significant differences between QNext and QSmart results in any group. APTT Study 2 - there was excellent agreement between QXpert and QNext with < 3% difference in all groups.

Conclusions: There was excellent agreement between results obtained on the 3 members of the Q family of analysers across a wide range of INRs and APTTs in all patient groups. DG-PT RecombiLIQ and Recombiplastin 2G INRs below 4.0 were < 5% different. DG-APTT Synth was more sensitive than SynthaSIL to some causes of prolonged APTT.

PB0621 | Genetic Analysis for Investigation of Heritable Bleeding and Thrombotic Disorders - Variable Interpretation of Data Identified by the UK National External Quality Assessment Scheme (UK NEQAS) Blood Coagulation Programme

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Background: Genetic analysis is important in the diagnosis of haemostatic disorders, and meaningful interpretation of results is an essential part of this process.

Aims: UK NEQAS distributes cases, providing a clinical scenario either with genetic analysis results, or with a blood sample for analysis. Laboratories produce an interpretation of the genetic variant detected in an informative report.

Methods: We describe here cases in which variable interpretation of genetic variants leads to different recommendations on submitted reports.

Results: Case 1: Patient with DVT after knee arthroscopy, with a family history of thromboembolism. Borderline antithrombin activity was noted. Sequence analysis of *SERPINC1* revealed heterozygosity for c.1246G>T. There was no overall consensus in reports on this variant ('Antithrombin Cambridge'), with some centres reporting the variant as causative of disease phenotype, others as a variant of uncertain significance. Half of reports contained no comments regarding the 'low' thrombotic risk associated with this variant.

Case 2: Patient with FVII deficiency, and history of epistaxis and easy bruising. Genetic analysis of *F7* revealed: c.1072A>T heterozygous; c.1285G>A heterozygous; c.1238G>A heterozygous. There was broad consensus when classifying the 3 variants; the majority of laboratories reporting both apparently compound heterozygous c.1072A>T and c.1285G>A variants (11/14 and 9/14 respectively) as causative of the disease phenotype and the c.1238G>A variant as a disease-associated polymorphism (DAP). However, in some reports increased importance was given to the DAP.

Case 3: Patient with severe haemophilia A, hemizygous for the *F8* intron 22 inversion, whose mother did not carry the causative variant. There was broad consensus that mother may be a germline mosaic for the variant, however several laboratories did not acknowledge this possibility, resulting in production of potentially damaging reports.

Conclusions: A lack of consensus in interpretation for some of these cases underlines a need for EQA, supporting standardisation of both genetic laboratory testing and interpretation.

PB0622 | Variability in Dabigatran and Apixaban trough Concentrations

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Background: DOACs show reproducible pharmacokinetics/dynamics. However, knowledge of between-patients and within-patient variability in trough concentration levels may be important for optimal management.

Aims: To evaluate the variability in trough concentrations (TC) of patients treated with dabigatran etexilate or apixaban.

Methods: Blood was drawn from 135 patients (61% men) affected by NVAF (53%) or VTE, on either dabigatran (44%) or apixaban treatment, 11–12 hours after last dosing. Patients on dabigatran (57% NVAF, 32% on low dose, 70±14 years) and on apixaban (49% NVAF, 59% on low dose, 74±12 years) had an average estimated creatinine clearance (ECrCl) of 87±28 ml/min and 65±25 ml/min, respectively. All patients had at least 3 blood samples (average 4.5) taken over a period of 1 year (overall 614). We measured Dabigatran and apixaban plasma levels with reagents from Stago (Asnieres sur Seine, France).

Results: Coefficients for between-patients variability were 43.0% for dabigatran (median TC=96 ng/ml) and 50.2% for apixaban (median TC=83 ng/ml). Gender, age, ECrCl and dose explained 35% of the between-patients variation in apixaban levels (r^2=0.348). Median within-patient variability was 25% for both dabigatran and apixaban (IQR 18–32% and 14–42%, respectively). Gender, ECrCl, and lower trough concentrations explained about 38% of the within-patient variability in dabigatran levels (r^2=0.376). Low-dose, indication (VTE), and the indication-dose interaction explained only about 13% of the within-patient variability in Apixaban levels (r^2=0.128).

Conclusions: Despite dose-adjustment for patients with NVAF or VTE based on ECrCl and age, the between-patients variability in dabigatran TC is still high, unpredictable, and comparable to that of apixaban with inclusion of patients on low dose prophylaxis. Based on a follow up of at least 1 year, within-patient variability is lower and partially predicted by gender and ECrCl for patients on dabigatran.

PB0623 | A Universal Quality Control Plasma for Anti-Xa and Anti-IIa Drugs

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Background: In our Institution, 24h measurement of DOACs plasma levels is available. Due to the relevant number of samples tested daily in our lab, an internal quality control (IQC) material is required for all DOACs and LMWH. If valid for all anti-Xa and anti-IIa assays, such IQC material may be highly cost-effective in the coagulation laboratory.

Aims: To produce and validate a universal IQC plasma for anti-Xa and anti-IIa assays.

Methods: We routinely use as control plasma citrated pooled plasma (NPP, ca. 2 liters) collected every 6 months from >100 apparently healthy volunteers who belong to the staff of our Institution. Before storing at −80°C, we added fondaparinux (Arixtra®, 625 ng/ml fc.) and dabigatran (Sigma, ca. 100 ng/ml f.c) to 100 ml of NPP, which, after thorough mixing, was separated in 0.4 ml aliquots. We report on the imprecisions observed with 6 lots of the IQC plasma used daily since 2017.

Results: A standard curve with commercially available calibrators (Stago, France) is obtained on average every 2 months on all coagulometers (STA-R Max®). Within-calibration imprecisions with all lots of the IQC plasma ranged from 11.2% to 15.3% for dabigatran (n=839, 90–121 ng/ml), 1.4% to 14.8% for apixaban (n=855, 62–93 ng/ml), 4.3% to 9.0% for rivaroxaban (n=811, 67–103 ng/ml), 9.4% to 10.5% for edoxaban (n=808, 58–88 ng/ml), and 3.3% to 10.0% for LMWH (n=663, 0.77–0.93 IU/ml). There was no clear trend for instability of the IQC material. The IQC plasma permits comparison of the potencies of the anti-Xa drugs. Thus, the anti-Xa activity of 0.5

IU/ml of enoxaparin appears to correspond to the activity of 45, 47, and 43 ng/ml of apixaban, rivaroxaban, and edoxaban, respectively.

Conclusions: Our universal IQC plasma for anti-Xa and anti-IIa commercial assays results in acceptable imprecision and permits comparison of the relative potency of anti-Xa drugs.

PB0624 | Hemostatic Profile: Is There Any Difference between Alzheimer's Disease and Frontotemporal Dementia?

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Background: Alzheimer's disease (AD) is the most frequent cause of dementia. Several hypotheses aim to explain its pathophysiology, which is complex and multifactorial. Hemostatic alterations have been reported in the pathogenesis of AD.

Aims: To investigate possible hemostatic alterations in patients with AD compared to behavioral variant frontotemporal dementia (bvFTD), another important cause of cognitive decline, and in elderly individuals with no cognitive impairment.

Methods: A total of 92 subjects were enrolled and distributed into three groups: AD (n= 34); bvFTD (n= 29) and controls (n=29). Hemostatic alterations were investigated by thrombin generation assays using thrombomodulin (TM) to investigate the resistance to this natural anticoagulant, in addition to plasma levels of ADMA (dimethylassimetric arginine), von Willebrand Factor (FvW), TM and ADAMTS-13 measurements. The study received approval from the local Ethics Committee.

Results: Higher plasma levels of ADAMTS-13 were observed in patients with AD and bvDFT compared to controls, however, no difference was found between the two dementias. ADMA, FvW and TM plasma levels and thrombin generation parameters were not different among groups.

Conclusions: There was no difference in the hemostatic profile between patients with AD and bvFTD, and between them and the controls. However, the higher plasma levels of ADAMTS-13 in patients with AD and bvFTD compared to controls encourage further studies on the role of this enzyme in the pathophysiology of dementias.

Ackowledgement: We are very grateful to CAPES, FAPEMIG (APQ 00512-14) and CNPq (426933/2016-8).

PB0625 | How Does Changing the Reagent/Instrument Affect the INR?

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Background: It is admitted that prothrombin time is affected by thromboplastin reagent. International normalized ratio (INR) was designed to standardize prothrombin time variation for a better monitoring of patients on antivitamin K (AVK) medication. However, several studies have shown that INR might vary with different reagent/instrument combinations.

Aims: To assess the agreement between INR values obtained with two different thromboplastin/instrument combinations.

Methods: INR was measured on plasmas from 331 patients undergoing AVK treatment using two reagent/instrument combinations: *Both Neoptimal* and *Neoplastine CI plus* on *STA-R* instrument from *Diagnostica STAGO, Asnières, France and Innovin* on *SYSMEX 2100i* instrument from *Siemens Health Care Diagnostics, Marburg, Germany.* The agreement for each reagent/instrument combination was evaluated using the Bland-Altman plot and Cohen's Kappa coefficient.

Results: When comparing INR values obtained with both *Neoptimal* and *Neoplastine CI plus*, the mean difference was −0.01 and the agreement limits were [−1.16 to 0.96] (figure 1). Cohen's kappa coefficient was 0.77. For INR values superior to 4.5, the mean difference was 0.26 [−1.25 to 1.77].

As for the second combination (*Neoptimal* vs *Innovin*), the mean difference was −0.28 and the agreement limits were [−2.16 to 1.6] (figure 2). When INR was superior to 4.5, the mean difference was −0.7 [−4.3 to 2.9]. Cohen's kappa coefficient was 0.76.

Conclusions: The agreement limits between *Neoptimal* and *Neoplastine CI plus* are small enough to consider that the reagent can

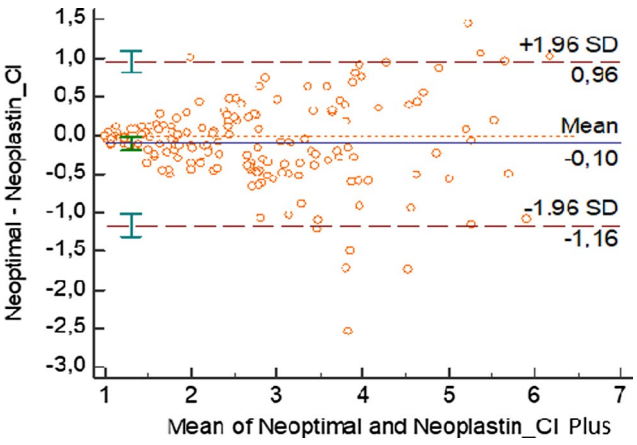

FIGURE 1 The Bland-Altman plot: difference against mean for INR values obtained with Innovin and Neoptimal

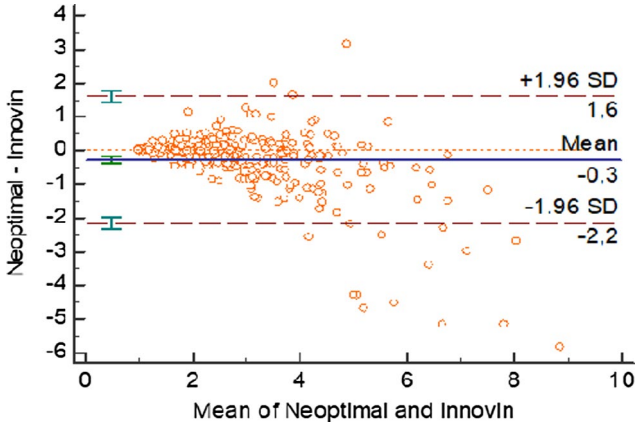

FIGURE 2 The Bland-Altman plot: difference against mean for INR values obtained with Neoplastine CI plus and Neoptimal

be used interchangeably which is supported by a substantial agreement as shown by Cohen's kappa coefficient.

This is not the case for the couple *Neoptimal* and *Innovin* in which the agreement limits are wider, particularly when INR values are superior to 4.5. An adjustment in anticoagulant dosage might be necessary.

PB0626 | An Evaluation of Treatment Monitoring in Patients with VWD Using a Chemiluminescent (VWF:GP1bR) Assay Compared to a Lyophilised Platelet Assay with Ristocetin (VWF:RCo)

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Background: Measurement of von Willebrand factor (VWF) activity levels following treatment with Desmopressin (DDAVP) or VWF containing concentrates in patients with VWD is recommended by various national and international guidelines. UKHCDO recommends during major surgery that factor VIII (FVIII:C) and VWF:RCo levels are used. Therefore, quick turnaround times of test results are essential.

Aims: Investigate a chemiluminescent VWF activity assay (VWF:GP1bR) against our current lyophilised platelet ristocetin based assay (VWF:RCo).

Methods: Residual post treatment samples (n=53, DDAVP n =6, Haemate P n= 26, Voncento = 1 Alphanate n= 3, Wilate n=3, Wilfactin n= 14) from 24 patients with VWD (types - 1 n=10, 2A n=15, 2B n=6, 2M n= 17, 3 n=2, acquired VWD n =1) were tested using chemiluminescent ACL AcuStar VWF Ristocetin Cofactor activity assay (HemoSII, USA) (VWF:GP1b:R). VWF:RCo was measured by a lyophilised platelet method with ristocetin (BC von Willebrand assay, Siemens, Germany) on a CS2100i (Sysmex, UK). Both assays were calibrated with standards traceable to the WHO international standard for VWF in plasma.

Results: Bland Altman (BA) analysis of all results calculated a bias of −29.9 (SD = 31.7) with 95% limits of agreement (LoA) −91.9-32.2. Additional BA analysis was done by separating the VWF:RCo results into group 1 (VWF:RCo levels < 100 IU/dL) and group 2 (VWF:RCo levels ≥100 IU/dL). Group 1 samples bias was −23.34 (SD =15.53) and 95% LoA −53.78 to 7.1 and group 2, bias was −34.6, (SD = 42.01) with 95% LoA −116.9 to 47.73. FVIII:C levels in 42 (79%) of samples were ≥100 IU/dL. Overall percent difference between results showed that the VWF:GP1bR assay was 31.9% higher with an SD of ±33%.

Conclusions: This study of post infusion monitoring samples showed that VWF:GP1bR levels were generally higher compared to VWF:RCo results. Further investigation is required to evaluate the differences.

PB0627 | Evaluation of the Succeeder SF-8200 Fully Automated Coagulation Analyzer

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Background: The SF-8200 is a new coagulation analyzer developed by Beijing Succeeder Technology Inc. China. SF-8200 able to perform clotting, chromogenic and immune-turbidimetric tests. System capability is a throughput of up to 360 tests per hour, 60 sample tubes can be loaded at any time and its reaction cuvette capacity is 1000. The analyzer also has an optional cap piercing module to reduce manual sampling time.

Aims: We aimed to perform an analytical performance comparison study between Succeeder SF-8200 and Stago Compact Max3 because of fully automated coagulation analyzers have become one of the most important components of clinical laboratories.

Methods: We assessed routine coagulation tests, which are the most ordered in laboratories such as PT, aPTT and fibrinogen. Stago Compact Max-3 was accepted as the reference instrument in the comparison study. The assay precisions were assessed using fresh and pooled plasma samples or consumer internal quality controls. Haemolysis, lipemia and icterus interferences were also verified.

Results: The coefficients of variation, assessed in the intra and inter-assay precision analyses were below 5% representatively for assessed parameters. The inter-analyser comparison demonstrated good results. Results, obtained by the SF-8200 showed a high comparability predominantly to used reference analyzers, with correlation coefficients ranging from 0.911 to 0.953. In our routine laboratory setting, SF-8200 reached a sample throughput rate of 360 tests per hour. No considerable influence on tests was found for elevated levels of free hemoglobin, bilirubin or triglycerides.

Conclusions: In conclusion, the SF-8200 was found to be an accurate, precise and reliable coagulation analyzer in routine testing. According to our study, the results demonstrated a good technical and analytical performance.

PB0628 | Evaluation of Hemostatic Markers in Patients with Sickle Cell Disease Using or Not Hydroxyurea

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Background: Hydroxyurea (HU) is the most successful pharmacological therapy with improvement in the quality of life of patients with sickle cell disease (SCD). Studies have reported hemostatic disorders in SCD, such as elevated plasma levels of plasminogen activator inhibitor type 1 (PAI-1) and D-dimer (D-Di).
Aims: To assess the association between the use of HU and plasma levels of PAI-1 and D-Di hemostatic markers in patients with SCD.
Methods: Cross-sectional study enrolling 40 patients with SCD from the Hemonúcleo Regional de Divinópolis / MG, Brazil, aged over 18 years, using (n = 16) or not (n = 24) HU, and 40 healthy controls matched by gender and age. The hemostatic markers PAI-1 and D-Di were determined by ELISA, whose results were expressed as mean ± SD and median (interquartile range), respectively, and analyzed by t Student's, Mann-Whitney, ANOVA and Kruskal-Wallis tests, with significance level < 0.05. The study received approval from the local Ethics Committee.
Results: Plasma levels of PAI-1 and D-Di were higher in patients with SCD [113.64 ± 24.89 ng/mL and 611.07 (402.82-155.25) ng/mL, respectively] than in the controls [65.00 ± 25.24ng/mL and 74.77 (49.55-122.65) ng/mL, respectively] (p < 0.001 for both). However, there was no significant difference in the plasma levels of these markers between patients with SCD using or not HU.
Conclusions: The use of HU was not able to favorably modify the hemostatic changes resulting from SCD, such as hypofibrinolysis and hypercoagulability, conditions related to the increase in plasma levels of PAI-1 and D-Di, respectively.
Ackowledgement: We are very grateful to the staff of Hemonúcleo Regional de Divinópolis, MG, Brazil, for the valuable help, as well as to CNPq, FAPEMIG and CAPES for the financial support.

PB0629 | The Effect of Haemolysis in Patient Samples on Coagulation Tests Performed on ACL TOP 550

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Background: Accurate result reporting is the cornerstone of laboratory diagnosis and haemolysis remains the leading cause of sample rejection. There is significant variation in the influence haemolysis has on coagulation tests likely due to the multifactorial causes of *in vitro* haemolysis.

TABLE 1 Effect of haemolysis on routine clotting tests performed on ACL TOP 550 (IL)

Test	Non-haemolysed samples (mean)	Haemolysed samples (mean)
PT (sec)	16.6	16.4
Derived Fibrinogen (g/L)	5.2	5.2
Clauss Fibrinogen (g/L)	4.5	4.4
Thrombin Time (sec)	15.3	14.8
APTT (sec)	32.3	31.6
Plasma Haemoglobin (mg/dL)	10	160

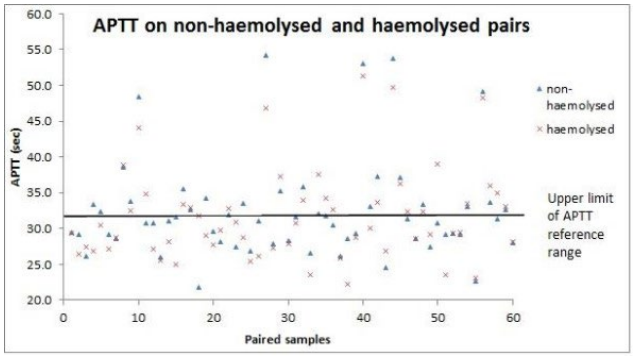

FIGURE 1 APTT on haemolysed and non-haemolysed paired samples on ACL TOP 550 (IL)

Aims: Evaluate the influence of haemolysis on routine clotting tests performed on the ACL TOP 550 (Instrumentation Laboratory).
Methods: Samples were collected in BD vacutainer tubes and centrifuged at 2000g for 10 minutes. Samples from patients rejected due to haemolysis with a replacement non-haemolysed sample sent within 4 hours constituted a paired set (n=60). Tests performed: Prothrombin time (PT) and derived fibrinogen (HemosIL® RecombiPlasTin 2G), activated partial thromboplastin time (APTT, HemosIL SynthASil), Clauss Fibrinogen (HemosIL Q.F.A Thrombin) and Thrombin Time (TT, HemosIL Thrombin Time) from Instrumentation Laboratory were tested on an ACL TOP 550 (Instrumentation Laboratory). Plasma haemoglobins were measured on Sysmex XN9000.
Results: PT: 4 pairs showed ≥10% change with a maximum change −4.2 sec, from a warfarinised patient. Derived Fibrinogen: 1 sample with ≥10% change, 2.92 to 3.92 g/L. Clauss Fibrinogen: 16 pairs with ≥10% change but maximum change −1.2 g/L. TT: 25 pairs with ≥10% change, but only 3 pairs showed > 4 sec difference. APTT: 12 pairs showed changes resulting in shifts above and below the upper limit of normal range resulting in false normalisation or prolongation of

APTT. Plasma haemoglobin was less than 220 mg/dL in all haemolysed samples.

Conclusions: Of the routine parameters, APTT is the most sensitive to haemolysis. In our study, 20% of paired samples showed changes in APTT that could be clinically relevant. In other assays, changes were unlikely to be of clinical significance.

PB0630 | Analysis of Platelet Functional Activity by Flow Cytometry: Reference Intervals and Cut-off Value for Evaluating the Efficiency of Antiplatelet Therapy

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Background: The main problem of flow cytometry for platelet testing is the lack of reference intervals for markers on the platelet surface. Moreover, there are no criteria for the effectiveness of antiplatelet therapy when using this method as a diagnostic test.

Aims: To determine the reference intervals for markers of platelet functional activity among healthy donors by flow cytometry, as well as to determine the cut-off value for evaluating the dual antiplatelet therapy (DAT) effectiveness in patients with peripheral artery diseases (PAD).

Methods: The study included 29 patients (over the 40 years) with PAD and 120 healthy donors (18-65 year). All patients on day 14 of DAT (75mg clopidogrel+100mg ASA) were determined by the expression of P-selectin on the platelet surface before and after activation of 20mkM ADP. This analysis was performed on CytomicsFC500 flow cytometer using CD41/61-FITC and CD62P-PE mAB (Beckman Coulter, USA). The light transmission aggregatometry and VerifyNow (Accumetrics, USA) were chosen as a comparison method.

Results: In the female, compared with male, the platelet count was significantly higher: $246\pm12\times10^9$ cells vs $208\pm12\times10^9$ cells, respectively (p=0.02), but did not go beyond the accepted reference interval $150\text{-}400\times10^9$ cells. For male, the reference interval for the number of platelets expressing P-selectin on its surface reached 0.1-1.5%/cell and 9.7-42.7%/cell before and after ADP activation, respectively. And for female this reference interval was 0.2-4.1%/cell and 13.9-50.5%/cell before and after ADP activation, respectively. The cut-off value for effective DAT in PAD patients was the number of platelets expressing P-selectin below 12.0%/cell (sensitivity 96.6%, specificity 98.5%).

Conclusions: We determined the reference intervals for male and female, and set the cut-off value to assess the effectiveness of antiplatelet therapy. This study facilitates the transfer of platelet function testing by flow cytometry from a scientific instrument to a diagnostic test for use in clinical laboratories.

PB0631 | Evaluation of IgG4 ELISA for the Screening of Factor VIII Inhibitors in Hemophilia A

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Background: Inhibitor development against factor VIII in patients with hemophilia A (PWHA) renders replacement therapies ineffective. IgG4 and to a lesser extent, IgG1 are the most abundant IgG subclasses in patients with FVIII inhibitors. Quantitative measurement of inhibitors is performed by Nijemegen Bethesda Assay (NBA) considered the gold standard. However requirement for large sample volumes, involved cost and requisite technical expertise make NBA challenging especially in developing countries. ELISA based screening would be more feasible to perform in resource poor setup.

Aims: To standardize and evaluate in-house IgG4 ELISA for diagnosis of inhibitor in PWHA.

Methods: It is a case series for diagnostic test evaluation. Study group comprises 30 PWHA with inhibitors (table 1), 33 PWHA without inhibitors, and 33 normal healthy controls. Complete coagulation workup was performed. In-house IgG4 ELISA was standardized. Checkerboard method for optimization of plasma-derived Factor VIII (HEMOFIL M Baxalta US Inc.], sample dilutions, and Antihuman IgG4-HRP conjugated (Southern Biotechnology Inc., Al, USA) concentration was performed. Multiple checks were exercised for consistency of results. Intra-plate, inter-plate and day to day variations of ELISA was evaluated by repeated analysis of all three study group samples. Samples were tested in triplicate. Cutoff optical density was calculated from normal healthy controls and PWHA without inhibitor (n=66) by calculating mean plus 2 SD. Sensitivity, Specificity, PPV, NPV and accuracy were calculated.

Results: The cutoff values of IgG4 ELISA derived by above method at 0.502 discriminates healthy controls and PWHA without inhibitors from PWHA with inhibitor with 93.3% sensitivity, 97.0% specificity, 97% NPV and 93.3% PPV with an accuracy of 95.83%.(table 2)

Conclusions: IgG4 ELISA is a promising tool for screening for PWHA with inhibitors. Sensitivity may be increased by using an enhancer with secondary antibody for improving the colour development.

TABLE 1 Inhibitor Titres in PWHA with inhibitors (n=30)]

Range Of Inhibitor Titre	Mean Inhibitor Titre	High Titre (>=5BU/ml) N (%)	Low Titre (<5BU/ml) N (%)
0.8-108.0 BU	17.04 BU	19(63.33%)	11(36.66%)

TABLE 2 2×2 table for estimating the sensitivity, specificity, PPV and NPV. Gold standard for Inhibitor status is NBA

	Cases (PWHA with inhibitor)	Control (PWHA without inhibitor + normal healthy controls)	
Elisa positive	28 (True positive)	2 (False positive)	30
Elisa negative	2 (False negative)	64 (True negative)	66
Total	30	66	96

Figure 1. Comparison of basic coagulation tests, lupus anticoagulant, thrombin time, anti-Xa and DOAC levels before and after the addition of DOAC-Remove™ using Mann-Whitney U test. Results are expressed by mean ± standard deviation. Values of $p<0.05$ were considered statistically significant. Five patients were true LA-positive: 2 on dabigatran, 2 on apixaban and 1 on edoxaban.

	LA positive DOAC patients (n= 16)											
	Dabigatran (n=5)			Rivaroxaban (n=4)			Apixaban (n=4)			Edoxaban (n=3)		
	Pre-DOAC Remove™	Post-DOAC Remove™	*p*	Pre-DOAC Remove™	Post-DOAC Remove™	*p*	Pre-DOAC Remove™	Post-DOAC Remove™	*p*	Pre-DOAC Remove™	Post-DOAC Remove™	*p*
PT (s)	14.04 ± 1.55	11 ± 0.47	<0.05	19.67 ± 5.61	10.85 ± 1.11	<0.05	16.47 ± 2.27	11.37 ± 1.31	<0.05	12.43 ± 2.22	12 ± 2.35	NS
aPTT (s)	55.9 ± 3.99	35.34 ± 3.34	<0.05	37.40 ± 6.14	32.50 ± 0.97	<0.05	39.93 ± 4.71	34.25 ± 6.80	<0.05	34.43 ± 3.99	31.53 ± 2.14	NS
dRVVT ratio	1.43 ± 0.24	1.08 ± 0.17	<0.05	1.91 ± 0.43	1.06 ± 0.09	<0.05	1.41 ± 1.04	1.23 ± 0.93	NS	1.29 ± 0.10	1.18 ± 0.04	NS
SCT ratio	1.14 ± 0.48	1.11 ± 0.36	NS	0.95 ± 0.13	1.06 ± 0.14	NS	1.48 ± 0.58	1.44 ± 0.63	NS	1.14 ± 0.16	1.09 ± 0.10	NS
Anti-Xa (iu/ml)				1.77 ± 1.01	0.04 ± 0.02	<0.05	2.57 ± 1.74	0.09 ± 0.08	<0.05	0.10 ± 0.09	0.01 ± 0.01	NS
TT (s)	195.83 ± 85.92	19.1 ± 3.11	<0.05									
DOAC plasma levels (ng/ml)	173.3 ± 107.1	2.23 ± 0.25	<0.05	377.32 ± 313.7	16.95 ± 10.55	<0.05	385.42 ± 275.7	13.10 ± 9.55	<0.05			

	LA positive patients not on a DOAC (n= 10)			LA negative patients not on a DOAC (n= 10)		
	Pre-DOAC Remove™	Post-DOAC Remove™	*p*	Pre-DOAC Remove™	Post-DOAC Remove™	*p*
dRVVT ratio	1.35 ± 0.31	1.37 ± 0.36	NS	0.87 ± 0.09	0.90 ± 0.09	NS
SCT ratio	2.33 ± 0.63	2.35 ± 0.71	NS	0.98 ± 0.08	1.01 ± 0.06	NS

PT= prothrombin time; aPTT= activated partial thromboplastin time; dRVVT= diluted Russell viper venom time; SCT= silica clotting time; Xa= activated factor X; s= seconds; TT= thrombin time; DOAC= direct oral anticoagulant; iu= international units; ml= milliliters; ng= nanograms

FIGURE 1 Comparison of basic coagulation tests, lupus anticoagulant, anti-Xa and DOAC levels before and after the addition of DOAC-Remove

PB0632 | Doac-Remove™ Can Neutralize the Effect of Direct Oral Anticoagulants on Lupus Anticoagulant Assay: A Single-centre Experience

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Background: Despite known effects of direct oral anticoagulants (DOAC) on plasma-based assays for thrombophilia screening or coagulation factors (Kitchen, 2014), these tests are frequently requested by clinicians for DOAC patients. Several studies have investigated the use of a simple charcoal product called DOAC-Remove™ (5-Diagnostics, Quadratech, Switzerland) to eliminate DOAC interference from patient samples undergoing coagulation tests (Cox-Morton, 2019; Jourdi, 2019).

Aims: To describe our haemostasis laboratory experience with DOAC-Remove™ in LA-positive samples of DOAC-patients.

Methods: This preliminary single-centre study included 10 LA-negative subjects, 10 LA-positive patients not on a DOAC or any other anticoagulant and 16 LA-positive patients treated with a DOAC. The presence of LA was determined according to guidelines of the *International Society on Thrombosis and Haemostasis (ISTH)*, using the diluted Russell Viper Venom time (dRVVT) and the Silica Clotting Time (SCT) integrated tests. PT, aPTT and LA tests were measured on ACL Top 700 analyzer (Instrumentation Laboratory, MA, USA). The results were expressed on normalized *ratios* and our cut-off values were 1.2 for dRVVT and 1.3 for SCT. All samples were treated with DOAC-Remove™ used according to the manufacturer's instructions. DOAC removal was confirmed by anti-Xa or thrombin time as appropriate, DOAC levels and the effect on basic coagulation tests.

Results: After adding DOAC-Remove™ to LA-positive plasma of DOAC patients, 11 of 16 (68.75%) tested negative for LA. Interferences in PT and aPTT were observed in 13 DOAC patients, 10 (76.92%) of which were neutralized. There were 5 (31.25%) true LA-positive samples. No effect was seen in LA-negative and LA-positive subjects not on a DOAC (Figure 1).

Conclusions: In our experience, false positivities of LA in DOAC patients can be neutralized by pre-treating their citrated plasma samples with DOAC-Remove™. This strategy has the potential of saving costs by avoiding both repeated testing and misinterpreted LA results.

PB0633 | Influence of DOAC Remove on Coagulation Assays During Thrombophilia Testing in Patients Treated with Rivaroxaban or Dabigatran

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Background: Direct oral anticoagulants (DOAC) are increasingly being used as an alternative to warfarin in the treatment of venous thromboembolism. DOAC administration has a significant impact on coagulation assays. This is especially important during thrombophilia testing.

Aims: To evaluate the effect of DOAC Remove on coagulation assays during thrombophilia testing in patients treated with DOAC.

Methods: We have used samples from 20 patients treated with DOAC. Two samples of venous blood on sodium citrate were taken for each patient. One was prepared by standard method for coagulation assays, while, the second one was prepared using DOAC Remove. According to the instruction the samples were mixed gently for 5 minutes with DOAC Remove tablet, and then centrifuged 2 minutes on 2000g. The supernatant after centrifugation is used to the coagulation assays. So prepared samples were used in the thrombophilia testing.

Results: Before DOAC Remove in 10 patients treated with Rivaroxaban average APTT of 31.5s; PT 104%; LA1 51.7s; LA2 40.3s; LA- R 1.28,

AT 104%, PC 104%, PS 87% and APC-R 4.1 were obtained. In Dabigatran patients APTT 40.5s, PT 104%; LA1 73.7s; LA2 53.7s; LA-R 1.37, AT 103% PC 98% and PS 82% were obtained. In relation to the APC-R test, in half of the investigated patients no result for APC-R was obtained, while, for the rest, average APC-R of 6.5 were obtained. After DOAC Remove in Rivaroxaban patients APTT 29.7s; PT 110%; LA1 37.3s; LA2 33.8s; LA- R 1.12, AT 96%, PC 102%, PS 88% and APCR 4.2 were recorded. In Dabigatran patients APTT 28.1s, PT 117%; LA 39.1s; LA2 33.8s; LA-R 1.15, AT 104%, PC 97.5%, PS 84.5% and APC-R 6.5 were recorded.

Conclusions: DOAC was practically inactivated after the addition of the DOAC Remove, which made it possible to perform analyzes for the LA and APC-R testing freely and obtain relevant results.

PB0634 | Impact of Centrifugation on Thrombin Generation Analysis Evaluated in Healthy Subjects and Patients Treated with Direct Oral Anticoagulants

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Background: Double centrifugation before freezing is recommended for thrombin generation (TG) analysis. However, this preanalytical procedure is often time consuming and not well managed in clinical practice.

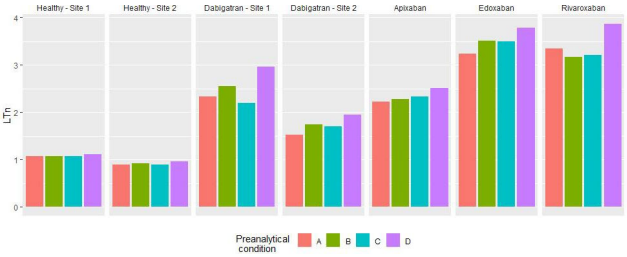

FIGURE 1 Normalized lag time (LTn) in the different centrifugation conditions (A, B, C and D; see text) for each DOAC

Aims: The aim of this study was to evaluate the impact of single and double centrifugation on TG results measured on frozen samples of healthy volunteers (HV) and patients treated with direct oral anticoagulants (DOAC).

Methods: Forty HV and 57 patients receiving a DOAC (apixaban, dabigatran, edoxaban or rivaroxaban) were included in this prospective double-center observational study. Blood was collected in 109mM citrated tubes and frozen at minimum −70°C before TG measurement using ST-Genesia® with STG-DrugScreen® reagent. Four pre-analytical conditions were studied: (A) single centrifugation (2000g, 15min) before freezing; (B) one centrifugation before freezing and another after thawing (2000g, 15min for both); (C) one centrifugation before freezing and another after thawing (2000g, 15 min then 2000g, 10 min); (D) double centrifugation (2000g, 15min) before freezing (reference). Normalized lag time (LTn), endogenous thrombin potential (ETPn) and peak height (PHn) were compared using the reference condition. Acceptable relative differences were defined at 6%, 8% and 10% for LTn, ETPn and PHn, respectively.

Results: TG parameters were significantly different between the two sites for dabigatran patients and for HV. Centrifugation conditions had no clinically significant impact on HV but single centrifugation always resulted in clinically significant reductions in LTn for DOAC samples (Figure 1). A second centrifugation after thawing permits the recovery of clinically acceptable differences for the three TG parameters for edoxaban and dabigatran (site 1) samples, but not for apixaban, rivaroxaban nor dabigatran (site 2) samples (Table 1).

TABLE 1 Differences of normalized thrombin generation parameters between pre-analytical conditions A, B, C and D (see text for description)

		Apixaban	Edoxaban	Rivaroxaban	Dabigatran Site 1	Dabigatran Site 2	Healthy volunteers Site 1	Healthy volunteers Site 2
	A vs. D	−6.5% **	−11.1% **	−11.2% ***	−22.5% *	−20.9% **	−3.7% **	−5.9% ***
LTn	B vs. D	−7.9% **	−5.8% **	−7.9% ***	−12.5%	−10.1% **	−3.5% **	−4.1% ***
	C vs. D	−6.6% *	−9.2%	−6.8% **	−25.6% *	−12.3% **	−4.0% **	−5.7% ***
	A vs. D	+14.0% ***	+9.1% **	+13.0% ***	+3.9%	+1.6%	+2.7% *	+1.2% *
PHn	B vs. D	+14.2% **	+2.2%	+3.0%	+4.8%	+2.3% *	+3.9% **	+2.8% ***
	C vs. D	+14.2% **	+9.6%	+7.3% **	+7.8% ***	+2.3% *	+2.8% *	+2.3% ***
	A vs. D	+8.4% *	+6.1% *	+15.1% ***	+3.3%	−1.7%	+1.7%	+1.5% *
ETPn	B vs. D	+4.2% *	+1.2%	+2.2%	−3.7%	+0.3%	+1.6%	+1.5% *
	C vs. D	+5.4% *	+2.6%	+7.5% *	+1.4%	+0.8%	+1.5%	+1.1%

Conclusions: Overall, single centrifugation before freezing is acceptable only for edoxaban samples and only if a second centrifugation is performed after thawing. Double centrifugation before freezing should remain the recommended pre-analytical condition for TG measurement.

PB0635 | Performance Evaluation of Automated Thrombin Generation Measurement on Ceveron® t100 with Normalized TGA Parameters

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Background: Fully automated thrombin generation on the Ceveron® t100 analyzer is a valuable tool for investigating blood coagulation activity, elevated thrombin activity suggesting a thrombotic tendency or depressed activity suggesting a bleeding tendency, when using standardized Ceveron® TGA kits. In addition to precise measurements between laboratories, easy result interpretation via normalization improves accessibility to diagnosticians.
Aims: Evaluate assay performance on the new Ceveron® t100 instrument regarding between-run imprecision for Peak Thrombin values and for the newly introduced normalization of Peak Thrombin values.
Methods: Two pathological samples (protein S deficiency and haemophilia A representing thrombotic and bleeding tendencies respectively) and a normal plasma for normalization were measured on 20 consecutive days on a Ceveron® t100. Two samples from patients on direct FXa inhibitors were also measured and normalized Peak thrombin (N-Peak) evaluated. Imprecision was calculated for Peak thrombin and N-Peak. A calibration curve was made at the beginning of the study using the standard analyzer settings for TGA calibration.
Results: CV for Peak thrombin of sample 1 improved from 7.12% to 6.72% when converted to N-Peak. Sample 2 had a Peak thrombin CV of 6.05%, improving to 5.38% for N-Peak. The normal sample had N-Peak value of 105%, the thrombophilic sample with protein S deficiency had 194% N-Peak and the haemophilic sample had 11.5% N-Peak. N-Peak and N-Lag ratio for the patient sample with 288ng/mL Apixaban were 41% and 2.0 respectively, and the sample with 322ng/mL Rivaroxaban 20% and 2.5 respectively.
Conclusions: These data demonstrate that automated TGA parameter normalization on Ceveron® series instruments improves analytical precision. Reference ranges for TGA parameters vary widely so normalization has potential to facilitate clearer understanding and interpretation of complex data sets. Further studies are required to generate reference ranges and anticipated ranges in thrombotic and bleeding disorders.

PB0636 | Quantification of Emicizumab in a Modified APTT and with Clot Growth Analysis

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Background: Quantification of modified coagulation factor VIII (FVIII), and the bifunctional monoclonal emicizumab (emi), in coagulation tests is complicated.
Aims: We evaluated a modified APTT for quantification of emi and the effects in clot growth analysis as pharmacodynamic model.
Methods: APTT with the STA and clot growth with Thrombodynamics. Commercial factor VIII, Haemate, and FVIII-deficient patient plasmas were used. Samples from three emi-treated patients were from the Stasey study (Roche).
Results: A modified APTT- based one-stage FVIII assay using advanced dilutions, showed a dose response curve of Haemate that paralleled a dose response curve of emi in FVIII deficient patient plasma: 1 U/ml FVIII was equivalent to 30-35 μg/ml emi. Importantly, FVIII deficient plasma supplemented with 50, 100 and 150% of recombinant FVIII as well as normal plasma, showed parallel curves for added emi and no contribution of FVIII in the assay. Thus, emi completely dominates the assay and can be quantified, also in the presence of FVIII. In three F VIII deficient patients treated with emi we detected no emi in the pre-treatment sample < LLOQ) and 56-66, 55-51 and 40-43 μg/ml emi in samples after 1- and 3-month protocol treatment.
In clot growth analysis correction with FVIII showed a non-linear response with a steep increase at low levels: 80% of correction was achieved at 0.2 U/ml Factor VIII. Emi showed a congruent pattern with 80% correction at 20 μg/ml emi. Analysis of clot growth in plasma of two patient treated with emi showed clot growth normalisation at 1- and 3-month treatment.
Conclusions: *In vitro* spiking in clot growth gave 80% correction with emi at relatively low concentrations (20 μg/ml). The APTT- based assay showed equivalence with I U/ml Factor VIII at 30-35 μg/ml. Both evaluations suggested adequate effects at lower emi concentrations than currently used in patient treatment.

PB0637 | Comparison of Different Stago Instruments for Fibrinogen Level Assessment with STA-liquid Fib

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Background: Fibrinogen assay is one pillar of routine coagulation screening and clotting Clauss method is established as the gold

standard, especially for patients with low fibrinogen levels (Miesbach W, Thromb Res 2010).

With the trend of laboratory services consolidation both through private organizations or within groups of public hospitals, and keeping in mind the need of ensuring patient medical care continuity, consistency of tests results whatever the analyzer is compulsory. Quality management plans within those pathology laboratories networks help verifying this consistency over time. Yet, manufacturers also have their responsibility in showing this agreement by design.

Aims: The objective of the study was to establish correlation and agreement of fibrinogen levels as measured with STA®-Liquid Fib on different Stago analyzers.

Methods: 65 frozen patients plasma samples, spread over the fibrinogen measuring range, were tested on STA® Satellite®, STA® Compact® Max and STA®-R Max analyzers, using the precalibrated STA®-Liquid Fib assay (Stago, France).

Each sample was tested on the 3 instruments in parallel and the whole set of samples was tested over 5 days to avoid any bias due to a single run.

Results: Linear regression was performed to evaluate correlations. Slope, intercept and Pearson correlation coefficients of those regressions are displayed in the table.

General agreement was confirmed through Bland and Altman analysis, with an overall mean bias lower than 0.03g/L for all comparisons.

Conclusions: Results confirm the high consistency of fibrinogen level assessment with STA®-Liquid Fib across Stago analyzers: As standardization of fibrinogen results is crucial for accreditation purposes, these good results also preclude an easy method validation process. Available under liquid format and precalibrated for all Stago analyzers, STA®-Liquid Fib is a flexible solution that can be used advantageously by any laboratory performing fibrinogen level determination with Clauss method while ensuring consistency of patients results within pathology networks.

TABLE 1 Summary of method comparisons of fibrinogen levels with STA®-Liquid Fib on 3 different types of Stago instruments

Analyzers comparison	Linear regression equation y = ax + b		Pearson correlation coefficient
STA®-Liquid Fib (g/L)	Slope (a)	Intercept (b)	(r)
STA® Compact® Max vs STA®-R Max	0.9573	0.1836	0.9894
STA® Satellite® vs STA®-R Max	1.0095	−0.0136	0.9982
STA® Satellite® vs STA® Compact® Max	1.0306	−0.1089	0.9860

PB0638 | Analytical Validation of a Novel Whole Blood Coagulometer for NOAC Testing

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[1]*Perosphere Technologies Inc., Danbury, United States;* [2]*Hofstra Northwell School of Medicine, Hempstead, United States*

Background: While use of the non-vitamin K antagonist oral anticoagulants (NOACs) does not currently require routine coagulation monitoring, this can be highly desirable in at-risk patients, including those suffering major trauma or requiring emergency surgery. However, a point-of-care (PoC) device for the rapid, bedside measurement of clotting times in patients on NOACs is currently not available.

Aims: To characterize the sensitivity of Perosphere Technologies' PoC Coagulometer to NOAC-induced anticoagulation, as well as instrument precision, via two studies: Study 1 - analytical measuring range, and Study 2 - a methods comparison to manual whole blood clotting time (mWBCT).

Methods: These studies were performed using healthy volunteers; for each study, whole blood samples were spiked with either 0 (sham), 30, 75, 150, 300 and 400 (apixaban, edoxaban) or 450 (rivaroxaban) ng/mL of a NOAC, in randomized order. In Study 1, a subject's divided blood sample was spiked with all concentrations and simultaneously tested on 24 PoC Coagulometers. In Study 2, a single concentration was tested per day, over 6 days, and samples were tested on 5 PoC Coagulometers and by 5 operators performing mWBCT, simultaneously, for comparison.

Results: In Study 1, PoC Coagulometer clotting times exhibited linearity across the measuring range, and sensitivity to even the lowest NOAC concentrations tested, as well as a high level of precision with notably low %CVs (Fig. 1). In Study 2, a strong linear correlation was observed for these methods, with R^2 values of nearly 1 (Fig. 2). Across all concentrations, the sensitivity of the PoC Coagulometer was significantly higher when compared to mWBCT, with absolute values of mWBCT measuring roughly twice those of the PoC Coagulometer.

Conclusions: These results suggest that this PoC Coagulometer could be ideal to assess the pharmacodynamic effects of the NOACs. The PoC bedside instrument delivers results within minutes, requiring only a drop of whole blood.

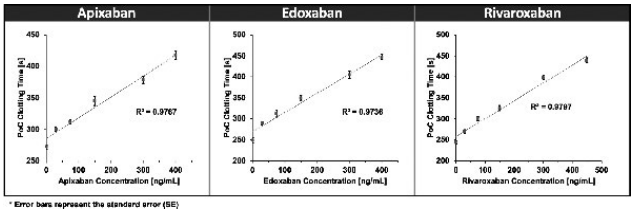

FIGURE 1

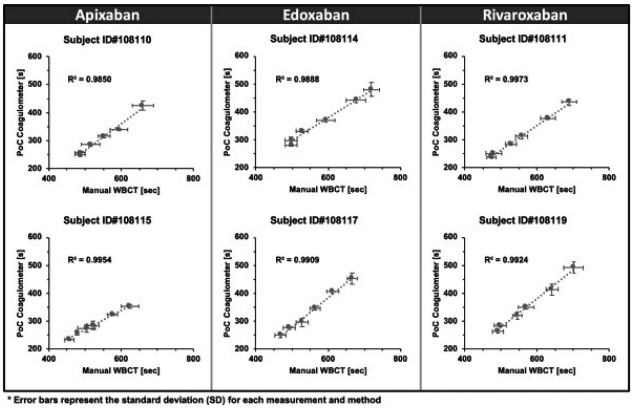

FIGURE 2

PB0639 | Rivaroxaban 20 mg Oce Daily Decreases Thrombin Generation in Patients with Venous Thromboembolism for up to 24 Hours

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Background: Common laboratory tests should not be used to monitor the anticoagulant effect of rivaroxaban (RIVA).
Aims: Thrombin generation assay (TGA) and calibrated anti-Factor X (anti-FXa) test were used to determine the effect of the RIVA 20 mg once-daily on coagulation in patients with venous thromboembolism (VTE).
Methods: Venous platelet-poor plasma (PPP) was obtained from 53 patients after 3 months of RIVA treatment. The first sample was collected 2 hours after drug ingestion (C_{max}) and the second collected 30 minutes prior to the next dose (C_{trough}). Control PPP was obtained from 178 healthy subjects without antithrombo-tics. Anti-FXa was measured using BIOPHEN® DiXal kit (Hyphen Biomed) and PT was tes-ted using Thromborel® S kit (Siemens Diagnostics) with BCS-XP® analyser (Siemens Diagnostics). TGA was determined using the kits for "thrombophilia tendency" testing (Technothrombin® TGA RC Low) and for "anticoagulant activity" testing (Technothrombin® TGA RC High) with Ceveron® alpha analyser (Technoclone). Results of the TGA were calculated for area under the curve (AUC). Paired t-test, Wilcoxon test and Pearson correlation test were used. Statistical significance was $p<0.05$.
Results: AUC TGA RC Low and AUC TGA RC High in patients treated with RIVA were lower at C_{max} and C_{trough} than those in the control group ($p< 0.001$, all). Negative correlations ($p< 0.05$, all) were observed between anti-FXa with AUC TGA Low and with AUC TGA High at C_{max} and at C_{trough}, except the negative correlation between anti-FXa and AUC TGA RC High ($r = -0.251$, $p = 0.069$, N.S.).
Conclusions: Once-daily 20 mg RIVA regimen is sufficient to decrease TG in patients with VTE in comparison with TG results in healthy persons. TGA (unlike anti-FXa) may be a useful tool for measuring inhibition of coagulation in patients receiving rivaroxaban for up to 24 hours.

PB0640 | Evaluation of Thrombogenicity of Apheresis-collected Platelet Concentrates under Blood Flow Condition

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Background: Platelet concentrates (PCs) are widely used for the treatment of thrombocytopenia and active platelet-related bleeding. However, detailed pharmacological efficacy of PCs to improve hemostatic ability is not fully understood.
Aims: To comparatively evaluate platelet reactivity for specific agonists and overall thrombogenicity of apheresis-collected PCs.
Methods: Three different apheresis-collected PCs were provided by Japanese Red Cross. Agonist-inducible platelet aggregation was evaluated by light transmittance aggregometry (LTA) and Multiplate, and total thrombus-formation analysis system (T-TAS) was used to evaluate thrombogenicity under flow conditions inside a microchip coated with collagen and tissue thromboplastin.
Results: White thrombi comprising activated platelets and fibrin fibers were formed inside the T-TAS microchip, and the occlusion time (OT) due to thrombus formation was 8.1±0.9 min. Thrombus formation was efficiently suppressed by SCH79797 (PAR-1-antagonist), OS-1 (GPIbα-antagonist) and abciximab (GPIIbIIIa-antagonist) in a dose dependent manner, whereas the effect of aspirin (100 μM) and AR-C66096 (P2Y12-antagonist; 1 μM) were limited even in combination (OT; 8.5±1.1 min).
Platelet aggregation induced by ADP and ristocetin was significantly impaired in the PC samples, whereas collagen and TRAP-6 induced aggregation was relatively preserved. Maximum aggregation of LTA with ADP (6.5 μM), ristocetin (0.77 mg/mL), collagen (3.2 μg/mL) and TRAP-6 (32 μM) were 13.0±6.9, 2.0±0.0, 50.8±9.3 and 42.1±16.0, and Multiplate-AUC were 1.4±1.4, 41.4±13.5, 100.7±2.9 and 66.9±11.1, respectively.
Conclusions: Platelet activation mediated by subendothelial collagen and thrombin might be important for PCs to achieve hemostasis, and thrombogenicity analysis by T-TAS might be a useful tool for assessing the functional hemostatic property of PCs.

PB0641 | A Novel Point-of-Care Whole Blood Coagulation Assay to Screen for Hemostatic Defects

S. Pourang[1]; M. Suster[1]; D. Maji[1]; S. Butala[2]; J. Nutt[1]; L. Nayak[1,3]; P. Mohseni[1]; S. Ahuja[1,2]

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Background: Screening coagulation assays (PT/PTT) have poor sensitivity and specificity for detection of hemostatic defects. We have developed a novel, point-of-care (POC), whole blood assay based on a dielectric microsensor (ClotChip) that provides global coagulation assessment in a miniaturized, portable measurement platform

Aims:

To assess the ClotChip sensitivity to detect hemostatic defects:

Methods: Patients (n=37) presenting with bleeding symptoms or with abnormal coagulation screening pre-operatively, and undergoing a bleeding disorder work-up, were enrolled. Blood samples were obtained by venipuncture in 3.2% sodium citrate. Coagulation was induced with $CaCl_2$. ClotChip was based on the electronic technique of dielectric spectroscopy (DS) integrated into a disposable microfluidic channel with < 10 µL sample volume. ClotChip curves were calculated as variation in blood permittivity at 1MHz, and time to reach a peak in permittivity (T_{peak}) was taken as coagulation time. Sensitivity and specificity of ClotChip were calculated for detection of a hemostatic defect

Results: ClotChip exhibited mean T_{peak} of 435 ± 22 sec (mean ± SEM) for patients with clinically normal hemostatic function. Mean T_{peak} for patients with a hemostatic dysfunction was 751 ± 159 sec and was significantly prolonged (P=0.002) compared to patients in the normal group. A receiver operator characteristic (ROC) curve (Fig. 1)
was generated to determine the true-positive rate (sensitivity) and false-positive rate (100% - specificity) for ClotChip T_{peak} to detect hemostatic dysfunction, and the area under the curve (AUC) for ClotChip T_{peak} was 0.88. The sensitivity and specificity of ClotChip T_{peak} with a threshold of 540 sec were determined as 78% and 93%, respectively (Table 1)

Conclusions: Our studies demonstrate a novel, microfluidic, dielectric sensor (ClotChip) was sensitive for detection of hemostatic defects. ClotChip has potential to fulfill an unmet clinical need as a POC tool to triage extensive work-up for bleeding symptoms or abnormal coagulation screening tests

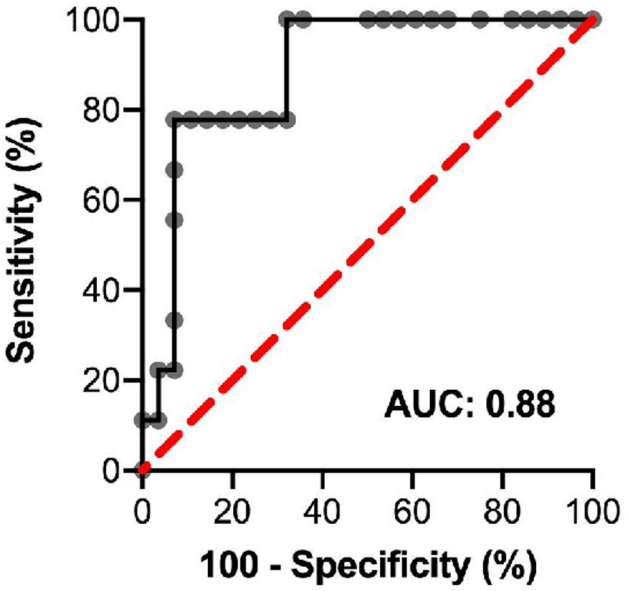

FIGURE 1 ROC Curve

PB0642 | Artificial Intelligence to Identify Neutrophil Extracellular Traps in Peripehral Blood Smears

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Background: Digitized microscopy capable of WBC differentials such as CellaVision® has revolutionized the laboratory. Smudge cells are usually seen in lymphoproliferative disorders representing remnants from degenerated lymphocytes (DLs). We discovered that the CellaVision® classifies not only DLs as smudge cells but also web-like remnants. We hypothesized that web-like remnants are Neutrophil Extracellular Traps (NETs). NETs are extracellular decondensed chromatin networks essential in immunity. Currently there is no clinical test available to identify and quantify NETs in blood.

Aims: To develop an *in-vitro* model for NET formation in blood and create a library of NET morphological changes at different maturation stages. Then develop an artificial intelligence (AI) platform for rapid and accurate detection of NETs on blood smears.

Methods: NET formation was induced with classic triggers (phorbol-12-myristate-13-acetate, lipopolysaccharide, ionomycin)

TABLE 1 Sensitivity and Specificity Results

	Hemostatic Defect Present	Hemostatic Defect Absent	Total
ClotChip Abnormal	7 (True Positive)	2 (False Positive)	9
ClotChip Normal	2 (False Negative)	26 (True Negative)	28
Total	9	28	37

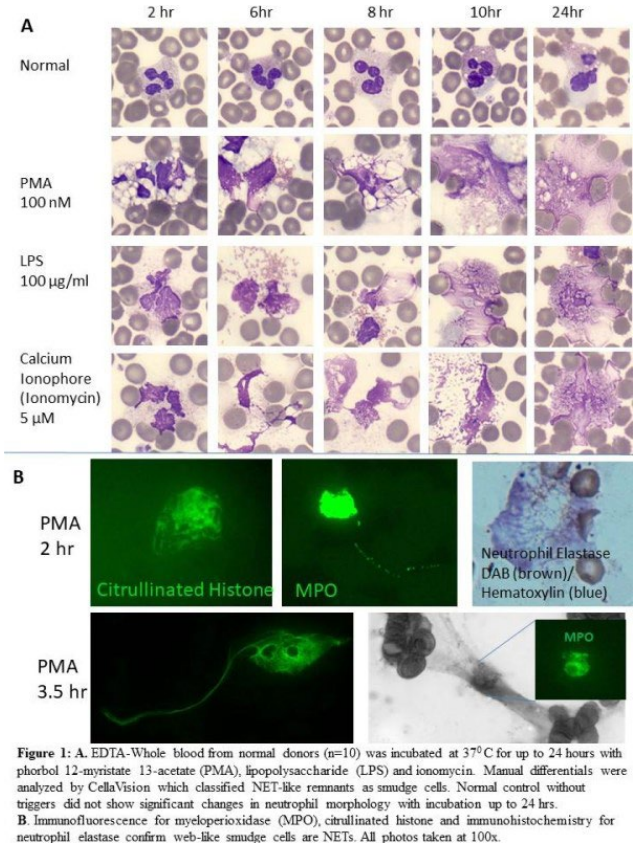

Figure 1: **A.** EDTA-Whole blood from normal donors (n=10) was incubated at 37^{0}C for up to 24 hours with phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide (LPS) and ionomycin. Manual differentials were analyzed by CellaVision which classified NET-like remnants as smudge cells. Normal control without triggers did not show significant changes in neutrophil morphology with incubation up to 24 hrs.
B. Immunofluorescence for myeloperioxidase (MPO), citrullinated histone and immunohistochemistry for neutrophil elastase confirm web-like smudge cells are NETs. All photos taken at 100x.

FIGURE 1 In Vitro time course of NET formation in whole blood

in EDTA-whole blood from normal donors. Smears were prepared at 30-minute intervals for 24 hours. Immunofluorescence and immunohistochemistry were used to identify NETs. WBC differentials were performed by CellaVision® to capture NETs at different stages and create a library of NET images. Since CellaVision® cannot differentiate NETs from DLs within smudge cells, we use this NET library to guide morphological selection of NETs from patients with high %

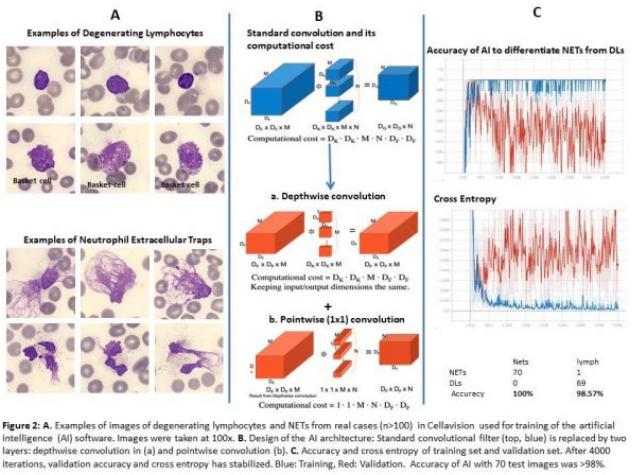

Figure 2: **A.** Examples of images of degenerating lymphocytes and NETs from real cases (n>100) in Cellavision used for training of the artificial intelligence (AI) software. Images were taken at 100x. **B.** Design of the AI architecture: Standard convolutional filter (top, blue) is replaced by two layers: depthwise convolution in (a) and pointwise convolution (b). **C.** Accuracy and cross entropy of training set and validation set. After 4000 iterations, validation accuracy and cross entropy has stabilized. Blue: Training, Red: Validation. Accuracy of AI with 70 test images was >98%.

FIGURE 2 Artificial Intelligence design to identify NETs in peripheral blood smears

NETs. AI was modified from an online phyton-based convolutional neural network (CNN).

Results: Neutrophil morphological changes were similar accross all triggers. NET formation followed a canonical order of morphological changes: vacuolation, nuclear decondensation, degranulation and chromatin ejection

The NET library aided selection of NETs from real cases. The training dataset included 706 NETs and 550 DLs, for a total of 1256 images. Using CNN, we obtained a sensitivity and specificity >98% to differentiate NETs vs. DLs among smudge cells

Conclusions: We developed an AI platform that is able to identify NETs on peripheral smears. This approach can provide a rapid, inexpensive and accurate tool to screen patients with infections.

PB0643 | Monitoring of Different FVIII Replacement Drugs Using the FVIII One-stage Clotting Assay on Cobas t 511/711 Analyzers

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Background: Reliable measurement of factor VIII activity is essential for monitoring patients with hemophilia A during FVIII replacement therapy. Factor VIII extended half-live products can lead to discrepancies when assayed with different one-stage clotting and chromogenic FVIII assays.[1] The effect of different FVIII replacement drugs on the analytical performance of the FVIII assay on cobas t 511/711 analyzers has been investigated.

Aims: To evaluate the performance of the FVIII one-stage clotting assay on cobas t 511/711 analyzers to measure samples containing different FVIII recombinant drugs.

Methods: Severe hemophilia A plasma samples (George King Bio-Medical) were spiked with recombinant FVIII products at defined concentrations (0.01-1.2 IU mL^{-1}). FVIII activity was determined using a cobas t 711 analyzer in combination with the FVIII assay kit (Roche Diagnostics). Both sample preparation and measurements were conducted in triplicates. To evaluate the result the following acceptance criteria were used [>25% ± 20% (relative); >25% ± 5% (absolute)].

Results: The correlation for most of FVIII replacement drugs between the measured FVIII activity and the theoretical activity was within the defined acceptance limits across the complete analyzed range. Values for Jivi® and ADYNOVI® were slightly higher than the upper deviation limit between 10-80% and a significant underestimation was observed for samples spiked with AFSTYLA® above 20% (Figure 1).

Conclusions: Overall, most of the FVIII replacement drugs can be reliably measured with the FVIII assay on cobas t 511/711 analyzers without the need for a product specific reference standard. The recorded data set allows the interpretation of FVIII assay results of samples containing different FVIII products on cobas t 511/711 analyzers.

References: 1 S. Kitchen et al., Semin Thromb Hemost 43 (2017): COBAS and COBAS T are registered trademarks of Roche. All other product names and trademarks are the property of their respective owners.

PB0644 | Distinguishing Patients Presenting Prolonged Activated Partial Thromboplastin Time with and without Bleeding Diathesis by Comparison of Immediate and post Incubation Mixing Test

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Background: With different mechanism underlying the impairment of artificial coagulation reaction in APTT assay, the impact on the clotting time by LAC and coagulation factor specific antibodies differs in mixing studies performed immediately and post incubation.

Aims: To establish laboratory differentiation diagnosis of acquired hemophilia A in patients presenting prolonged activated partial thromboplastin time (APTT) with and without bleeding diathesis by comparing the outcome of correction test performed immediately and post incubation.

Methods: The coagulation function profile of 186 patients enrolled in the single center from March 2013 through November 2019 with prolonged APTT, including 42 acquired hemophilia A (AHA) patients with bleeding symptoms and 144 asymptomatic individuals, was retrospectively investigated. The association of bleeding manifestation and outcome of APTT correction tests, both immediate and post-incubation as represented by Rosner index (RI 1,2), were analyzed. Novel parameter, Shanghai index (SI 1,2), calculated by minus or dividing post incubation Rosner index with immediate Rosner index was also adopted in the study and its performance in laboratory differentiation diagnosis of AHA was also evaluated.

Results: The correction of APTT immediately after mixture was more prominent in AHA patients as represented by correction index RI 1 (median18.23; IQR:12.72-25.21vs 37.49 ± 11.70, p=0.000). The magnitude of correction was similar in AHA patients and individuals without bleeding complications when the clotting assay was performed after 2 hours incubation as shown by index RI 2 (43.42 ± 14.38vs 41.62 ± 11.60, p=0.277). Both the novel correction index SI 1 and SI 2 had excellent performance with Youden Indexover 0.9.

Conclusions: The kinetics of coagulation inhibition differs between AHA and asymptomatic individuals with prolonged APTT; the blood coagulation interreferences of the two conditions can be better characterized by index Shanghai concerning outcome of both immediate and post-incubation mixing study.

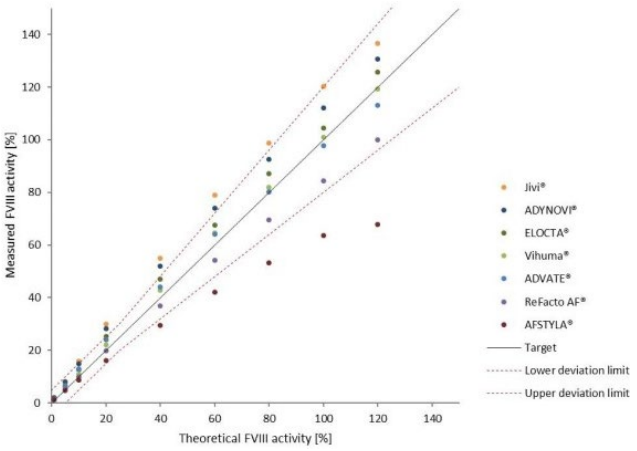

FIGURE 1 Recovery of different FVIII replacement drugs in the FVIII assay on cobas t 711

PB0645 | Multimer Analysis in von Willebrand Disease Diagnosis: the Experiency of Use

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Background: Von Willebrand disease (vWD) is the most common congenital bleeding disorder caused by quantitative or qualitative deficiencies of von Willebrand factor (vWF). There are no difficulties in diagnosis type I and type III of vWD by screening tests while subtypes IIA IIB, IIM, IIN require additional tests including multimer analysis. The existing techniques for analysis the multimer structure of vWF are manual, complicated, non-standard and time consuming.

Aims: Evaluate diagnostic capabilities of new automated method, vWF multimer screening assay.

Methods: 30 children with vWD, 15 with Acquire von Willebrand Syndrome (aVWS) and 8 healthy donors as a control group were enrolled in this study. To diagnosis vWD von Willebrand factor antigen (vWF Ag); ristocetin cofactor activity (VWF:Rco); Collagen Binding (VWF:CB); Ristocetin-Induced Platelet Aggregation (RIPA); Factor VIII clotting activity (FVIII:C);Factor VIII Binding Activity (vWF:FVIIIb) were performed. Multimer analysis was carried out using the commercial HYDRAGEL5 von Willebrand Multimers kit on semi-automatic gel electrophoresis instrument, HYDRASYS (SEBIA).

Results: The samples from control group have 9-12 bands of vWF multimers with the same distribution as control plasma; 13 patients with type I vWD have proportional decrease in the intencity of the bands with preservation of the normal distribution of the bands; 3 patients with type III vWD have complete absence of multimers on the gel. Multimer analysis in 9 cases shows the absence of HMW multimer bands 5 of them with increase RIPA (type IIB), 4-without (type IIA). In other 5 patients the distribution of vWF multimers was

normal against the changes in functional properties of vWF (IIM,N); 15 children with aVWS also showed normal distribution of vWF multimers.

Conclusions: Multimer analysis allows the visualization of multimers in various types of von Willebrand disease. The method is easy to perform and can be useful for classification of the subtype of vWD. But only full test panel including genetic tests will allow the classification of vWD type with high precision.

PB0646 | Establishing Reference Range and Validating Precision Performance of the TES-6s System

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Background: Viscoelastic assay is a real time analyzer of whole blood for coagulation. Thromboelastography (TEG), one of the viscoelastic assays, provides in vitro assessment of global coagulation from begging of clot formation to fibrinolysis and provides a better understanding. The TEG6s (Haemonetics Corporation, Braintree, MA) is the sequel of the TEG5000, which has the limitation of labor-intensive. The TEG6s is a newly developed cartridge based system. It is a fully automated and the first true point-of-care TEG.

Aims: In this study, we evaluated the precision of TEG6s and established reference ranges for TEG6s in Korean population.

Methods: TEG assays were performed on the blood of healthy donors (n=149) to determine TEG6s reference ranges and on the QC level 1 and level 2 materials for precision testing. The study design was developed in accordance with Clinical and Laboratory Standards Institute Guidelines (EP28-A3c, EP05-A3). Healthy subjects >18 years of age were included.

Results: TEG6s precision testing yielded low variability. Within run, between run, and total precision ranged between 0.2% and 8.6% except R, which was 11.8% due to a low value for the mean (1.02). The reference ranges 149 healthy subjects of R, MA and LY30 were similar to the manufacturer's reference range. Some were different from those of the manufacturer, showing hypocoagulable tendency: short K and increased α (Table 1).

Conclusions: The new TEG6s has been overcome the limitations of the TEG5000. This study has shown that TEG6s has high precision for all parameters. According to the results of our population, it is thought that each institution should validate the manufacturer's reference range before adopting TEG and set the reference range if necessary. It will be necessary to confirm whether the differences in the reference ranges are due to ethnicity and to conduct further studies on the wide range of reference ranges available in Korea.

PB0647 | Who May Benefit from Direct Oral Anticoagulant Level Monitoring? A Systematic Literature Review

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Background: Direct oral anticoagulants (DOACs) are frequently used due to their rapid action, fixed dosing schedule and limited interactions. The question for which patients DOAC level monitoring might be beneficial, is still unanswered.

Aims: We performed a literature search to evaluate whether DOAC monitoring of at risk patients could be beneficial in comparison to no monitoring at all.

Methods: We systematically searched the Pubmed and Medline databases for DOAC studies (Dabigatran, Edoxaban, Rivaroxaban, Apixaban or Betrixaban) in combination with at risk patients suffering from bleeding or thrombotic complications published in the past 5 years. Relatively new literature was searched as discussion about monitoring patients under DOACs due to bleeding or thrombotic adverse events only started recently. The patient groups were selected based on indication of DOAC use (i.e. atrial fibrillation/venous thromboembolism) and other possible vulnerable groups of anticoagulant users. Special attention was given to DOAC monitoring studies.

Results: From 474 studies, 50 met the inclusion criteria (only 5 showed data on monitoring). Different characteristics were identified:

TABLE 1 Reference values for Kaolin assay of TEG6s (Due to the regulation of the table, the limited results are shown

	R	K	α	MA	LY30
Shapiro-Wilk test	0.138	0.002	0.529	0.956	<0.0001
Mean ±2SD	5.07-9.33	0.81-2.86	58.92-77.22	51.21-66.27	0-2.38
Median	7.2	1.8	68.3	58.7	0
Min./Max.	4.6/9.9	0.8/3.5	52.8/79.3	48.9/69.2	0/10.5
95% CI	5.2-9.6	0.98-2.93	58.65-76.35	50.95-65.83	0-2.35

Population demographics: body mass index (10%), age (10%): Specific clinical conditions: chronic kidney disease (10%), liver disease (4%), pregnancy (2%), atrial fibrillation (28%), cancer (10%) or venous thromboembolism (4%)

High risk interventions: before surgery (4%) or admission to an emergency room (2%):

Recommendations which might be of practical value were extracted from 8 studies (16%) : Monitor renal function using the Cockroft-Gault formula every 3-6 months depending on degree of renal failure (chronic kidney disease study).

Measure DOAC level once within the first 3 months after therapy has started (non-valvular atrial fibrillation study).

Routine monitoring without an indication did not show beneficial effects (monitoring study).

Conclusions: There is limited evidence to properly answer whether active monitoring of DOAC levels in at risk patients would result in less thrombotic or bleeding complications.

PB0648 | Performance of the DP-Filter to Remove DOACs from Plasma. An Analytical Validation Demonstrating its Efficiency and Innocuity

J. Favresse[1]; C. Bouvy[2]; R. Sirriez[1]; P. Hainaut[3]; H. Yildiz[3]; A.-S. Delvigne[1]; E. Montay[4]; J.-M. Dogné[1]; J. Douxfils[1,2]

[1]UNamur, Pharmacy, Namur, Belgium; [2]QUALIblood s.a., Pharmacy, Namur, Belgium; [3]Cliniques Universitaires Saint Luc, UCL, Internal Medicine, Brussels, Belgium; [4]UCLouvain, Student, Brussels, Belgium

Background: The impact of direct oral anticoagulants (DOACs) on hemostasis parameters is a well-known issue and may cause false-positive and -negative results. Therefore, the correct interpretation of tests performed in patients taking DOACs is mandatory to prevent misclassification and the subsequent clinical consequences.

Aims: We aimed at evaluating the efficiency of the DP-Filter (Qualiblood, Belgium) to overcome the effect of DOACs on various hemostasis parameters (prothrombin time (PT), activated partial thromboplastin time (aPTT), dRVV Screen, dRVV Confirm, and PTT-LA).

Methods: Normal pooled plasma (NPP) were mixed with either apixaban, dabigatran, edoxaban, or rivaroxaban at 0, 100, 300, and 1000 ng/mL. Plasma DOAC concentrations were assessed before and after filtration with DP-Filter using calibrated STA-ECAII or STA-Liquid anti-Xa.

Results: The use of the DP-Filter device in plasma spiked with DOACs significantly reduced their concentrations under 25 ng/mL (< LOQ). The DP-Filter was also effective to overcome the effects of DOACs on every hemostasis parameters and had no impact on the samples not containing DOACs (Table 1).

Conclusions: The DP-Filter is an effective, simple and ergonomic device that allows to overcome the interference of DOACs on coagulation tests. This is particularly relevant for the hemostasis testing in patients under DOACs.

PB0650 | Validation of a New Point-of-Care Platelet Counter

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[1]Maastricht University, Cardiovascular Research Institute Maastricht (CARIM), Maastricht, the Netherlands; [2]2M Engineering, Valkenswaard, the Netherlands; [3]Thrombinoscope BV, Maastricht, the Netherlands

Background: Platelet count can be altered in various diseases and treatments and that may provide better insight into the expected outcome. So far, determination of platelet count is done within laboratory conditions by using established hematology analyzers, while a point-of-care device could be used for this purpose to speed up this process.

Aims: Our aim was to validate a newly developed point-of-care platelet counter device (PC100) by determining the bias between PC100 and a reference method (Sysmex XP-300).

Methods: Whole blood was taken from 60 healthy individuals into EDTA or ACDA. Basic platelet count in EDTA whole blood was measured by PC100 and compared to Sysmex XN-900LT hematology analyzer. Platelet rich plasma (PRP) was prepared from ACDA whole blood and platelet count was adjusted to the range 250-3600 platelets (plts) /nL and measured with the PCI-100 and Sysmex XP-300. The PC100 platelet counter consists of a clinical analyzer and disposable glass slides, performing automatic enumeration of the platelets in two independent adjacent chambers (chamber A, B) providing two replicates as a result.

Results: Platelet count measured in whole blood correlated well between PC100 and the reference method. Platelet count determined in PRP by PC100 showed 1.42% median difference in comparison to the reference method. While the difference between two methods increased with concentration of platelets in PRP, a strong linear relationship remains throughout the whole interval (250-3600 plts/nL) with high correlation coefficient (r=0.99). The slope obtained from linear regression analysis was 0.98 (95% CIs: 0.97-0.99), representing a 2% proportional difference between the two methods. Comparability assessment of the predicted bias at extreme concentrations of measuring interval, where the highest variations are expected, showed that the bias falls within the 10% acceptable range.

Conclusions: The PC100 platelet counter provides equivalent results compared to the reference method, Sysmex XP-300 hematology analyzer.

PB0651 | DP-Filter, a Medical Device to Remove Edoxaban and Edoxaban-M4 from Plasma of Patients Taking Edoxaban

J. Favresse[1]; C. Bouvy[2]; R. Sirriez[1]; P. Hainaut[3]; H. Yildiz[3]; A.-S. Delvigne[2]; M. Hardy[4]; F. Mullier[5]; J.-M. Dogné[1]; J. Douxfils[1,2]

[1]UNamur, Pharmacy, Namur, Belgium; [2]QUALIblood s.a., Pharmacy, Namur, Belgium; [3]Cliniques Universitaires Saint Luc, UCL, Internal Medicine, Brussels, Belgium; [4]UCLouvain, Anesthesiology, Yvoir, Belgium; [5]UCLouvain, Laboratory Medicine, Yvoir, Belgium

Background: The impact of direct oral anticoagulants (DOACs) on hemostasis parameters is a clinical issue. The use of devices or techniques to overcome this type of interference is needed for the correct interpretation of hemostasis tests in patients taking DOACs.

Aims: This study aims at evaluating the efficiency of DP-Filter (Qualiblood, Belgium), a new and simple device to remove edoxaban and its more relevant M4-metabolite from patient samples.

Methods: Seventy-eight plasma samples obtained from 52 patients taking edoxaban were included in this study. DP-Filter was used to filter plasma. Edoxaban and edoxaban-M4 were then measured in both filtered and unfiltered plasma using a recently validated UHPLC-MS/MS method.

Results: The mean edoxaban and edoxaban-M4 concentrations measured in the unfiltered plasma were 128.0 ng/mL (95% CI 98.0-158.1 ng/mL) and 8.4 ng/mL (95% CI 5.8-11.0 ng/mL), respectively. The mean concentrations of edoxaban and edoxaban-M4 measured in the filtered plasma decreased to 0.0 ng/mL (95% CI 0.0-0.0 ng/mL) and 0.2 ng/mL (95% CI 0.1-0.3 ng/mL), respectively (< LOQ of the assay).

Conclusions: The DP-Filter is an effective, simple and ergonomic device to completely remove edoxaban and edoxaban-M4 from plasma of patients taking edoxaban.

PB0652 | The Effect of DOAC-Stop® on Several Oral and Parenteral Anticoagulants

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Background: DOAC-Stop® is a recently manufactured product which can neutralise the direct oral anticoagulants (DOAC) in plasma samples *in vitro*. However, there are still some grey areas regarding its use.

Aims: To investigate the effect of DOAC-Stop® on several oral and parenteral anticoagulants using standard and research-based coagulation assays.

Methods: We analysed pools of normal platelet poor plasma (PPP), warfarinised PPP (INR 2.1, 3.2, 4.2), normal PPP spiked with direct factor Xa inhibitors (apixaban 182, 265 ng/mL; edoxaban 151, 220 ng/mL; rivaroxaban 241, 339 ng/mL), direct thrombin inhibitor (dabigatran 203, 318 ng/mL), indirect factor Xa inhibitors (enoxaparin 0.93, 1.68 U/mL; fondaparinux 1.62, 2.16 µg/mL). Samples treated with DOAC-Stop® were compared with untreated samples. The following assays were performed: anti-Xa, diluted thrombin time, APTT, PT/INR, lupus anticoagulant (LA), factor assays (I, II, VII, VIII, IX, X, XI, XII), calibrated automated thrombography (CAT) with TF 5pM, thromboelastography (TEG).

Results: After DOAC-Stop® treatment, the DOACs showed undetectable anticoagulant concentrations and complete normalisation of APTT and PT/INR. The false positive LA results obtained with rivaroxaban were normalised.

Direct factor Xa inhibitors were completely normalised on the CAT, while for dabigatran only lag time and time-to-peak were normalised (Table 1). On the TEG, the MA of all DOACs was normalised, whereas

TABLE 1 CAT results before and after DOAC Stop® treatment

	Lag time (ref. 3.54-4.34 min)		ETP (ref. 1852.7-2225.4 nM*min)		Peak (ref. 243.45-349.02 nM)		Time to peak (ref. 6.86-8.53 min)	
Sample	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
Normal PPP	4.17 (0.23)	3.84 (0.23)	1911.0 (52.3)	2013.0 (12.7)	270.33 (5.48)	301.69 (4.96)	8.00 (0)	7.33 (0)
Warfarinised PPP INR 4.2	11.67 (0)	10.84 (0.23)	275.0 (12.7)	296.5 (19.1)	46.67 (1.62)	51.17 (3.17)	15.00 (0)	14.00 (0)
Apixaban 265 ng/mL	8.92 (0.47)	3.91 (0)	643.0 (0)	1855.5 (23.3)	27.57 (2.54)	262.72 (3.20)	14.43 (1.65)	7.58 (0)
Edoxaban 220 ng/mL	11.59 (0)	3.74 (0.24)	643.0 (5.7)	1945.5 (132.2)	31.25 (0.64)	282.46 (11.67)	24.78 (0.24)	7.42 (0.23)
Rivaroxaban 339 ng/mL	12.09 (0.23)	3.91 (0)	467.5 (20.5)	1956.0 (123.0)	15.66 (0.33)	259.95 (11.79)	28.28 (0.47)	7.91 (0)
Dabigatran 318 ng/mL	18.50 (0.71)	3.84 (0.23)	748.0 (2.8)	1652.5 (23.3)	148.06 (3.37)	224.60 (2.05)	20.83 (0.71)	7.84 (0.23)
Enoxaparin 1.68 U/mL	Flat CAT traces							
Fondaparinux 1.62 µg/mL	14.00 (0)	11.50 (0.24)	242.5 (0.71)	274.5 (3.54)	12.90 (0.20)	16.56 (0.11)	24.84 (0.23)	20.33 (0)

TABLE 2 TEG results before and after DOAC Stop® treatment

	R time (ref. 9.23-15.01 min)		K time (ref. 1.56-4.12 min)		Angle (ref. 40.27-63.44 deg)		MA (ref. 28.83-36.57 mm)	
Sample	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
Normal PPP	10.05 (0.64)	12.55 (0.49)	3.05 (0.21)	3.15 (1.06)	51.45 (2.05)	47.30 (9.48)	30.80 (0.42)	32.15 (2.19)
Warfarinised PPP INR 4.2	18.15 (0.21)	23.80 (1.41)	6.60 (0.57)	5.90 (0.28)	26.80 (3.25)	35.50 (2.69)	32.35 (0.49)	33.65 (0.35)
Apixaban 265 ng/mL	17.70 (0.14)	16.55 (1.91)	8.55 (0.21)	4.50 (1.84)	28.25 (2.47)	37.90 (14.42)	23.45 (0.64)	29.05 (1.77)
Edoxaban 220 ng/mL	25.35 (3.18)	16.50 (0.42)	8.90 (0.14)	3.65 (0.49)	25.05 (3.89)	40.15 (5.59)	25.95 (1.63)	30.55 (2.05)
Rivaroxaban 339 ng/mL	29.80 (2.40)	16.30 (0.14)	13.25 (0.49)	3.70 (0.42)	17.40 (0.71)	43.75 (0.21)	23.30 (1.84)	31.0 (1.27)
Dabigatran 318 ng/mL	47.75 (1.34)	17.80 (1.27)	10.20 (7.35)	4.15 (0.92)	25.60 (15.27)	41.05 (6.01)	33.55 (2.62)	35.35 (0.49)
Enoxaparin 1.68 U/mL	Flat TEG traces							
Fondaparinux 1.62 μg/mL	Flat TEG traces							

a variable degree of normalisation of the other parameters was observed (Table 2).

Normal and warfarinised PPP on the CAT showed shortening of lag time and time-to-peak and prolongation of ETP and peak; on the TEG they showed an increase of R time. There showed also a trend towards a reduction of factors VIII, IX, X, XI, XII levels.

Conclusions: Our preliminary findings indicated that while basic coagulation assays were normalised by DOAC-Stop®, there might be potential for reduction of plasma levels of several coagulation factors, suggesting a certain level of DOAC-Stop® binding which could affect other assays.

PB0653 | Results of Anti-Xa Activities for Monitoring Therapeutic and Prophylactic Doses of Low Molecular Weight Heparin in Children

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Background: Low molecular weight heparin (LMWH) is widely used in prevention and treatment of thromboembolic events in children. Children receiving LMWH can be monitored using LMWH-calibrated anti-FXa assay. Generally accepted range for therapeutic doses is defined within 0.5 and 1.0 IU/mL whereas that for prophylactic doses of LMWH is 0.2 to 0.5 IU/mL.

Aims: To evaluate LMWH-calibrated anti-FXa activities for prophylactic and therapeutic doses of enoxaparin in children and to compare measured values with predefined target values.

Methods: Study included 122 measurements in plasma samples of children on therapeutic (n=75) and prophylactic (n=47) doses of enoxaparin. Venous blood samples were collected 4 hours after last dose. LMWH anti-FXa activity was measured using LMWH-calibrated anti-FXa method (Innovance heparin, Siemens Healthineers, Germany) on Sysmex CS2500 analyzer. Results are presented as median (95% CI) and interquartile range (IQR) due to not normal data distribution. Differences between groups were tested using Mann-Whitney test. Chi-squared test was used for comparison of proportions.

Results: In 75 plasma samples of 10 children (10 months to 22 years) on therapeutic LMWH doses anti-FXa activities ranged from 0.10 to 1.50 IU/mL (median 0.65 IU/mL) and in 47 plasma samples of 7 children (2 weeks to 13 years) on prophylactic LMWH doses ranged from 0.10 to 0.78 IU/mL (median 0.27 IU/mL) with significant difference of anti-FXa activities between therapeutic and prophylactic doses (P< 0.0001) (Table 1). Higher proportion of measurements were within target values for therapeutic doses than for prophylactic doses (P< 0.0001), whereas there were no significant differences between proportions of measurements below and above target values for both therapeutic and prophylactic LMWH doses (Table 1).

TABLE 1 Results of low molecular weight heparin-calibrated anti-FXa activities in children on therapeutic and prophylactic doses of enoxaparin

Low molecular weight heparin (LMWH) enoxaparin	N; age (range)	Dosing regimen	LMWH-Anti-FXA activity (IU/mL) Median (95%C), IQR	Within target range therapeutic: 0.5-1.0 IU/mL prophylactic: 0.2-0.5 IU/mL	Below target range therapeutic: <0.5 IU/mL prophylactic: <0.2 IU/mL	Above target range therapeutic: > 1.0 IU/mL prophylactic: >0.5 IU/mL	P
Therapeutic dose	75; 10 months-22 years	2x5 mg/kg a day	0.65 (0.59-0.74) 0.45-0.83	51/75 (0.68)	20/75 (0.27)	4/75 (0.05	Within/below: P <0.001 Below/above: P = 0.002 Within/above: P <0.001
Prophylactic dose	47; 2 weeks-13 years	2x2.5 mg/kg a day	0.27 (0.15-0.33) 0.10-0.38	28/47 (0.60)	16/47 (0.34)	3/47 (0.06)	Within/below: P = 0.01 Below/above: P = 0.007 Within/above: P < 0.001
P	/	/	<0.001	<0.001	0.412	0.812	/

Conclusions: Our results suggest that both therapeutic and prophylactic target LMWH ranges are appropriately predefined based on the previous studies. Monitoring of prophylactic and therapeutic doses seems to be justified because of significant proportion of measurements outside target values.

PB0654 | Fibrinolysis Detection by TEG and ROTEM Viscoelastic Analysers: An *in vitro* Comparison

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Background: Hyperfibrinolysis is a critical component of coagulopathy with poor outcomes if not diagnosed and treated early. Successful hemostasis management requires close monitoring of fibrinolysis and its targeted treatment with anti-fibrinolytics (where applicable, in addition to initial TXA).
Aims: The study objective was to explore and compare the fibrinolysis detection capabilities of viscoelastic hemostatic analyzers (VHA) under controlled conditions.
Methods: Previous (TEG® 5000, ROTEM® Delta; n=140) and new generation (TEG® 6s, ROTEM® Sigma; n=160 (duplicate measurements)) viscoelastic analyses were performed on healthy volunteers tPA (0-140ng/ml) contrived whole blood samples. Fibrinolysis was measured with TEG® at 30 minutes after maximum amplitude (LY30) for RapidTEG® (CRT) and Kaolin (CK) assays and with ROTEM® at 30 minutes after clotting time (LI30) for INTEM and EXTEM assays. Device relationships to tPA were studied in linear or nonlinear models and measurement variances were studied with Bland-Altman analyses.
Results: Both analyzers showed a monotonic relationship with increased tPA concentrations in log-logistic four parametric models. Differences were observed between TEG and ROTEM on the probability of tPA induced lysis detection. The lowest study concentration with sensitivity levels above 70% for Lysis prediction versus reference range was 60ng/ml for CK.LY30 and CRT.LY30, 80ng/ml for EXTEM.LI30 and 100ng/ml INTEM.LI30. In comparison, current commonly used VHA-guided thresholds for fibrinolysis treatment corresponded to ~80ng/ml or higher. All devices had specificity levels >80% at most studied concentrations. Finally, the new generation devices variance of measurements ranged from 1.6% (CRT.LY30) and 3.7% (CK.LY30) for the TEG®6s to 11.9% (EXTEM.LI30) and 15.8% (INTEM.LI30) for the ROTEM® Sigma.
Conclusions: Important differences were found between fibrinolysis detection capabilities of the VHA analyzers *in vitro*. The clinical importance of these findings needs to be further investigated. VHA-guided fibrinolysis treatment algorithms could potentially be adjusted in line with the sensitivity of the new VHA analyzers.

TABLE 1 Model based minimum detectable tPA induced lysis

Device	Assay.Parameter	Reference Range (ng/ml)	Negative control (ng/ml)
TEG® 5000	CK.LY30	72.6	59.1
ROTEM® Delta	INTEM.LI30	107.0	95.9
TEG® 6s	CK.LY30	71.7	62.4
TEG® 6s	CRT.LY30	89.2	84.0
ROTEM® Sigma	INTEM.LI30	114.6	110.9
ROTEM® Sigma	EXTEM.LI30	100.1	100.7

PB0655 | Real-time Intra-site and Multi-site Correlations and Evaluation of Time Stability with the Same Frozen Plasmas Kits which Cover the Full Measuring Range for Routine Tests and Anticoagulant Monitoring Tests

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Background: In order to ensure compliance with local regulatory agencies (COFRAC guideline in France) inter-instrument comparison must be performed during initial validation and on an ongoing way. For intra and inter-sites comparison, access to available samples which cover the full measuring range is a challenge.
Aims: The aim of this study is to evaluate the same frozen plasmas kits for instruments comparisons in the same laboratory and in two differents laboratories distant from 30km.
Methods: Four set of plasmas were tested at the same time on both instruments on site 1 and 2 at day 0 and 7 and on site 1 at day 15. Prothrombin time (2 reagents), activated partial thromboplastin time (4 reagents), fibrinogen and factor V were tested on set A (n=30 samples); INR on set B (n=5, 2 reagents); unfractionated heparin (UFH) and low molecular weight heparin anti-Xa activities on set C (n=10) and D-dimer and fibrin monomers on set D (n=20). The Bland and Altman plot and the laboratory criteria (2.8*SD) were used for comparisons.
Results: All parameters were consistent except for UFH anti-Xa activity on day 0. Lack of correlation on the UFH set could be related to plasma stabilization after thawing, a good correlation was then observed on day 7 and 15.

Conclusions: Using frozen plasmas limit some matrix biases when quality control are used for correlation and allows being compliant with regulations that make difficult to use patient samples or answer to the impossibility for some structures to obtain samples in extreme values. The 4 sets are appropriate for methods validation and comparison of intra and inter-sites instruments. For each assay, chronometric, chromogenic or immunoturbidimetric, samples cover the whole measuring range, except for fibrin monomers. These samples remain to be evaluated at day 60 and 90 to allow for a more accurate comparison and a prospective follow-up.

PB0657 | Evaluation of the Atellica® COAG 360 (Siemens Healthineers) at Caen-Normandy University Hospital (FRANCE)

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Background: As part of the process for the renewal of our routine analyzers, we evaluated the analytical performance of Atellica® COAG 360. The Atellica COAG 360 system (Siemens Healthineers) was installed and tested for a period of 10 days in our laboratory.
Aims: The objective of this evaluations was to assess the usability and to evaluate analytical performances of the automate under routine conditions.
Methods: We selected parameters reflecting panel of tests performed routinely in our laboratory : clotting assays (PT, APTT, Fibrinogen and selected factors: FV, FII, FVIII, FIX), chromogenic assays (Anti-Xa activity of UF & LMW Heparin) and one immunochemical assay (D-Dimer). The performance evaluation for repeatability and reproductibility was done using internal quality control tested three times per day (minimum of 15 measurements).
Results: The analytical performance evaluation (precision) under routine conditions were in alignment with the requirements of GFHT (*Groupe Français d'étude de l'Hémostase et de la Thrombose*) and were comparable to our current routine analyzers (ACL-Top® 700 CTS, WERFEN).
For the intra-assay imprecision, all coefficients of variation (CV%) were less than 3% .
Inter-assay imprecision is presented on Table.

Conclusions: During this period of test, Atellica COAG 360 system appeared intuitive, reliable, easily accessible with an optimized reagent management (continuous loading and reduced dead-volume) and advanced traceability gestion for calibration, controls and patients.
The pre-analytic module for HIL have not been compared due to the lack of equivalent system on our current automates.

PB0658 | Identifying and Reducing the Cause of Haemolysis in Coagulation Blood Samples due to Sample Transport in a Pneumatic Tube System

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Background: Pre-analytical errors represent 70-90% of laboratory errors. Of these 40-80% are due to in vitro haemolysis, which may occur during sample transport.
Aims: To assess if a pneumatic tube system (PTS) used to transport samples contributed to haemolysis, and whether packaging could reduce vibration and haemolysis experienced by samples during transport.
Methods:
Quadruplet blood samples from healthy volunteers (n=30) were
1) transported from the distant location to the laboratory via PTS in a transport carrier packaged with bubble wrap,
2) same journey in an unpackaged carrier,:
3) hand-delivered and:
4) transported from a nearby location via PTS in an unpackaged carrier.
All samples were analysed for haemolysis on a CS5100, and for concentration of plasma haemoglobin. Data loggers measured the cumulative number of shocks above 2g as an area under the curve (Streichert et al Clin Chem 2011:57;1390) during transport across the different PTS lines, packaging conditions, and manual transport.
Results: Three of 30 samples transported unpackaged from the distant location were flagged for haemolysis by analyser (10%). No other samples were flagged as haemolysed confirming that the haemolysis had occurred in vitro, and that adequate packaging

TABLE 1 Inter-assay imprecision (CV%) on Atellica COAG 360 during the period of test

	PT	INR	APTT (Actin FS)	Fib	DDi	Anti-Xa Heparin (UF)	FII	FV	FVIII
CV (%) Normal Level	3.6	1.5	0.9	1.8	5.7	4.9	2.1	3.5	3.7
CV (%) Pathological Level	1.3	0.95	1.2	2.2	1.5	3.6	2.8	2.6	4.7
CV (%) recommended by GFHT	4.8	5.3	N=3.9 P=4.7	6.5	n/a	n/a	5.7	6.7	7.9

TABLE 1 Results

Transport	number of subjects	samples rejected for haemolysis	data logger runs (n)	Cumulative shocks > 2g (AUC)	CV of shock measurements (%)
Hand Delivered	30	0	19	18.2	93%
Nearby location unpacked	30	0	20	954	7%
Distant location packed	30	0	20	926	14%
Distant location unpacked	30	3	19	1044	14%

prevented haemolysis caused by long distance PTS transport. The cumulative vibration experienced by samples in the PTS was significantly different between all the transport conditions ($p< 0.05$). Packaging significantly reduced total vibration in the carrier compared to no packaging.

Conclusions: Packaging carriers significantly reduces vibration experienced within carriers in PTS and reduced in vitro haemolysis as detected by CS5100 analyser. Each laboratory should evaluate their own PTS for the induction of haemolysis in haemostasis samples. The use of data loggers is useful to quantify the extent of vibration and assess options to reduce this during transport via PTS with associated improvemetns in sample quality.

PB0659 | Calibrated Automated Thrombogram II: A New Thrombin Generation Test

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Background: Thrombin generation (TG) assessed by Calibrated Automated Thrombogram (CAT-I) reflects the overall capacity of plasma to generate thrombin, thus evaluating the balance between the anti- and procoagulant processes. However, with this method the calibrator curve is usually not measured until completion which has a severe impact on the calculation of the TG parameters, especially under conditions where almost all substrate is consumed. Additionally, direct thrombin inhibitor (DTI) cannot be present in the calibration sample due to inhibition of the calibrator.

Aims: Development of a modified TG assay (CAT-II) and its comparison head to head with the CAT-I method using the same fluorometer.

Methods: TG was assessed with CAT-I and CAT-II in control plasma and plasma containing different anticoagulants (dabigatran, rivaroxaban, apixaban). In CAT-I, measurements were calibrated against the same plasma containing calibrator in the presence of fluorogenic substrate (Z-GGR-AMC). In contrast, CAT-II method used a standard concentration of thrombin in buffer and AMC in a separate plasma sample for calibration thereby not being influenced by the presence of DTI.

Results: TG obtained from CAT-I and CAT-II using anticoagulant free plasmas appeared to be identical with intra-assay variations less than 5% concerning all measured parameters (lag time, peak height, endogenous thrombin potential). When comparing the two different calibration methods in the presence of different anticoagulants, a high correlation was seen in the presence of rivaroxaban and apixaban (R^2>0.97), but not with dabigatran, a direct thrombin inhibitor. CAT-II method showed dose-dependent inhibition of TG in the presence of dabigatran, while CAT-I was not able to detect it. Furthermore, hemoglobin or bilirubin does not interfere with the results of CAT-II.

Conclusions: Our results showed that there is no difference between the results of CAT-I and CAT-II method when it is applied in control plasmas, while the CAT-II method is able to correctly measure samples containing direct thrombin inhibitor.

PB0660 | A Novel Quantitative Chromogenic Fibrinogen Assay Adaptable to Centralized Laboratory Analyzers

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Background: Available fibrinogen assays have some limitations. The Clauss fibrinogen assay is influenced by fibrin(ogen) degradation products (FDPs). Immunological assays with monoclonal antibodies are also affected by degraded forms of fibrin(ogen) and cannot differentiate between fibrinogen and fibrin and between intact and fragmented molecules. On this knowledge, we have developed a novel chromogenic method of measuring plasma fibrinogen levels without interference by FDPs, not dependent on a clotting reaction or antibodies, and adaptable to all chromogenic or fluorogenic microplate readers. The test principle is based on a competition between fibrinogen and a synthetic peptide substrate for cleavage by the snake venom derived thrombin-like enzyme batroxobin.

Aims: To compare the new chromogenic fibrinogen assay with the Clauss fibrinogen assay in citrated plasma from healthy individuals.

Methods: Citrated plasmas from healthy donors (n=15) were tested by Clauss fibrinogen assay performed on BCS XP using Multifibren

U reagent and by our new chromogenic fibrinogen assay performed on a microplate reader, using Ci-Trol 1 plasma (specification: 2.4-3.0 g/L) as calibrator. Samples were diluted to cover wider fibrinogen concentration range. FDPs of known concentrations were spiked into the enzyme kinetic reaction to determine interfering effects of FDPs.

Results: Fibrinogen levels of healthy patients closely match when the two assay methods are compared (Clauss assay mean = 2.6±0.3 g/L; chromogenic assay mean = 2.9±0.2 g/L) and a comparison plot was constructed with R^2=0.92. While observable interference in fibrin clotting was detected, FDPs at 57 μg/mL did not affect the novel chromogenic assay.

Conclusions: Fibrinogen levels measured in healthy plasma by the novel chromogenic fibrinogen assay and the Clauss assay were similar. However, on the contrary of the clot-based method, the novel chromogenic method was not interfered by FDPs. Our new fibrinogen assay allows the fast detection of intact (non-proteolysed) fibrinogen.

PB0661 | Possibilities of Thromboelastography in Patients with Severe Burns

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Background: Severe burns cause profound hemostasis. An objective assessment of the state of the hemostatic system is the key to effective treatment of patients.

Aims: To study the relationship of thromboelastogram indices with generally accepted coagulological tests in patients with severe burn injury.

Methods: The state of the endothelial, coagulation, anticoagulant and fibrinolytic links of the hemostatic system (ACL TOP 500 analyzer), the viscoelastic properties of the blood (thromboelastograph TEG 500, tests using a coaline activator and heparinase), were determined in 233 patients with burns over 20% of the body surface and in 20 healthy people.

Results: The studies have shown that the hemostatic system reacts with a sharp increase in procoagulant activity, endothelial dysfunction, oppression of anticoagulant mechanisms as a response to severe thermal damage. The highest sensitivity was revealed to the following tests: von Willebrand factor (FW) activity was 100%, XIIa-dependent fibrinolysis (XIIa-df) - 95%, fibrinogen (Fg) - 72%, antithrombin III (AT III) - 65%, TEG (alpha, MA and G) - 44%, APTT, thrombin time, prothrombin time, TEG (R and k) - less than 20%. Analysis of the correlation between TEG indices and standard coagulogram tests revealed a moderate relationship between APTT and R (r = 0.42, p = 0.001), Fg and MA (r = 0.47, p = 0.001), Fg and G (r = 0.48, p = 0.001). There was no correlation with FW, ATIII, XIIa- df.

Conclusions: Thromboelastography is not an alternative to advanced coagulological studies. At the same time, it is an indispensable tool for verifying the cause of hypocoagulation in patients with severe burn injury.

PB0662 | Feasibility of a Universal Calibrator for Direct FXa Inhibitor DOACs

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Background: Increasing use of direct oral anticoagulants has led to rising numbers of requests for drug level measurement in routine laboratories. Although the same test is used, the anti-Xa assay, individual calibrator and control sets are required for each direct FXa inhibitor (DFXaI).

Aims: Proof of concept study to test usability of a single calibrator set with assigned values for edoxaban, rivaroxaban and apixaban.

Methods: A calibrator set composed of normal pooled plasma spiked with different levels of edoxaban was assigned calibrated values for edoxaban, rivaroxaban and apixaban traceable to LC-MS/MS of each DFXaI. On the new Ceveron c100 automated analyzer, TECHNOCHROM® anti-Xa assay was calibrated using the universal calibrator set separately for each DFXaI. For all drugs a high and low range application using different plasma pre-dilutions can be employed. Plasma samples from patients receiving one of the respective drugs were assessed using the appropriate LC-MS/MS method and TECHNOCHROM® anti-Xa assay calibrated for the respective drug.

Results: DFXaI-specific calibration curves were established using standard Ceveron c100 applications. As expected, correlation of anti-Xa determined edoxaban to mass spectrometry values for *ex-vivo* samples was 0.98. For *ex-vivo* rivaroxaban samples, Passing and Bablok regression between anti-Xa and LC-MS/MS exhibited a slope of 0.95 and intercept of < 2 ng/mL (pearson r 0.95). Similarly, the regression for *ex-vivo* apixaban samples generated a slope of 1.1 and correlation of 0.94.

Conclusions: Here we demonstrate the feasibility of a universal calibrator set which can be used to calibrate respective applications for edoxaban, rivaroxaban and apixaban using a chromogenic anti-Xa assay on a routine coagulation analyser. In addition, a similar approach can be used for a universal anti-Xa control set. A universal calibrator and control set for all anti-Xa assays has potential to reduce the cost of DFXaI measurement and improve throughput without compromising quality and traceability.

PB0664 | Comparison of Thromboelastography with T2MR Data

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Background: Simple and rapid assessment of the coagulation system is essential for an optimal management of bleeding and thrombotic disorders. Thromboelastography is a well-established point-of-care method to investigate the cellular as well as plasmatic components of the hemostasis. T2 Magnetic Resonance (T2MR) was also demonstrated to be capable of rapid platelet function testing. T2MR quantitatively monitors 1H proton relaxation over time. The T2 value is directly correlated to the water mobility within the sample. During the clotting process the mobility of the water molecules is progressively inhibited by the clot but increased in the serum environment. Thus, two curves of T2 values characterizing the clot and the serum components can be monitored and analyzed. Static and dynamic process parameters can be derived and compared to benchmark methods like thromboelastography.

Aims: Establishment of a workflow to conduct T2MR measurements with thromboelastography activators, data analysis optimization and comparison of T2MR parameters with thromboelastography results.

Methods: Citrated blood samples were collected from healthy donors and activated using specific agonist to initiate the intrinsic (IN) and extrinsic (EX & FIB) clotting pathway. Thromboelastography measurements were performed as recommended by the manufacturer. T2MR was performed with a Carr-Purcell-Meiboom-Gill (CPMG) sequence on a 1 T benchtop spectrometer.

Results: T2 value generation by triexponential fitting was optimized. T2MR curves and thromboelastography data were then analyzed by asymptotic values and curve fitting. Characteristic parameters were calculated and compared with standard thromboelastography parameters. For example the difference of the final T2 values of serum and clot for the extrinsic fibrinogen test (FIB) are lower than for the other two executed tests.

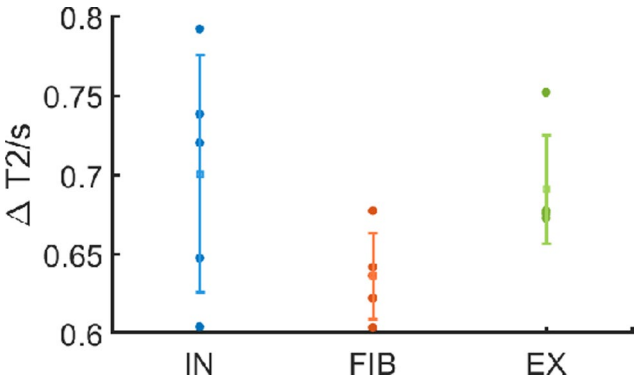

FIGURE 1 The difference of the serum and clot T2 values of 15 healthy donors and three activators

Conclusions: The shape of the T2MR can be compared to thromboelastography and characteristic parameters can be defined to check platelet function. In the next step, samples of patients with impaired platelet functions are investigated to further refine parameter definition and their clinical relevance.

PB0665 | Evaluation of a New Device to Remove Direct Oral Anticoagulants (DOACs) from Plasma Samples

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Background: Dabigatran (Da), rivaroxaban (Ri), apixaban (Ap) and edoxaban (Ed) can interfere with coagulation assays, especially on thrombophilia assessment. To avoid these interferences, a new device, DOAC Filter, allows the specific removal of DOACs from plasma.

Aims: To show that DOAC Filter efficiently removes DOACs and to ascertain the absence of impact on regular coagulation assays.

Methods: The DOAC Filter (Stago, Asnières-sur-Seine, France) is a single-use device in which DOAC molecules are trapped by non-covalent binding while plasma is filtered through a solid chemical phase.

Normal plasma pools spiked with DOACs around 300 ng/mL, 18 plasmas from DOAC-treated patients (6 each for Da, Ri and Ap) and patient's samples tested for lupus anticoagulant (LA) but without DOAC were tested before and after filtration onto DOAC Filter. DOAC levels were determined with STA-Liquid anti-Xa assay for direct FXa inhibitors and with STA-ECA II assay for Da, with specific calibrators and controls. Coagulation assays were performed on STA-R Evolution, with Stago reagents.

Results: 27 to 35 replicates of filtration were done for spiked plasmas. All post-filtration levels of DOAC were below the detection thresholds (LoD) (Da< 15, Ri< 25, Ap< 23, Ed< 20 ng/mL).

All individual patient's plasmas reached concentrations below the LoD after filtration, except one for Ap, initially at 298.0 ng/mL which gave a concentration at 25.1 ng/mL.

Regular coagulation assays results varied between −4 and + 8% after filtration. Such limited variations are not expected to have clinical impact.

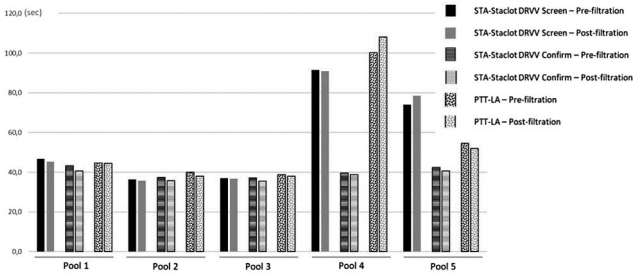

FIGURE 1 LA detection before and after filtration with DOAC Filter

The differences in clotting times for LA determination are between −6 and + 8% (Figure 1). LA diagnosis of post-filtration plasma is respected in 100% of cases compared to initial diagnosis.

Conclusions: The DOAC Filter efficiently removes DOACs from plasma and achieves concentrations under the LoD of specific assays. Integrity of plasma is respected and the cartridge seems to not impact the diagnosis of LA.

PB0666 | Performance Evaluation of a New Fibrinogen Assay Reagent Using Recombinant batroxobin

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Background: In Clauss fibrinogen assay excess thrombin is added to the plasma to measure the time until clot is formed and convert it to the concentration of fibrinogen in comparison with the standard. Clauss assay generally shows a good agreement with antigen testing, but the presence of thrombin inhibitors such as heparin affects the outcome. In addition, in the aspect of reagent stability and standardization thrombin reagent has limitations. Recently, we developed a new fibrinogen assay reagent using recombinant batroxobin.

Aims: We evaluated the performance of the new fibrinogen assay reagent using recombinant batroxobin, ANYFIB.C (NC bit Inc., Seongnam, Korea).

Methods: ANYFIB.C (NC bit Inc.) reagent was evaluated for its precision, lower detection limit, interference, and stability and compared to HemosIL fibrinogen-C for clinical samples using ACL TOP 700 coagulation analyzer (Instrumentation Laboratory, Lexington, MA, USA).

Results: Within-run, between-run, and total coefficients of variation for ANYFIB.C were 1.14%, 0.07%, and 4.62% at the low level (mean, 71.4 mg/dL), respectively, 0.60%, 1.40%, and 3.59% at the mid-level (mean, 292.4 mg/dL), respectively, and 0.73%, 0.96%, and 4.29% at the high level (mean, 561.2 mg/dL), respectively. Lower detection limit was measured to be 46.4 mg/dL as the mean value of 20 repeated measurements. The presence of hemoglobin 500 mg/dL, bilirubin 40 mg/dL, or heparin 10U/mL in a specimen did not affect the fibrinogen concentration. Lot 1 and 2 ANYFIB.C reagents were shown to be stable keeping their activity at least more than one week in room temperature. Regression analysis between ANYFIB.C and HemosIL fibrinogen-C showed an excellent correlation (R^2=0.991).

Conclusions: A new fibrinogen assay reagent, NCbit ANYFIB.C is considered to replace Clauss assay fibrinogen reagents.

PB0667 | Improving Comparability of Optical Aggregometry Results from Automated Coagulation Analyzer Systems Compared with a Manual Aggregometer

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Background: Light Transmission Aggregometry (LTA) is still the gold standard method for platelet function testing - to monitor dual antiplatelet therapy (DAPT) and to uncover platelet dysfunction. To achieve comparable results between different laboratories, recommendations for the standardization of LTA were published by different societies. New automated protocols allow to perform Gustav Born's method on redesigned coagulation analyzers, reducing turnaround-time, and the amount of blood needed.

Aims: To make platelet function data compareable independently of the used method (routine coagulation analyzer versus optical aggregometer)

Methods: Platelet Rich Plasma (PRP) samples from 64 patients on DAPT or with bleeding disorders were tested. Routine LTA was performed on the Chronolog 700 aggregometer (Chronolog Corporation; PRP volume 450µl) and compared with 2 Atellica COAG coagulation analyzers (Siemens Healthineers; PRP volume 140µl) using different stirring rates. LTA was performed using standard platelet activating reagents (i.e. ADP, Arachidonic Acid, Collagen, Epinephrine, TRAP, and Ristocetin) in PRP with normal platelet Counts. PRP was tested within 2 hours on all three devices.

Results: Interpretation of Aggregation results showed no significant differences when using the same stirring rates but different agonist concentrations e.g. 2µg/ml Collagen (Horm) on the manual aggregometer, and 0.5µg/ml on the automated coagulation analyzer, respectively (Fig. 1a).

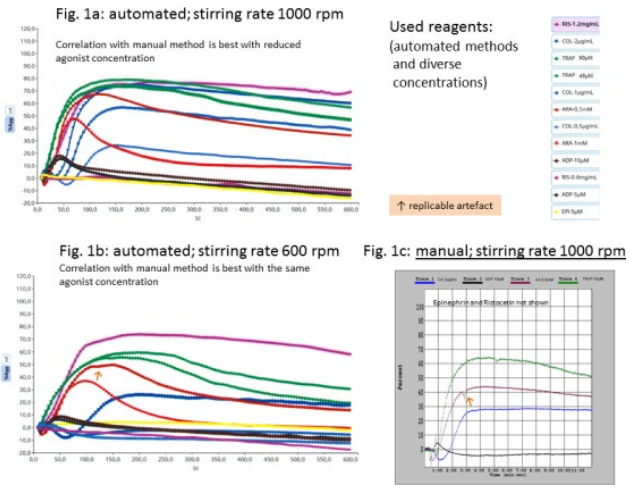

FIGURE 1 Comparison of automated and manual aggregation testing

Using the same agonist concentrations, corresponding interpretation was assured when using adopted stirring rates (automated analysis 600rpm (Fig 1b) versus manual method 1000rpm, (Fig. 1c).

Conclusions: Automated LTA on routine coagulation analyzers corresponds with the gold standard manual method and will allow for broad-based access in future patient care overcoming the limitations of the present manual method: 24/7/365 availability, standardized testing procedures, and minimized sample volumes.

However recommendations how to standardize LTA have to consider opto-physical differences (i.e. sample volume, stirring rate, wavelength) in the used analyzers in order to achieve reproducible results independent of the performing laboratory and platform.

PB0668 | Altered FIBTEM/Fibrinogen Correlation in Liver Transplant Patients Relative to Cardiac Surgery Patients

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Background: Viscoelastic tests, whole blood-based assays that allow for continuous monitoring of clot development and dissolution, are increasingly utilized in liver transplant and other surgical settings. Correlation with conventional coagulation assays among these different populations remain largely unknown.

Aims: We hypothesized that the tenuous hemostatic state of liver failure patients may alter correlations between viscoelastic test parameters and traditional coagulation assays.

Methods: We performed a retrospective review of all FIBTEM-Maximum Clot Firmness (MCF) and Clauss fibrinogen (FIBR) assays and all EXTEM-Clotting time (CT) and Prothrombin time (PT) assays performed in parallel in liver transplant and cardiac surgery populations over a 3-year period.

Results: Ninety-five and 421 instances of parallel FIBTEM-MCF/FIBR testing were identified for liver transplant and cardiac surgery patients, respectively. For both groups, there was a strong positive correlation between FIBTEM-CT and FIBR (R=0.89, p =< 0.00001 for liver transplant; R=0.86,p=< 0.00001 for cardiac surgery). Linear regression showed FIBTEM-MCF[mm] = (0.0526)Fibrinogen[mg/dL] for liver transplant patients and FIBTEM-MCF[mm] = (0.0615) Fibrinogen[mg/dL] for cardiac surgery patients. Interestingly, linear combinations of estimators analysis of the linear coefficients showed the two patient population correlations were statistically significantly different (p=0.005).

One-hundred-and-eleven and 476 instances of parallel EXTEM/PT testing were identified for liver transplants and cardiac surgery patients, respectively. In contrast to FIBTEM-MCF/FIBR, there was a lack of correlation between the EXTEM-CT and PT in both patient populations (R= 0.30 for liver transplant; R=0.32 for cardiac surgery). No discernable difference in this lack of correlation between the two patient populations could be identified.

Conclusions: Overall, these data suggest that FIBTEM-MCF correlation with Clauss fibrinogen is altered in liver transplant patients. Additional studies to assess clinical impact are needed.

PB0669 | Effects of Anticoagulants and Common Interfering Substances on Four Commercially Available dRVVT Assays

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Background: dRVVT (dilute Russell's Viper Venom Time) is a popular assay for detection of lupus anticoagulants (LA), a marker of thrombophilia. Anticoagulant-free plasma samples are ideal for LA testing but not always feasible because anticoagulant therapy is a common thrombosis treatment. Anticoagulant interference may impact dRVVT results, affecting diagnosis.

Aims: We evaluated effects of numerous anticoagulants and common interferents on four commercial dRVVT assays to compare assay-specific impacts to LA diagnosis.

Methods: Precision BioLogic Inc. (PBI) dRVVT screening and confirmatory assays were performed on three analyzers - Stago Evolution (mechanical), IL ACL-TOP (optical) and Siemens BCS XP (optical) - and compared with the respective manufacturer's dRVVT assay. Plasma samples spiked with matrix blank or interferent were tested in quintuplicate according to the manufacturer's test application. Interferents were: Heparins (UFH, LMWH, Enoxaparin; 1 U/mL), Argatroban 1.5 µg/mL, Fondaparinux 1.25 µg/mL, Rivaroxaban 0.4 µg/mL and VKA plasma from patients on warfarin (INR< 3, N=10). HIL spiked samples (Hemoglobin 250 mg/dL, Bilirubin 2.5 mg/dL, Intralipid ~500 mg/dL) were also tested.

Results: Heparins and HIL did not affect results. However, both optical analyzers failed to detect clots in intralipid-spiked samples. FIIa inhibitor argatroban had no diagnostic impact, except it produced errors with IL ACL TOP, precluding results. FXa inhibitor fondaparinux did not affect results, but rivaroxaban caused false positive results with Siemens, Stago and IL reagents while PBI diagnostic results were unaffected. VKA (warfarin) had a similar impact; using Siemens, Stago and IL reagents, false positive results were observed in some samples while PBI results were correctly identified as LA negative.

Conclusions: Due to differences in test systems, different influences by DOACs and warfarin on dRVVT results can be expected. Notably, samples containing rivaroxaban or VKA would be correctly diagnosed with PBI reagents but might be incorrectly diagnosed false positive with Siemens, Stago or IL dRVVT reagents.

PB0670 | A Correlation of Thrombin Generation Assay and Clot Waveform Analysis in Patients on Warfarin

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Background: Thrombin generation assays, such as Calibrated Automated Thrombogram (CAT), are fairly well recognised global haemostatic tests with much research and clinical data available. Data on clot waveform analysis (CWA) as a measurement of overall haemostatic function are emerging recently but its performance against other established assays is yet to be ascertained.

Aims: To study the correlation between CWA and CAT parameters across a range of INR in warfarin-treated patients.

Methods: Plasma samples were taken from patients on warfarin and their venous INRs were measured. TGA was performed using CAT platform with PPP Reagent, Thrombin Calibrator and FluCa kit (Diagnostica Stago). aPTT-based CWA parameters were generated using CS2100i coagulometer (Sysmex) with Dade Actin FSL reagent (Siemens). Both raw and adjusted (adjusted for baseline transmittance) CWA data were collected: Min1 (maximum velocity), Min2 (maximum acceleration) and Max2 (maximum deceleration). Pearson's correlation test was used.

Results: 54 samples were included with a median INR of 4.27 (range: 1.55-7.38). For CAT, INR correlated negatively with endogenous thrombin potential (ETP) ($r = -0.316$, $p = 0.003$) and to peak thrombin ($r = -0.426$, $p = 0.0005$). For CWA parameters, INR correlated negatively to Min1 ($r = -0.378$, $p = 0.004$), Min2 ($r = -0.363$, $p = 0.006$) and Max2 ($r = -0.406$, $p = 0.002$). CWA parameters demonstrated positive correlation with CAT results (Table 1), with the strongest correlation noted between adjusted CWA parameters and peak thrombin.

Conclusions: aPTT-based CWA parameters correlated negatively to INR and most of these parameters demonstrated moderate to strong correlation to CAT parameters, with the strongest correlation noted between adjusted CWA parameters and peak thrombin. These results affirm that aPTT-based CWA is a potential valuable tool to assess global haemostatic function.

TABLE 1 Correlations between CWA and thrombin generation parameters

	aPTT-CWA Parameters					
	Adjusted CWA			Unadjusted CWA		
Thrombin Generation Parameters	Min1	Min2	Max1	Min1	Min2	Max2
ETP	r = 0.609 p < 0.0001	r = 0.563 p < 0.0001	r = 0.641 p < 0.001	r = 0.230 p = 0.087	r = 0.326 p = 0.016	r = 0.437 p = 0.0009
Thrombin Peak	r = 0.803 p < 0.0001	r = 0.772 p < 0.0001	r = 0.822 p < 0.0001	r = 0.435 p = 0.001	r = 0.562 p < 0.0001	r = 0.648 p < 0.0001

PB0671 | Diagnostic Performances Evaluation of LIAPHEN Free Protein S on CS-2500

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Background: Protein S (PS) is a vitamin K-dependent glycoprotein present in plasma under free active form (FPS) or complexed to C4b-binding protein, which availability regulates the proportion of the different PS forms. Congenital or acquired PS deficiencies are a risk factor for venous thrombosis. PS levels are influenced by age, sex, oral contraceptive use, anti-vitamin K therapy and PS gene polymorphism. PS levels may decrease in various contexts such as pregnancy, hepatic disorders, early stages of inflammatory diseases. PS deficiency is diagnosed using PS antigen assay and functional PS activity assay. A new, fully automated, immunoturbidimetric assay for FPS is developed for use on any coagulation analyzer.

Aims: To report diagnostic performance characteristics of this new FPS latex immunoassay on CS-2500.

Methods: LIAPHEN Free Protein S is an immunoturbidimetric method, based on antigen-antibody reaction: FPS antigen of the sample reacts with Latex particles sensitized with two mouse monoclonal anti-FPS antibodies, leading to latex particles agglutination. Performances are evaluated using the commercial lyophilized plasma calibrator and controls on CS-2500, and comparison studies with other commercially available assays are performed using 89 plasma samples from healthy subjects (n= 41) or PS-deficient individuals with various pathologies (n= 48).

Results: Between-run precision coefficients of variation (CV%, n=20), evaluated using lyophilized plasmas with normal and pathological FPS levels are 1.6% and 1.9% respectively. The LIAPHEN Free PS antigen measurement on patients' plasmas is well correlated with STA Staclot Protein S functional assay (n=54, r=0.916, p< 0.0001). An excellent correlation is obtained in comparison with STA-Liatest Free Protein S on STA-R: y=0.96x-1.35, r=0.953, p< 0.0001.

Conclusions: This new FPS assay is highly sensitive, specific, reproducible and standardized to rapidly assess Free PS concentrations in human citrated plasma. It is a useful tool for the diagnosis of PS deficiencies.

PB0672 | Top 50 Cited Papers in Developmental Hemostasis: A Scoping Review

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[1]*McMaster University, Faculty of Health Sciences, Hamilton, Canada;* [2]*Thrombosis and Atherosclerosis Research Institute, Hamilton, Canada;* [3]*Ryerson University, Biomedical Sciences, Toronto, Canada;* [4]*Monarch Park CI, Toronto, Canada;* [5]*McMaster Children's Hospital, Division of Pediatric Hematology/Oncology, Hamilton, Canada*

Background: Developmental hemostasis is known as the evolution with age of the coagulation system throughout childhood. While an understanding of the normal fluctuations in the levels of hemostatic proteins is vital in the prevention, diagnosis and treatment of hemostatic problems, the knowledge of the hemostatic system in neonates and children remains inadequate.

Aims: The present study reports findings from a citation analysis of the top 50 most cited papers in developmental hemostasis to describe the research trends in the field.

Methods: A keyword search of papers indexed in the Scopus database using the terms "developmental hemostasis" and "developmental haemostasis" yielded 81 papers. Key data of the top 50 cited articles were retrieved and analyzed using Excel 2016. If any papers in the top 50 were deemed to be irrelevant, they would be discarded and replaced by the next most highly-cited articles in the pool considered to be relevant.

Results: The total number of citations for the top 50 cited papers in developmental hemostasis ranged from 3 to 305, published in 32 journals, from 1995 to 2018. The most frequently cited papers appeared in Thrombosis and Haemostasis (n = 5, Impact factor in 2018 = 4.733) and Seminars in Thrombosis and Hemostasis (n = 5, Impact factor in 2018 = 3.401). There is an increasing trend in published articles in the last decade. Highly-cited papers tended to investigate the reference range of hemostatic proteins in large cohorts of healthy infants.

TABLE 1 Journals of the 50 most cited papers in developmental hemostasis (record ≥ 2)

Journal	Record	Impact Factor in 2018
Seminars in Thrombosis and Hemostasis	5	3.401
Thrombosis and Haemostasis	5	4.733
Journal of Thrombosis and Haemostasis	4	4.662
Thrombosis Research	4	3.266
Blood Coagulation and Fibrinolysis	3	1.120
British Journal of Haematology	2	5.206
Seminars in Fetal and Neonatal Medicine	2	3.846

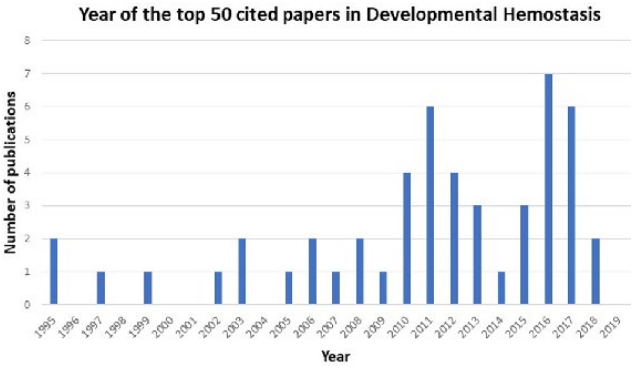

FIGURE 1 Year of the 50 most cited papers in developmental hemostasis

Conclusions: Cohort studies, often focused on reference range, appear to have the highest impact in the field of developmental hemostasis. Knowledge of the pediatric hemostatic system may be important when the use of novel anticoagulants is being considered, since age-related variations in protein levels and structures might affect the efficacy of the drug. However, research in the field remains inadequate and further research is needed.

PB0673 | Health Outcomes after the Implementation of POCT Rotational Thromboelastometry in Patients Undergoing Cardiac Surgery

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Background: Viscoelastic tests (rotational thromboelastometry, ROTEM®), together with the implementation of a specific algorithm for coagulation management in cardiac surgery, enable perioperative coagulopathy to be better controlled.

Aims: The present study aimed to compare transfusion rates, the main associated cardiac surgery complications and other clinical outcome parameters, in order to assess the impact of the implementation of ROTEM® viscoelastic point-of-care tests with algorithm-based coagulation management in cardiac surgery with cardiopulmonary bypass.

Methods: Retrospective cohort study including 675 patients who underwent cardiac surgery with cardiopulmonary bypass. The incidence of allogeneic blood transfusions and clinical postoperative complications were analyzed before and after ROTEM® implementation.

Results: Following viscoelastic testing and the implementation of a specific algorithm for coagulation management, the incidence of any allogeneic blood transfusion decreased (41.4% vs 31.9%, p=0.026) during the perioperative period. In the group monitored with ROTEM®, decreased incidence of transfusion was observed for packed red blood cells (31.3% vs 19.8%, p=0.002), fresh frozen

plasma (9.8% vs 3.8%, p=0.008), prothrombin complex concentrate administration (0.9% vs 0.3%, p=0.599) and activated recombinant factor VII (0.3% vs 0.0%, p=0.603). Increased incidence was observed for platelet transfusion (4.8% vs 6.8%, p=0.530) and fibrinogen concentrate (0.9% vs 3.5%, p=0.066), tranexamic acid (0.0% vs 0.6%, p=0.370) and protamine administration (0.6% vs 0.9%, p=0.908). Similar results were observed in the postoperative period, but with a decreased incidence of platelet transfusion (4.8% vs 3.8%, p=0.813). In addition, statistically significant reductions were detected in the incidence of postoperative bleeding (9.5% vs 5.3%, p=0.037), surgical reexploration (6.0% vs 2.9%, p=0.035), and length of Intensive Care Unit (ICU) stay (6.0 days vs 5.3 days, p=0.026).

Conclusions: The monitoring of hemostasis by ROTEM® in cardiac surgery, was associated with decreased incidence of allogeneic blood transfusion, clinical hematologic postoperative complications and lengths of ICU stay.

PB0674 | How Does the Availability of Thromboelastography Influence Clinical Reasoning in a Paediatric Intensive Care Unit? A Narrative View

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Background: The monitoring of haemostasis and anticoagulation in paediatric patients receiving Extra-corporeal Life Support (ECLS) is vital for safe patient care, yet there is a lack of evidence underlying practice and no consensus on the best approach. All established methods have weaknesses and have been shown to correlate poorly with each other, and with heparin activity. Viscoelastic haemostatic assays (VHA) are increasingly used to direct treatment for patients at high risk of bleeding. Research to date has primarily focused on how VHA compares to standard laboratory tests, or on specific variables, such as the use of transfusion products. There is a paucity of qualitative research on how physicians incorporate this technology into clinical decision making in 'real world' acute scenarios.

Aims: This study aims to explore how the availability of TEG 6S influences clinical decision making for children on ECLS in a paediatric intensive care unit (PICU).

Methods: This qualitative study took a phenomenological approach. Purposive sampling was used to identify consultants using thromboelastography (TEG) to manage children in the PICU at Bristol Royal Hospital for Children. Semi-structured interviews invited the participants to discuss their 'lived experience' of how TEG is incorporated into clinical decision making. Thematic analysis of interview scripts identified a collection of core themes.

Results: Final analysis identified several themes which are evidenced by quotes and short narratives. End users emphasised the importance of real-time supportive data and increased confidence in clinical decisions, particularly when other assays showed discrepant results.

Conclusions: The addition of TEG into the monitoring of haemostasis and anticoagulation in children requiring ECLS gives clinicians the ability to unpick complex situations in "real time" and leads to greater confidence in clinical decision-making.

PB0675 | Laboratory Validation of a Novel Hexagonal Phase Phospholipid Neutralization Assay for Lupus Anticoagulant Detection

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Background: Numerous assays exist to detect lupus anticoagulants (LA), varying greatly in their principle, sensitivity, and specificity. The hexagonal phase phospholipid neutralization test (HPNT) is an integrated and automatable assay that detects LA by measuring shortened activated partial thromboplastin times (aPTT) in the presence of excess hexagonal phase phospholipid. The HPNT involves both a screening ("Start") and confirmatory assay ("Correct").

Aims: The goal of this work is to characterize the performance of our HPNT on three different models of automated coagulation analyzers manufactured by Diagnostica Stago, Siemens Healthcare, and Instrumentation Laboratory.

Methods: To gauge assay performance, we executed a 20-day precision study and a three-site reproducibility study using three pooled plasma controls, performed using three different lots of reagents. We also examined the stability of the assay reagents on board the analyzers and looked at the effects of common coagulation interferents on the performance of the assay. Lastly, we measured the normal range of our HPNT using 120 venipuncture plasma samples collected in 3.2% citrate.

Results: We observed excellent precision and reproducibility across all three study sites, with overall clot time %CVs below 10%. The assay was able to correctly diagnose each control with no false positives or negatives. The reagents themselves were stable on board the instrument for a full eight hours, with no impact on the diagnostic accuracy of the assay. Hemolytic, icteric, and lipemic samples showed no interference using mechanical endpoint detection.

Conclusions: We observed excellent performance and stability of our newly developed HPNT on three common coagulation analyzers. The precision and reproducibility are excellent across sites and the reagents are stable enough to be used without replacement throughout a typical working day. Our HPNT's convenient packaging and pre-frozen format allows labs to cut down on preparation time and reagent spoilage without compromising performance.

PB0676 | Determination of Serotonin Concentration in Single Human Platelets by Single-entity Electrochemistry

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Background: Serotonin, a well-known neurotransmitter is quickly taken up by platelets in blood, stored in dense granules, and released by platelet activation. Thus, measuring the serotonin content of individual platelets is a useful way to assess platelet activation.
Aims: We introduce a method to directly detect serotonin in a single platelet through single-entity electrochemistry.
Methods: We determined oxidation potential of platelet serotonin by cyclic voltammetry and detected serotonin in individual platelets by current-time measurements.
Results: We combined the previously introduced method to measure serotonin in a platelet fixed on the surface of an electrode and single-entity electrochemistry (SEE) suitable for analysis of soft particles. First, we conducted cyclic voltammetry (CV) on a bulk Pt electrode coated with platelet rich plasma (PRP) diluted with normal saline (NS) and observed serotonin oxidation peak around +0.4 V. Based on this, the current-time (*i-t*) curve experiment was conducted at an applied potential of +0.5 V to induce complete oxidation of serotonin in platelets with an ultramicroelectrode (UME) where platelets and serotonin acted as tiny reactors and redox species, respectively. When diluted PRP was applied to UME, anodic current spikes were observed while no spikes occurred with blank samples (NS, diluted platelet poor plasma). As the concentration of platelets increased from 10.0 to 20.1, 40.2, and 100 fM, the collision signals also increased in frequency. The potential dependency was also confirmed by observing no peaks on i-t curve at +0.2 V. The concentration of serotonin was determined by integrating each current peak. The calculated serotonin concentration of individual platelet was between 50 and 950 μM with the average of 178 μM. In addition, this method can be used to determine the platelet concentration.
Conclusions: We developed a quick and simple electrochemical method to measure platelet count and serotonin concentration of individual platelets.

PB0677 | Point-of-Care Method in Determining the Activated Partial Thromboplastin Time

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Background: The point of care test (POCT) for monitoring hemostasis has been increasingly used in patient care in the hospital and outpatient setting. In a hospital environment, the POCT must be chosen, validated, approved and managed by professionals in the Clinical Laboratory of the Institution where they will be implanted.
Aims: To evaluate the results of the Activated Partial Thromboplastin Time (APTT) of POCT equipment and additionally to compare the results of the POCT with the conventional methodology used in our routine.
Methods: 69 samples were obtained from normal individuals (n = 36), inpatients and outpatients using anticoagulants (n = 33). Among anticoagulated patients, 9 were using AVK, 13 using unfractionated heparin, 8 using Eliquis and 3 using Xarelto. The samples for this study were obtained by capillary puncture (fingertip) and citrated plasma (3.2% sodium citrate). The POCT test was performed immediately on the sample by capillary puncture (fingertip) using Roche Coagucheck Pro II equipment. The APTT test in citrated plasma was performed on the ACL Top 750 equipment, using the APTT-SP liquid reagent, both from Werfen.
Results: When comparing the APTT results obtained by POCT (capillary samples) with the reference method (samples collected in citrate), we obtained a correlation coefficient (r) of 0.77. Evaluating normal individuals, we observed a 94.4% agreement of results between the conventional methodology and the POCT. For anticoagulated patients, agreement was 75.8%.
Conclusions: The results of the comparison between the POCT and the conventional method were quite satisfactory, with great benefits for patients with collection difficulties, pediatric patients and patients included in hemorrhage protocols where speed in the release of the result is essential for the continuity of the procedure clinical care.

PB0678 | Development of a Flow Cytometry-based Assay for the Detection of ITP Autoantibodies

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Background: ITP is a common autoimmune bleeding disorder in which anti-platelet autoantibodies target one's own platelets. Most ITP autoantigens have been localized on αIIbβ3 (GPIIbIIIa) integrin and GPIbα. We were among the first to demonstrate ITP mediated by anti-GPIbα-antibodies is distinct from anti-αIIbβ3 (Blood. 2006); many anti-GPIbα antibodies mediate Fc-independent ITP, (Nat. Commun. 2015, Blood. 2018) leading to resistance of first-line therapies. The gold standard for ITP-autoantibody detection is the monoclonal antibody immobilization of platelet antigens (MAIPA) assay. However, MAIPA is rarely clinically utilized due to the necessity of a large amount of patient blood, fresh donor platelets and three different monoclonal antibodies, furthermore, MAIPA takes 3-4 days to perform, lacks standardization and is prone to false positives/negatives. Thus, leaving clinicians to rely on empirical treatment.

Aims: Development of functional αIIbβ3- and GPIbα- bound monolayer coatings that can be applied to magnetic-silica beads for the rapid detection of ITP mediating autoantibodies to improve the diagnosis and treatment of ITP.

Methods: Surface- and bio-chemistry synthesis.

Results: Both coatings are based on a self-assembling-monolayer which facilitates oriented (binding-site upward) covalent linkage of either αIIbβ3 or GPIbα and is resistant to non-specific protein binding. These two coatings were synthesized on 0.6-3μm silica beads. Immobilized GPIbα bound both conformational and linear-epitope specific anti- GPIbα mAbs. αIIbβ3 coated surfaces bound fibrinogen and both conformational and linear-epitope specific mAbs. Furthermore, αIIbβ3 coated beads were incorporated into platelet aggregates. Using these beads, we developed a flow cytometry-based assay for anti-platelet autoantibodies. Our assay produced a limit of detection one order of magnitude more sensitive than MAIPA, while only requiring ~6 h to complete.

Conclusions: Our facile flow cytometry assay is more rapid and sensitive than MAIPA, likely due to the higher antigen density than on a platelet surface. A study evaluating our assay against MAIPA with ~50 ITP patient samples is ongoing.

PB0679 | New Ready-to-Use Liquid Multiplatform Global Screening Assays with Extended Stabilities to Facilitate Laboratory Practice

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Background: Clinical laboratories increasingly need simple, rapid, highly stable routine coagulation assays. A complete line of fully automated liquid global screening assays was developed to facilitate routine tests on citrated human plasma, on any laboratory instrument.

Aims: To report performance characteristics of new liquid clotting Fibrinogen (Fbg), Thrombin Time (TT), Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT) assays on CS series, STAR, ACLTOP, BCSXP.

Methods: Performances were evaluated using proposed plasma calibrators and controls by testing within (n=40) and between-run (n=20 to 30) precision, interferences, stability. Comparison studies with commercial predicates were performed on CS-5100 using plasmas samples from healthy subjects or various pathologies.

Results: All ready to use reagents generate highly accurate results, with low within- and between-run coefficients of variation on all analyzers: 0.3% to 5.0%. Extended dynamic range 0.25-12 g/L Fbg is obtained on multiple analyzers, without Heparins and DOACs interferences at usual therapeutic concentrations. TT assay is very sensitive to low levels of anti-IIa drugs and heparins. Combined APTT reagents demonstrate good sensitivity and specificity for screening patients with Lupus anticoagulant; PT/APTT are sensitive to low factors concentrations. Ready to use recombinant PT with ISI about 1.0 offers an easy tool for VKA monitoring. Reagents can be used within 5 minutes, and are highly stable up to 3 months at 2-8°C and 15 days onboard. Excellent correlations are obtained with predicates (r 0.918 to 0.999, p< 0.0001, CS-5100).

Conclusions: Ready to use liquid FIBRIPHEN LRT, HEMOCLOT TT LRT, CEPHEN and PTPhen LRT are fully automatable, standardized, highly sensitive global screening/monitoring assays, safe and effective on citrated plasma. They provide enhanced practicability tools to optimize laboratory practice, with low CVs, extended stabilities, good sensitivity and limited interferences. Those polyvalent multiplatform assays can be used within few minutes on all available instruments.

PB0680 | Updated CLOTSref: A Mobile Application for Standardized Reference Ranges in Developmental Hemostasis

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Background: Developmental hemostasis is known as the evolution with age of the hemostatic system starting in fetal life and throughout childhood. The process involves a dynamic change in the levels of procoagulation and anticoagulation proteins. Developmental hemostasis creates a challenge for clinicians in the diagnosis and treatment of coagulation disorder in the pediatric population. Therefore, specific age-related reference ranges are necessary for an accurate diagnosis and management. However, reference ranges are analyzer and reagent dependent; and it is estimated that only a small number of laboratories worldwide use adequately established age-, reagent- and analyzer-specific reference ranges. Inappropriate clinical use of reference ranges leads to both over diagnosis and missed diagnosis of the child.

Aims: We aimed to create a platform for practitioners to compare their patients′ results with appropriate age-, reagent- and analyer-specific reference ranges. In addition, a platform for clinicians to contribute data towards a worldwide harmonized reference ranges for hemostatic parameters was established.

Methods: Data on developmental hemostasis from the literature was extracted and pooled into age-, reagent- and analyer-specific reference ranges. A mobile application was created as a platform to present the data conveniently. In addition, a user-friendly form was embedded for clinicians to contribute data towards a worldwide harmonized reference ranges for hemostatic parameters.

Results: Two clinically relevant proteins, alpha-2-macroglobulin and protein C were analyzed. Data from xx identified articles in the

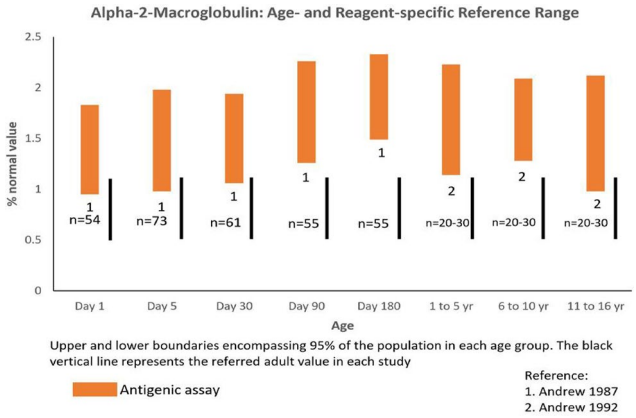

FIGURE 1 Alpha-2-Macroglobulin development across lifespan

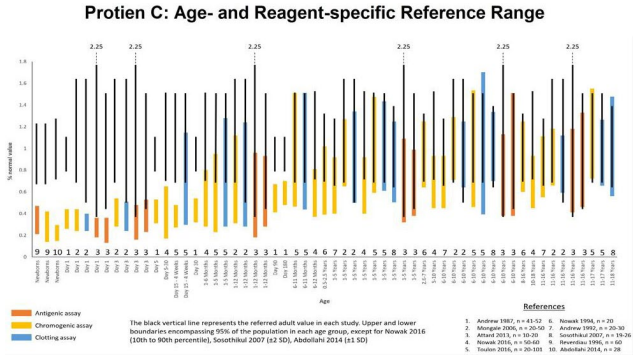

FIGURE 2 Protein C development across lifespan

literature was collected and presented in the developing mobile application. Inadequate sample size of each age group and differences in analyzer use were noticed. Link to the app prototype: http://bit.ly/prototype_clotrefs

Conclusions: The CLOTSref mobile application is a promising platform for practicioners in pediatric hematology to provide the best possible standard of care based on appropriate reference ranges. In addition, it allows clinicians to contribute data towards a worldwide harmonized reference ranges for hemostatic parameters.

PB0681 | Unique Ready to Use, Highly Stable Multiplatform Chromogenic Assays to Facilitate Laboratory Practice

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Background: Clinical laboratories increasingly need rapid, highly stable routine diagnostic assays, easy to use on any analyzer, without additional variability due to reconstitution laboratory practice. Liquid fully automated chromogenic assays were developed to facilitate routine tests on citrated human plasma, and explore the patients' thrombotic, pre-surgery and coagulation/fibrinolytic status on any automated laboratory instrument.

Aims: To report performance characteristics of liquid chromogenic Antithrombin (AT), Protein C (PC), Plasminogen (Plg) and A2Antiplasmin (A2AP) assays on various platforms: CS series, STAR, ACLTOP and BCSXP.

Methods: Performances were evaluated using proposed plasma calibrators and controls qualified against WHO international standard, by testing within- and between-run precision, and stability. Comparison studies with commercial predicates were performed on CS-5100 using plasmas from healthy subjects or plasma at various pathological levels.

Results: Highly accurate results are generated with all ready to use reagents, with low within- and between-run coefficients of variation (eg 0.8 to 4.8% on optical detection analyzers). Dynamic range is extended from about 5% to 150% for A2AP and Plg, and to 200% for AT and PC.

Reagents can be used without reconstitution within few minutes, and are highly stable up to 30 months in original vial, for 5 weeks at 2-8°C or 7 days at 18-25°C after opening, and up to 10 days onboard, which is of strong interest in daily laboratory practice. Excellent correlations are obtained with each predicate (r from 0.945 to 0.995, CS5100).

Conclusions: Ready to use liquid AT anti-h-Xa, Protein C, Plasminogen and A2Antiplasmin LRT assays are fully automatable, standardized chromogenic assays, safe and effective for measurement on citrated plasma. With low CVs, high stabilities, and extended shelf life, they provide enhanced practicability tools of strong interest to facilitate laboratory practice, polyvalent for use within few minutes on multiple available instruments.

PB0682 | Establishment of Reference Intervals for Automated Light Transmission Aggregometry

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Background: Transition from time consuming and labor intensive manual to automated light transmission aggregometry (LTA) requires the establishment of agonist specific reference intervals.

Aims: The aim of the study was to establish reference intervals for the standard panel of agonists on the coagulation analyzer Sysmex CS-2500 (Siemens Healthineers, Germany).

Methods: At least 20 healthy volunteers with normal platelet values were included in the study. Maximal aggregation response was measured in platelet rich plasma samples according to manufacturer instructions (acquisition time of 600 seconds), using the following

agonists and final concentrations: adenosin diphosphate (ADP, 2 and 5 μM), arachidonic acid (AA, 1 mM), collagen (2 and 4 mg/L), epinephrine (5 and 10 μM) and ristocetin (1.2 g/L). All agonist were from HYPHEN Biomed (Neuville sur Oise, France). For each agonist concentration duplicate measurements were performed and the mean maximal aggregation (%) was used for the establishment of reference intervals, by finding the 2.5th to 97.5th percentile for each agonist and agonist concentration. The MedCalc program version 9.3.2.0 for Windows (MedCalc Software, Mariakerke, Belgium) was used for statistical analysis.

Results: The following reference intervals were obtained: 69.8-92.4% for AA (N=21), 63.4-90.9% for 2 μM ADP (N=26) and 67.1-93.0% for 5 μM ADP (N=26), 62.3-94.1% for 2 mg/L collagen (N=26) and 70.9-93.4% for 4 mg/L collagen (N=21), 63.1-92.1% for 5 μM epinephrine (N=22) and 63.4-95.6% for 10 μM epinephrine (N=21), while the obtained reference interval for ristocetin (N=20) was 71.3-91.7%.

Conclusions: Reagent specific reference intervals for automated LTA on Sysmex CS-2500 were established for the standard agonist panel and introduced into routine practice for our patient population.

PB0683 | HFLC and IPF, Can these Two Parameters Reliably Distinguish between Dengue and ITP?

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Background: Dengue virus has become endemic in Pakistan with thousands of cases reported yearly. Thrombocytopenia is the commonest cytopenia associated with dengue; however it is challenging to distinguish it from the cases of ITP on the basis of platelet counts only, before embarking upon other diagnostic tests including peripheral film. High flourecence lymphocyte counts and Immature platelet fractions are relatively new parameters, that may be of help in differentiating between these two disorders in resource constraint settings.

Aims: To identify whether HFLC and IPF can reliably differentiate between dengue fever and ITP before doing further investigations.

Methods: In order to check the diagnostic reliability of HFLC and IPF parameter of automated hematology analyzer, a total of 124 patients of dengue and ITP (62 each) were included in the study. CBC was estimated using SYSMEX XN 1000. Dengue cases were diagnosed by serology and ITP as diagnosis of exclusion. Data was analyzed using SPSS version 22.

Results: Total of 124 patients were recruited in the study, 62 patients each of Dengue and ITP group respectively. M:F ratio in Dengue was 41:21, while in ITP it was 40:22. Mean age was 25.11±13.432 yrs and 34.44±20.82yrs in dengue and ITP groups respectively. Mean platelet counts were comparatively higher in dengue than in ITP i.e. 120.59±99.78 and 41.03±39.7 1 X10^{9}/L respectively. Mean IPF% of dengue was less than the mean IPF in ITP i.e. 8.078± 23.082 ($p<0.000$). HFLC was higher in dengue than in ITP i.e. 5.01±0.193, ($p<0.000$).

Conclusions: On the basis of above findings it can be concluded that dengue fever can be reliably distinguished from ITP, on the basis of HFLC and IPF before embarking upon further investigations. These findings are useful in dengue endemic resource constraint settings.

PB0684 | Institutional Review of Reflex Factor Coagulation Testing in Patients with an Unexplained Prolonged aPTT

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Background: A diagnostic algorithm for reflex coagulation investigations (CI) with factor testing for prolonged lupus insensitive activated partial thromboplastin time (aPTT) is routinely done at London Health Science Center (LHSC), Ontario.

Aims: We aim to determine the clinical utility of the CI diagnostic algorithm.

Methods: We retrospectively reviewed all potential CI [lupus insensitive aPTT of ≥ 32s (normal range 20s-29s)] from April 2014 to June 2019 in our institution. The diagnostic algorithm requires completion of CI only if deemed clinically necessary by the hemostasis technician via chart review to ensure no interfering medications and meets the predetermined clinical criteria (preoperative sample or unexplained bleeding). If the criteria for CI are met, we complete a one-stage factor assay activity for Factor VIII(FVIII), IX(FIX) and XI(FXI). Data were obtained through the electronic medical records to capture clinical characteristics, laboratory findings, prophylactic hemostatic replacement and bleeding outcomes.

TABLE 1 Review of 55 patients with abnormal CI

Characteristics	N; (range)
FVIII deficiency	7; (4%-48% FVIII activity)
FIX deficiency	2; (6%-49% FIX activity)
FXI deficiency	42; (4.5%-65% FXI activity)
Diagnosed Etiology for factor deficiency	N; (notes)
Congenital FXI deficiency	3; (1 severe, 2 partial deficiency)
Congenital mild FVIII deficiency	3
Congenital mild FIX deficiency	1
Factor inhibitor	1 FVIII inhibitor (8 BU), 1 FIX inhibitor (6.4 BU)
other	5

TABLE 2 Types of surgical procedures in patients with abnormal CI including bleeding complications

Surgical Bleed Risk	No. of patients based on Factor Deficency		Range of Factor Activity Level	Post-op bleeding, N	Comments
Major risk	**FVIII**	**3**	**25-48%**	**0**	**Temporal lobe lobectomy; total knee replacement; major urologic procedure - no hemostatic prophylaxis**
	FIX	0	-	-	
	FXI	23	29-65%	2	Combination of major abdominal and urologic procedure in patient with FXI of 29% - no bleeding; Factor activity in bleeding patient: 38%, 62%
Moderate risk	FVIII	0	-	-	
	FIX	0	-	-	
	FXI	7	9-63%	1	FXI 7 9-63% 1 Factor activity in bleeding patient: 27%; Colonoscopy with biopsy in patient with FXI of 9% - no bleeding
Low risk	FVIII	3	27-42%	2	Factor activity in bleeding patient: 27%, 42%
	FIX	0	-	-	
	FXI	2	43-46%	0	

Results: 3317 samples from 2940 patients were identified as potential CI during the study period. 263 distinct patients met the criteria for CI and had factor assays completed. Of those, 55 (21%) patients had abnormal factor testing with potential clinical implications (Table 1). Most had single factor deficiency (95%) with FXI deficiency as the most common deficiency (73%). Only 9 patients received formal consultation with hematology, and 14 patients had confirmation of etiology for factor deficiency on subsequent testing (Table 1). Table 2 lists the type of surgery including bleeding outcomes. Only 1 patient received prophylactic hemostatic therapy.

Conclusions: Reflex coagulation factor testing is unique to our institution at LHSC. Through the application of a diagnostic screening algorithm, we were able to identify rare bleeding disorders such as acquired hemophilia earlier through screening aPTT alone. The low follow up rates for further testing of an identified factor deficiency suggests potential lab alert fatigue from ordering physicians.

PB0685 | Validation of the Atellica COAG 360 Coagulation Analyzer

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Background: The coagulation tests are primarily used to assess the blood clotting function in patients. Modern analyzers allow measurement of the components responsible for coagulation in order to contribute to the diagnosis and monitoring of bleeding disorders.

Aims: The aim was to evaluate the analytical performance of Atellica COAG 360 (Siemens Healthineers) for prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen, antithrombin (AT), D-dimer and anti-Xa activity.

Methods: We used citrate blood samples collected from randomly selected patients. The validation was performed within two weeks. Patient samples (n = 410) were used for comparison analyses with the routinely used analyzer BCS XP1 (Siemens Healthineers). First, we tested the samples on the routinely used BCS XP 1 analyzer as a reference method and then on the Atellica COAG 360 analyzer as a test method. On the new Atellica COAG 360 coagulation analyzer, using the reference material (positive, negative control) we performed an experimental model 5x5 (repeatability test and intermediate inaccuracy-ANOVA analysis) for the following tests: prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen, antithrombin (AT), D-dimer and anti-Xa activity.

Results: The results of statistical analysis of the reproducibility and inaccuracy of the control material within one day (5 measurements for each test) and between 5 days showed good repeatability and precision, since the coefficient of variability (CV) between batches and within batches ranges within very narrow limits. The results of the statistical analysis of the method of comparing the test results between the analyzers showed a high correlation between the analyzers, because the results of the correlation coefficients range between 0.922 and 0.996.

Conclusions: Based on the results, we find that Atellica COAG 360 is suitable for routine use for all of these tests.

PB0686 | Rapid Latex Enhanced Immunoassay (LIA) for Heparin Induced Thrombocytopenia and False Positivity

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Background: Serotonin release assay (SRA), the gold standard for diagnosis of heparin-induced thrombocytopenia (HIT), is mostly performed in reference laboratories with unacceptable turn-around-times (TAT). HIT- ELISAs are performed in most laboratories, usually with 24-hour TAT. HIT IgG antibody is more specific for HIT-thrombosis; IgA and IgM antibodies are not pathogenic. Recently, a fully automated, rapid assay latex enhanced immunoassay (LIA) (HemosIL, HIT-Ab $_{(PF4-H)}$, Instrumentation Laboratory, Bedford, MA) was FDA approved, which replaced our manual ELISA (PF4 IgG assay, Immucor, Waukesha, WI). LIA-positive samples reflex to SRA (Versiti, Milwaukee, WI).

Aims: We evaluated performance characteristics of LIA test compared with SRA and clinical outcome.

Methods: We reviewed all 65 positive LIA tests (≥ 1 U/ml per manufacturer) between October 2017 and September 2019. Fifty-nine tests from 52 patients had corresponding SRA; 44 had clinical data available. Statistical analyses were performed using Stata and Microsoft Excel.

Results: All patients had a 4Ts score ≥ 3. LIA had a 60% false positive rate (31 of 52 patients negative by SRA); positive predictive value (PPV) was 0.40 (95% CI 0.27, 0.54). Seven SRA-negative patients had repeat SRAs; 2 (29%) became positive. One of these positives had initial LIA of 1.7 U/ml and repeat result of 1.4 U/ml after 5-day interval. The second patient had initial and repeat LIA of both 7 U/ml at 6-day interval. Of the 28 SRA-negative patients with clinical data, 25 (89%) were treated with alternate anticoagulation.

Conclusions: Test performance in our institution was concordant with the package insert PPV. The high false positive rate (PPV 0.40) may be due to detection of IgM or IgA HIT antibodies that do not activate platelets. However, LIA shows increased sensitivity, becoming positive before SRA as demonstrated in two cases. To reduce false positives by LIA and its attendant treatment consequences, we are revising our testing algorithm (Figure 1).

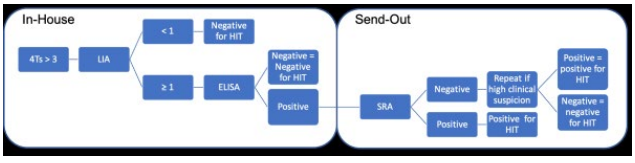

FIGURE 1

PB0687 | Advancing the Understanding and Significance of Genetic Testing Results in Hemostasis/Thrombosis

K. Lee[1]; D. DeMille[2]; K. Gomez[3,4]; W. Kahr[5]; M. Lambert[6]; D. Lillicrap[7]; D. McDougal[1]; S. Mohan[1]; P. Morange[8]; A. Mumford[9]; J. Ross[1]; J. Zehnder[10]; J. Berg[1]; N. Key[11]; on Behalf of the ClinGen Hemostasis/Thrombosis Clinical Domain Working Group Executive Committee

[1]*University of North Carolina at Chapel Hill, Department of Genetics, Chapel Hill, United States;* [2]*ARUP Laboratories, Salt Lake City, United States;* [3]*University College London, London, United Kingdom;* [4]*Royal Free Hospital, London, United Kingdom;* [5]*The Hospital for Sick Children, Toronto, Canada;* [6]*Children's Hospital of Philadelphia, Philadelphia, United States;* [7]*Queens University, Ontario, Canada;* [8]*Inserm, Marseille, France;* [9]*University of Bristol, Bristol, United Kingdom;* [10]*Stanford University, Stanford, United States;* [11]*University of North Carolina at Chapel Hill, Department of Medicine, Chapel Hill, United States*

Background: Genetic testing is increasingly utilized in hematology as a first line tool for diagnostic purposes. While genetic testing can aid in determining disease etiology without the need for multiple confirmatory laboratory tests, reporting of results may be imprecise and lead to confusion or mis-diagnosis.

Aims: The Clinical Genome Resource (ClinGen) is a consortium project funded primarily by the National Human Genome Research Institute of NIH to promote genomic knowledge in order to improve patient care. The Hemostasis/Thrombosis Clinical Domain Working Group (CDWG) within ClinGen was established in 2018 to address the need to improve the utility of genetic testing, while leveraging previous work by the ThromboGenomics project.

Methods: The CDWG convened an executive committee of clinicians and researchers with expertise in hemostasis/thrombosis who prioritized the curation of genes and variants within several domains: platelet disorders, coagulation factor deficiency disorders, von Willebrand disease, thrombosis and hereditary hemorrhagic telangiectasia. Expert panels were developed to tackle both gene curation (assessing the strength of association between a particular gene and a disease phenotype) and variant curation (assessing the pathogenicity of variants within selected genes with respect to a specific phenotype).

Results: Gene curation results are publicly available on the ClinGen website (https://clinicalgenome.org/) and can be used to assess the validity of genes within testing panels, and determine which disease phenotypes are associated with genes of interest. Variant curation results, which will be submitted to the ClinVar website for public use, will provide expert-reviewed variant classifications in genes included in clinical test panels.

Conclusions: By assessing the strength of gene/disease associations, standardizing gene-specific variant curation guidelines, sharing of genomic data amongst expert members and the incorporation of data from existing disease databases, the number of variants of

uncertain significance will decrease, thereby improving the value of genetic testing as a diagnostic tool.

PB0688 | Factor V inhibitor in a patient with Strongyloides Stercolaris and antibiotic therapy

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Background: There are reports of patients with Factor V inhibitor in exposure to bovine thrombin, surgery, antibiotic therapy (β-lactam or aminoglycoside group) and oncohematological diseases.
Aims: To report a case acquired Factor V inhibitor with Strongiloidiasis and antibiotic therapy.
Methods: Patient78-year-old male, with a history of partial prostactectomy, chronic renal failure, choledocytic and vesicular lithiasis, cholangitis, biliary focus sepsis in empirical treatment with Imipenem, fibrillation and atrial flutter initially anticoagulated with rivaroxaban and subsequently with antivitamin K (AVK). Because of suspiction of psoas hematoma AVK was suspended and thromboprophylaxis with low molecular weight heparin was started. Hematoma was ruled out and right psoas abscess (Echerichia coli associated with pleural effusion) was confirmed. A treatment for 28 days with ceftriaxone and Strongyloidiasis diagnostic were carried out.
Retrospective analysis of the patient′s medical history. Coagulation laboratory studies: Prothrombin time (PT): Thromborel, Siemens. Partial Activated Thromboplastin Time (APTT): Pathromtin SL, Siemens. Fibrinogen: Dade Thrombin Reagent (Clauss), Siemens. Waveform analysis. Platelet count: Advia 2120. Thrombin time: Thrombin Time, Siemens. Factor measurement in 3 dilutions: Deficient plasma and Siemens calibrator. Lupus Anticoagulant: LA1 and LA2, Siemens; Silica Clotting Time: Instrumentation laboratory. Coagulometer: Sysmex CS-2500.
Results: See Table
Conclusions: Due to the suspicion that the development of the inhibitor might have followed to strongyloidiasis and antibiotic therapy, ceftriaxone was suspended and rotated to endovenous Ertapenem until 6 weeks are completed and treatment for Strongyloides with Ivermectin is performed. Then the patient normalizes the PT (%) and negativizes the FV inhibitor, with later normalization of FV levels. This inhibitor could be associated with the use of antibiotics, in an anticoagulated patient with strongiloidiasis.

TABLE 1 Results

Assay (Reference Value)	04/2019	01/ 07/2019	30/07/19-02/08/19	26/ 08/2019	02/ 09/2019	11/2019
Prothrombin Time PT: (70-120%) / APTT (24-42 sec)	27 / 38	32 / 38	86 / 31	<5 / 169	36 / 51	79 / 35
Fibrinogen (200-400 mg/dL) / Thrombin Time TT: (12-20 sec)	* / *	* / *	* / *	445 / 16	378 / 16	* / *
waveform análisis TP, APTT / Platelets (200-400 10^{-3} uL)	* / *	* / *	* / 277	Alteraded / 252	Normal / 243	* / 290
PT (%), Normal Pool / APTT, Normal Pool	* / *	* / *	* / *	93 / 30	90 / 33	* / *
PT (%), Mix Pat+Normal / APTT, Mix Pat+Normal	* / *	* / *	* / *	25 (fail corrected) / 52 (fail corrected)	65 / 36	* / *
Factors II, VII, IX, X, XI, FVIII one stage and chromogenic	*	*	*	Normal (increase activity with dilution)	Normal	*
Factor XII (50-150%) / Factor V (70-120%)	* / *	* / *	* / *	30 / <5	40 / 20	* / 90
Lupus Anticoagulant	*	*	*	Not determinable by inhibitor interference	Negative (tested on the mixture P1:N1)	*
Inhibitor aFV (<0.6 UB/ml)	*	*	*	5.0 (Positive)	0.2 (Negative)	Negative

PB0689 | Clinically Significant Disagreement between Point-of-Care INR and Central Laboratory-assessed INR in Acute Stroke Patients

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Background: Current guidelines indicate that an INR > 1.7 is a contraindication to thrombolysis therapy in acute stroke patients. Guidelines also recommend a patient arrival-to-thrombolysis-start time of 60 minutes. However, assessment of INR in acute stroke patients can lead to treatment delay, if result turnaround time is prolonged. Point of Care (POC) coagulometers have been proposed as a potential solution, since they allow for rapid, bedside assessment of INR. Yet, POC coagulometers are susceptible to several pre-analytical and analytical variables and, data on the safety of these devices in the acute stroke emergency care setting are limited.

Aims: We sought to assess the reliability of POC-INR results relative to central laboratory-assessed INR (CL-INR).

Methods: We performed a retrospective review of all POC-INR results obtained in parallel with CL-INR over a 1-year period in acute stroke patients presenting to our emergency department.

Results: Thirty-eight parallel testing instances were identified. There was a strong positive correlation between POC-INR and CL-INR (P = 0.84). When the prothrombin time was within the normal range on the POC device (40% of cases), agreement with the CL-INR around the INR=1.7 cutoff for thrombolysis therapy was 100%. However, the POC prothrombin time was prolonged in 60% of cases with 16% of these having clinically significant disagreement with the CL-INR. In 3 cases, the POC-INR was >1.7 while the CL-INR was < 1.7. Likewise, in 3 other cases, the POC-INR was < 1.7 while the CL-INR was >1.7.

Conclusions: In 16% of cases, over a 1-year period, assessment of INR with a POC device alone could have led to the wrong treatment approach, potentially increasing the risk of adverse events in our emergency department acute stroke patients.

PB0690 | Inherited Coagulopathies Study by Massive Sequencing (NGS) Experience of a Diagnostic Portuguese Center

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Background: Next-generation sequencing (NGS) with targeted gene panels introduced substantial benefits in the clinical practice regarding definitive diagnosis in certain haematological diseases in which the candidate gene is not obvious. It changed study algorithms in diagnostic centres and contributes to a broader view of the molecular causes of diseases, identifying genetic heterogeneity and allowing a better genotype/phenotype correlation.

Aims: To improve diagnostic capacity with NGS workflow using a panel of Thrombosis and Haemostasis (TH) which includes 43 genes for the diagnosis of Inherited Bleeding Diseases, Thrombosis and suspected Platelet disorders (IBD, T and PD).

Methods: 496 patients were studied (mean age 34.5 years (1M-78); 257F:239M, since may/2017, from several central hospitals with clinical data of IBD (n=311), T (n=133) and PD (n=52), for elucidation of molecular diagnosis with the panel PTH/NGS IONS5. Study algorithm includes, in severe haemophilia A, initial exclusion of F8 inversions and posterior detection of deletions/duplications by MLPA.

Results: In 300/496 patients were identified variants that justified the phenotype and were consistent with functional studies. A total of 146 different pathogenic/likely pathogenic/UVS variants in 28/43 genes were found, 42 not previously described. In 226/311 (73%) IBD patients (haemophilia, VWD, fibrinogen and other factor deficiencies) variants were detected in *F5*,*F7*,*F8*,*F9*,*F10*,*F11*,*F12*,*F13A1*, *LMAN1*, *FGA*, *FGB*, *FGG*, *GGCX*, *VWF*; In 29/52 (56%) PD patients (thrombocytopenias and functional platelet disorders), variants were identified in *GP1BA*,*GP6*, *PLAU*, *TBXA2R*, *ITGA2B*, *ITGB3*, *NBEAL2*, *ANKRD26* and *TUBB1*; only in 45/133 (34%) patients with thrombosis, variants were identified in *PROC*, *PROS1*,*F3*, *FGG*, *PLG* and *SERPINC1*. Double and compound heterozygous has explained different intra/interfamilial patient's phenotypes.

Conclusions: Sensitivity in mutation detection was different between the 3 groups, being the thrombosis group the one revealing lower diagnostic efficacy. These data show the need of exome study in some patients. NGS reveals to be a valuable tool in our clinical practice and provides more accurate diagnosis helping to determine the most effective treatment in these complex diseases.

PB0691 | Identifying DOACs in Mixing Study Samples

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Background: The College of American Pathologists accreditation standards require that laboratories have a procedure to detect antithrombotic drugs that inhibit coagulation in samples with positive mixing study results. With increasing use of direct Xa inhibitors (DOAC) in our patient population, our laboratory is increasingly, perhaps inadvertently, receiving mixing study samples from patients with recent DOAC use. While review of medication history can reveal recent DOAC use, sometimes, the only clue that a DOAC is present is an inhibitor pattern in the mixing study results.

Aims: We sought to determine whether an inhibitor pattern is a reliable indicator of DOAC presence.

Methods: We performed a retrospective review of mixing studies performed on patients with recent Rivaroxaban use and for which a concurrent anti-Xa Rivaroxaban level was available.

Results: Nineteen test results met the study criteria. We found that the difference between the predicted mixing study result based on factor X prothrombin time reagent sensitivity and the actual prothrombin time mixing study result significantly correlated with the Rivaroxaban level in a linear fashion (P=0.66, p=0.0076).

Conclusions: A prothrombin time mixing study inhibitor pattern indicated by the difference between predicted and actual mixing study results based on factor X reagent sensitivity can provide a clue that a DOAC may be present in a mixing study sample.

PB0692 | Validation of the ROTEM PLATELET® Module as an Impedance Platelet Aggregation Test Compared to the Gold Standard Test: Conventional Platelet Aggregation

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Background: Platelet aggregation testing using platelet-rich plasma is the gold standard test for evaluation of platelet function. Despite being considered the most important test in the diagnosis of platelet disorders, this technique can be affected by different pre-analytical and analytical conditions. ROTEM PLATELET® allows simultaneous analysis of thromboelastometry and platelet aggregation using impedance methodology, including the effect of different anti-aggregating drugs through different agonists: ARA-TEM for cyclooxigenase inhibitors, ADP-TEM for ADP receptor blockers.
Aims: The present study aims to investigate the reliability of the ROTEM PLATELET module® in evaluating platelet function in ARATEM and ADPTEM tests.
Methods: Samples of 40 outpatients who would electively do a conventional platelet aggregation test were collected upon a signed consent form. Included 10 healthy volunteers as a control group. The results of PLATELET ROTEM®, were analyzed in the form of three parameters: A6, MS, AUC and the results of platelet aggregation performed in Chronolog by impedance or optic method using ADP agonists (3 Micromoles and 1 Micromol) and achidonic acid. The analysis was performed qualitatively (normoaggregating x hypoaggregating x hyperaggregating) according to the reference values already established in the laboratory. Statistical analysis was performed through Kappa agreement test, and calculated Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV).
Results: When a concordance analysis was performed between the results of the 49 samples, it presented Kappa agreement of 0.58, demonstrating a moderate agreement between the two methodologies. However, using only healthy volunteers and patients using antiaggregants (ASA and clopidogrel), totaling 25 samples, the agreement test Kappa obtained was 0.91. Specificity and Negative Predictive Value were 100%.
Conclusions: From the validation of the ROTEM PLATELET®, it was possible to offer a laboratory test - Point Of Care to assess platelet function quickly and effectively, allowing the identification of patients with high hemorrhagic risk (using antiplatelet drugs).

PB0693 | Two Cases in which Measuring Dabigatran Plasma Levels Is Indicated and Recommended

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Background: P-Glycoprotein (P-gp) inhibitors are contraindicated in association with dabigatran etexilate due to an increase of its absorption, ultimately increasing AUC and Cmax of dabigatran, the active drug. Patients in clinical trials are older than 18 years, therefore no guideline gives indications on how to treat juvenile deep vein thrombosis (VTE). Tests for the measurement of dabigatran's blood concentrations utilizing the diluted thrombin time are available: in patients taking 150 mg dabigatran twice daily, the 90th percentile of plasma concentrations measured at trough is about 146 ng/ml.
Aims: We report the case of two patients treated with dabigatran 150 mg b.i.d. in whom the measure of plasma dabigatran levels confirmed the therapeutic choice.
Methods: The first case concerns an adult male patient with atrial fibrillation complicated by cerebral ischemic stroke. He had to start therapy with mirabegron, a P-gp inhibitor prescribed for overactive bladder. We measured our patient's dabigatran plasma concentration at trough at baseline and after three and six weeks of mirabegron treatment. Dabigatran plasma levels were 125 ng/mL, 187 ng/mL and 164 ng/mL, respectively. Anticoagulant treatment was continued safely.
Results: The second case concerns a young girl aged 16, affected by extensive spontaneous DVT. The choice of dabigatran etexilate rested on the pharmacodynamic data available and the availability of the antidote (idarucizumab). We prescribed 150 mg twice daily, considering the high CrCl (95.6 mL/min) associated with the young age which could result in underdose with 110 mg twice daily. We measured dabigatran's plasma concentration at one week, one month and three months of treatment. Plasma levels were 54 ng/mL, 88 ng/mL and 77 ng/mL, respectively, confirming that with full dose dabigatran her trough plasma levels allowed continuation of treatment.
Conclusions: Our cases show that it is possible to improve dabigatran treatment in patients with the help of plasma concentrations measurement.

PB0694 | Quantra Reported Stiffness Values Have a Strong Linear Relationship with Clot Max Stiffness

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Background: The Quantra® is a newly introduced cartridge-based viscoelastic testing device designed to monitor coagulation at the point-of-care (POC). The system directly measures a series of clot stiffness-based parameters which include total Clot Stiffness (CS)

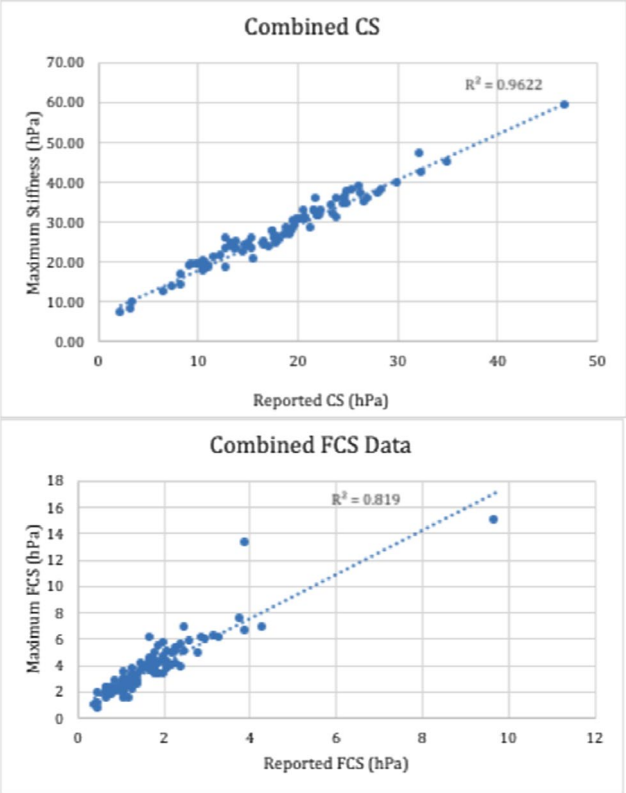

FIGURE 1

and the Fibrinogen Contribution (FCS) to clot stiffness. From the measurements of CS and FCS, the Platelet Contribution to stiffness (PCS) is calculated. CS and FCS represent the clot stiffness (shear modulus) measured 7 minutes after clot initiation.

Aims: To establish the relationship between the measurements of CS and FCS determined 7 minutes after clot initiation and the maximum clot stiffness values measured by the Quantra System.

Methods: The Quantra System uses multichannel cartridges with each channel containing unique combinations of lyophilized reagents. CS is measured in the presence of thromboplastin, whereas FCS is measured in the presence of thromboplastin with inhibition of platelet aggregation/contraction by abciximab. In the experiments presented here, clot stiffness vs time curves were generated for a time period of roughly 60 minutes and maximum (i.e., peak) clot stiffness values were recorded off-line using MATLAB (Mathworks Inc, Natick, MA).

Results: Data were collected using an Investigational Use Only (IUO) version of the Quantra and QStat cartridges from whole blood samples obtained from normal healthy volunteers (N = 32) and trauma patients enrolled at two sites in the United States (N = 56). The data indicated a strong correlation between FCS, CS and maximum clot stiffness measurements. The r value for the combined relationship is above 0.9 indicating that the 7 min reading used by the Quantra is directly proportional to maximum stiffness. (See Figure)

Conclusions: The relationship between maximum clot stiffness and Quantra-based stiffness parameters is highly linear indicating that reporting clot stiffness 7 min after clot initiation not only establishes physiologically based representative data but also more rapid information than max stiffness readings.

PB0695 | Interference of C - Reactive Protein Level in Activated Partial Thromboplastin Time

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Background: The C - reactive protein (CRP) is an acute-phase protein with an affinity to phospholipids. Some studies correlate a prolongation of the Activated Partial Thromboplastin Time (APTT) in proportion to the increase in the CRP concentration depending on the type of APTT reagent used in the routine.

Aims: To evaluate the interference of the levels of CRP in the reagent used for the determination of APTT performed routinely in our laboratory.

Methods: Samples from 1859 patients were included in this study. APTT assays were performed on the ACL TOP 750 instrument using the APTT-SP HemosiL (Werfen). Normal samples showed normal APTT ratio up to 1.19 and the abnormal APTT ratio were higher than 1.19. CRP measurements were performed on VITROS 5600® using the hs-CRP and CRP reagents (Ortho Clinical Diagnostics). The CRP results were classified according to the patient's cardiovascular risk (Low: < to 1.0 mg / L; Medium: between 1.0 and 3.0 mg / L; High: > to 3.0 mg / L). The Spearman test was used to assess the correlation between the levels of CRP and APTT ratio.

Results: In total samples evaluated, 18.61% had high risk CRP and prolonged APTT ratio; 57.27% had high risk CRP with normal APTT ratio, 1.24% medium risk CRP and prolonged APTT ratio; 10.99% CRP medium risk and normal APTT ratio; 0.73% CRP low risk and prolonged APTT ratio and 11.16% CRP low risk and normal APTT ratio. There was no correlation between the levels of CRP with abnormal APTT ratio (Spearman r = 0.27; r2 = 0.055)

Conclusions: The data obtained in this study show that high levels of CRP did not influence the prolongation of APTT assay in our routine, not showing in this study interference with the phospholipids present in the evaluated reagent.

PB0696 | Clinical Utilization of Lupus Anticoagulant Testing in a Large Hospital System

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Background: Lupus anticoagulant (LA) laboratory testing is mostly requested by internists. Testing for the presence of a LA is performed after screening samples for anticoagulants that interfere with testing. Tests for antiphospholipid antibodies (APA) are performed independently. Our approach is to assess for the presence of

LA/APA based on the Sydney criteria for the diagnosis of antiphospholipid antibody syndrome (APS)

Aims: The goal of this retrospective review is to evaluate the appropriate clinical utilization of the LA testing in our hospital system.

Methods: Six months of LA and APS testing results performed in 2019 were retrospectively reviewed for:

1. Dual LA testing - PTT/lupus anticoagulant (PTT LA) screen followed by confirmation using the hexagonal phospholipid neutralization test (HXN), and simultaneously performing the Dilute Russell viper venom test (DRVVT) screen followed by confirmation with the DRVVT normalized ratio(DRVVTNR),
2. Concurrent orders of APA - anticardiolipin, and/or beta 2 glycoprotein antibodies,
3. Positive APS test results,
4. repeat testing for positive results
5. overall sample anticoagulant triage outcome

Results: 544 LA tests reviewed. Results were negative for LA in 90% and confirmed positive in 10%. 43% of the confirmed LA tests were identified solely based on PTTLA, 27% based on DRVVT and 30% were confirmed positive for both. Of the 38% samples with concurrently ordered APAs, 18% also tested positive for LA. Testing was withheld on 77.5% of testing due to the patient being on anticoagulation.

Conclusions: Triaging decreased the number of equivocal results due to anticoagulation interference. Confirmed positive results were obtained when performing dual testing for LA. Less than one fifth of the positive LA or APAs were repeated. Even with the widely disseminated Choosing Wisely campaign, improper laboratory testing after a thrombotic event persists. Using resources such as Data Management Team as proposed by Clinical Pathology experts may improve appropriate testing practices.

PB0697 | Prevalence and Pattern of Pseudo-thrombocytopenia in a Nigerian Tertiary Hospital

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Background: Pseudo-thrombocytopenia (PT) refers to falsely low platelet count usually due to improper counting of platelet on automation platform. This has a huge implication on patient care, leading to delay in surgeries, chemotherapy and other interventions. It also leads to wastage of resources on investigation of 'thrombocytopenia' and unnecessary usage of blood component particularly in low resource settings like ours.

Aims: The aim of this study is to determine the prevalence and pattern of thrombocytopenia and pseudo-thrombocytopenia in our hospital.

Methods: We retrospectively reviewed the Full blood count data of 2000 patients received over a period of 3 months. Peripheral blood film report of all cases of thrombocytopenia was accessed and in few cases fixed smears were stained and reviewed. The patients clinical summary, red cell indices and total WBC were also reviewed and analysed.

Results: The prevalence of Peudo-thrombocytopenia amidst our study population was found to be 0.6%, accounting for 4.8% of all cases of Thrombocytopenia. No cases of malignant disease have pseudo thrombocytopenia, in the PT group, the mean corpuscular volume is higher. We found no statistically significant correlate with occurrence of PT and age, degree of anaemia, mean corpuscular haemoglobin, red cell distribution width, and total white blood cell count.

Conclusions: This study shows that one out of twenty cases of thrombocytopenia are Pseudo-thrombocytopenia. This further emphasizes the role of peripheral blood film review in all cases of thrombocytopenia in order to avoid unnecessary intervention and wastage of scarce blood component in our environment

PB0698 | Comparison of Thromboelastometry by ROTEM® Delta and ROTEM® Sigma in Obese Patients

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Background: Rotational thromboelastometry (ROTEM®) is a point-of-care device that has been used to evaluate hypercoagulability in obese patients who were candidates for bariatric surgery. Two devices are commercially available to perform the thromboelastometric analysis, ROTEM® Delta and Sigma. ROTEM® Delta requires manual pipetting of blood samples and liquid reagents by trained personnel while ROTEM® Sigma is a cartridge-based fully automated system and no pipetting is needed.

Aims: To determine whether these two systems give similar results, we compared ROTEM® parameters using the ROTEM® Delta and Sigma devices in a group of 18 III grade obese subjects before bariatric surgery.

Methods: After informed consent venous blood samples were collected from patients in sodium citrated tubes and run in parallel using both systems with the tests EXTEM, INTEM and FIBTEM. The following parameters were analyzed: CT, CFT, alpha-angle and MCF. Results were expressed as medians with interquartile ranges (IQR)

TABLE 1 Difference between ROTEM® Delta and Sigma for CT, CFT, alpha-angle and MCF

	EXTEM			INTEM			FIBTEM		
	ROTEM® Delta	ROTEM® Sigma		ROTEM® Delta	ROTEM® Sigma		ROTEM® Delta	ROTEM® Sigma	
	Median (IQR)		p value	Median (IQR)		p value	Median (IQR)		p value
CT (s)	68 (61-71)	62 (59-68)	<0.05	187 (170-207)	186 (179-192)	0.77	62 (60-67)	61 (57-73)	0.50
CFT (s)	68 (64-81)	76 (68-94)	0.01	65 (53-75)	54 (51-70)	0.15			
Alpha angle (°)	76 (74-78)	74 (72-76)	<0.01	77 (75-79)	79 (78-80)	< 0.01			
MCF (mm)	68 (65-70)	67 (65-70)	0.46	66 (65-68)	67 (64-69)	0.55	22 (20-24)	19 (17-23)	<0.01

and the Wilcoxon signed rank test was used. *P* values < 0.05 were considered statistically significant.

Results: Median CT, CFT and alpha-angle values in EXTEM were significantly different between the two devices while median MCF values were similar (Tab. 1). In INTEM, median alpha-angle was significantly increased on Sigma analyzer compare to Delta ($p < 0.01$) while no significant difference was detected for all other parameters considered (Tab. 1). In FIBTEM, median MCF obtained from Sigma analyzer was significantly reduced compare to Delta ($p < 0.01$) while median CT values were comparable (Tab. 1).

Conclusions: In our group of obese patients a consistent variation was found between EXTEM tests performed on both devices, especially for CT, CFT and alpha-angle parameters. Therefore, results from EXTEM test should be interpreted in relation to the reference ranges established for each device.

PB0699 | On the Measurement of Direct Anti-Xa-Inhibitors

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Background: Anti-Xa assays are the standard of care for the estimation of plasma levels of anti-Factor Xa (FXa) inhibitors. Laboratories have to establish a calibration for each of the anti-FXa-inhibitors, which is expensive and cumbersome to handle.

Aims: The aim of this study is to show that the DOAC plasma levels can be estimated independently from the anti-Xa-assay and the specific calibrator used in the test. It would be beneficial to compare DOAC levels based on their actual anti-Xa activity rather than the non-comparable mass concentrations.

Methods: A total of 303 blood samples containing apixaban (n = 122), edoxaban (n = 85) or rivaroxaban (n = 96) from patients at defined time points after DOAC intake were included into the analysis. Plasma levels were analyzed using six anti-Xa-assays were calibrated against each available anti-Xa calibrator. A consensus value for each analyte and sample was calculated from all combinations of assay and calibrators. Results from individual combinations of assays and calibrators were compared to the consensus value.

TABLE 1 Correction factors for the determination of DOAC levels using a non-corresponding analyte and calibration

Calibrator (column) analyte (rows)	Apixaban	Edoxaban	Rivaroxaban
Apixaban	1	1.05	0.75
Edoxaban	0.95	1	0.71
Rivaroxaban	1.33	1.41	1

Results: The variation of results between different assays and calibrators does not exceed the measurement error of anti-Xa assays. Furthermore, the bias obtained by using the "wrong" calibration (e.g. determination of an edoxaban level using an apixaban calibration) is constant between assays and calibrators using correction factors.

Conclusions: DOAC levels can be determined with just one calibrator using correction factors for a non-corresponding drug. While apixaban and edoxaban show similar anti-Xa activity based on their mass concentration, rivaroxaban differs significantly.

Based on our data it would be possible to define a standardized calibrator for all DOACs using a concept similar to LMWHs. It is conceivable, that comparing DOAC levels based on their anti-Xa activity is preferable over a comparison based on mass concentration. As anti-Xa assays are incapable of identifying a DOAC in a sample, providing solely the anti-Xa activity constitutes a superior approach.

PB0700 | Use of Liquid Low Molecular Weight Heparin Anti-Xa Assay for Detection of Apixaban

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Background: Apixaban use has been increasing as an alternative to VKA and even other direct oral anticoagulants. Although monitoring is not required, detection of drug effect is important in determining need for anticoagulant reversal in bleeding patients. Andexanet

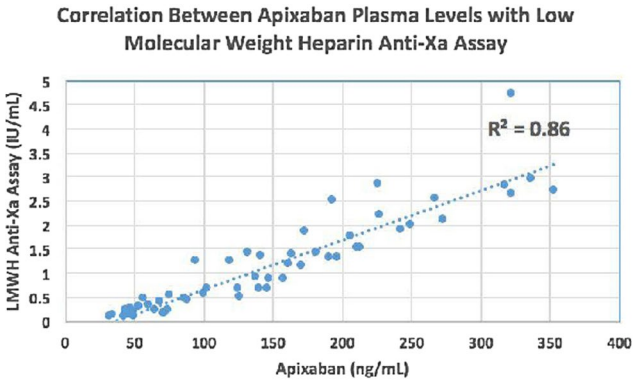

FIGURE 1

Alfa (Portola Pharmaceuticals, San Francisco, CA), a reversal agent for Apixaban and Rivaroxaban, carries a boxed warning for thromboembolic and ischemic risks due to inhibition of tissue factor pathway inhibitor and Annexa full study report excluded patients from hemostatic efficacy with Apixaban levels < 75 ng/mL. Anti-Xa assays with Apixaban calibrators are not routinely available and, thus, there is a clinical need for a routine test to detect Apixaban effect/level because PT is insensitive.

Aims: To determine if a ready to use liquid heparin anti-Xa assay can be used as a surrogate for Apixaban anti-Xa.

Methods: Forty discarded samples from 30 patients on Apixaban were frozen between May-October 2019. Low molecular weight heparin (LMWH) anti-Xa assay (Innovance Heparin, Siemens, Marburg, Germany) was performed on each sample. If results were > 1.5 IU/ml, the upper limit of detection, samples were diluted 1:5 with standard plasma and run to obtain discrete values. Apixaban anti-Xa assay (Hyphen, Neuville-Sur-Oise, France), a previously validated laboratory developed test, was concurrently run on these 40 samples. To obtain additional lower end values, 20 samples were selected in which Apixaban levels were between 100-200 ng/mL. These samples were diluted 1:1; both Apixaban and LMWH assays were then run. All assays were run on the BCP XP (Siemens). Linear regression analysis was used to assess degree of correlation between LMWH anti-Xa activity and Apixaban levels.

Results: LMWH anti-Xa shows good correlation with Apixaban assay (R^2=.86) (see Figure 1).

Conclusions: LMWH anti-Xa assay can be used as a surrogate to detect clinically relevant amounts of Apixaban.

PB0701 | Impact of Anticoagulant and of Time Elapsed Since Blood Collection on Platelet Function Assessed by Multiple Electrode Impedance Aggregometry

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Background: Preanalytical phase is the most critical part of the analyzing process in the haemostasis laboratory. For multiple electrode aggregometry (MEA), the manufacturer recommends to use hirudin tubes to preserve calcium levels; and to perform the analysis between 30 (taken as the reference here) and 180 min after blood collection. However, in clinical practice, citrated tubes and various time-intervals are often used without adjustment of clinical decision thresholds.

Aims: To assess the impact of two anticoagulants and five time-intervals since blood collection on ADP- and TRAP-MEA.

Methods: MEA was performed with a Multiplate® analyzer 5 min, 30 min, 60 min, 120 min and 180 min after blood collection from 20 healthy volunteers into hirudin 15 μg/mL and sodium citrate 109 mM tubes. Hirudin samples were diluted in NaCl 154 mM 1:1 v/v, and citrate samples with either NaCl 154 mM or $CaCl_2$ 3 mM solutions, 1:1 v/v. Blood samples were activated either with ADP (final concentration: 6.5 μM) or TRAP (provided by Roche; final concentration: 32 μM). Statistical analysis: Wilcoxon signed-rank test.

Results: Area under aggregation curves were significantly lower when citrate was used instead of hirudin ($p < 0.001$; Figure 1). The differences were significantly reduced when $CaCl_2$ solution was used as diluent for citrate samples, even though remaining significantly lower.

There was a statistically significant reduction of platelet reactivity when tests were performed within 5 min after blood drawing, by comparison with tests performed 30 min after ($p < 0.001$). Platelet function then remained quite stable until 60, 120 or 180 min after

TABLE 1 Mean differences of MEA between the different time points (30 min being the reference). Percentage difference (%) and 95% confidence interval for the m

		5 min (vs. 30 min)	30min	60min (vs. 30 min)	120min (vs. 30 min)	180min (vs. 30 min)
ADP	Citrate + CaCl2	−24.2 (−35.5%) [−30.3; −18.2] ***	0	0.9 (2.6%) [−5.1;7.0]	4.2 (7.5%) [−1.8;10.3]	−1.6 (1.0%) [−7.7;4.4]
	Hirudin	−7.5 (−10.0%) [−13.6; −1.5] *	0	4.40 (7.0%) [-1.61;10.41]	7.0 (11.6%) [1.0;13.1] *	6.0 (10.0%) [−0.01;12.0]
	Citrate + NaCl	−9.1 (−19.9%) [−15.1; −3.1] **	0	0.8 (3.0%) [−5.2;6.8]	−0.2 (0.1%) [−6.3;5.8]	−9.0 (−17.1%) [−15.0; −3.0] **
TRAP	Citrate + CaCl2	−36.4 (−29.5%) [−43.6; −29.3] ***	0	−6.8 (−5.8%) [−14.0;0.3]	−6.0 (−4.9%) [−13.2;1.1]	−14.7 (−12.1%) [−21.9; −7.6] ***
	Hirudin	−11.0 (−8.1%) [−18.2; −3.9] **	0	−2.3 (−1.2%) [−9.5;4.8]	−9.8 (−6.8%) [−17.0; −2.7] **	−13.6 (−9.2%) [−20.9; −6.4] ***
	Citrate + NaCl	0.8 (1.3%) [−6.3;7.9]	0	−10.3 (−9.2%) [−17.5; −3.2] **	−12.7 (−11.8%) [−19.8; −5.6] ***	−18.3 (−17.2%) [−25.5; −11.2] ***

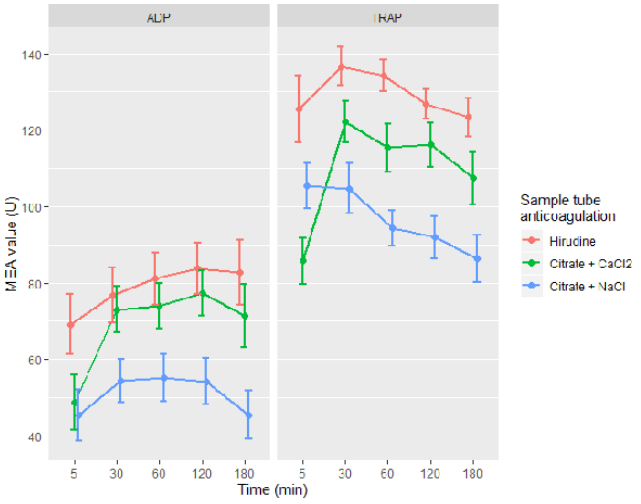

FIGURE 1 Mean areas under aggregation curves (U) according to the time elapsed between blood drawing and analysis

blood drawing, depending on the activator and the anticoagulant used (Table 1).

Conclusions: Overall, ADP- and TRAP-MEA should be performed between 30 and 120 min after blood drawing. If citrate tubes are used, dilution with $CaCl_2$ instead of NaCl should be considered to reduce differences with hirudin tubes. Clinical decision thresholds should be accompanied with the preanalytical conditions under which they apply, and used only under those specific conditions.

PB0702 | APTT Mixing Studies in the Preoperative Setting

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Background: Mixing studies have been classically used as the first step in the investigation of a prolonged APTT.

Aims: We aimed to investigate the information provided by APTT mixing studies performed prior to surgery from August 2018 to December 2019.

Methods: Abnormal APTT were investigated by mixing studies.

Results: Eleven cases with abnormal APTT in a preoperative screening were investigated. In two cases the mixing study did not correct APTT. After a 2 hours incubation period, 4 additional cases showed APTT inhibition. Six cases had positive lupus anticoagulant (LAC), five of them with prolonged mixing studies, and one with normal mixing study. Low levels of factor XII were found in this patient. Regarding surgery outcome, within the 6 positive LAC patients one major bleeding event was observed after spinal metastases resection in the only case with normal mixing study, three cases were operated without complications (bariatric surgery, anal fistula, and arteriovenous fistula), while two cases have not been operated (renal transplantation and hysterectomy). One patient with a prolonged mixing study and normal levels intrinsec pathway factors was not investigated for LAC and successfully underwent an appendicectomy. In three cases with normal mixing studies, lupus anticoagulant tests were negative. Two of them had low factor XII levels and underwent a knee arthroplasty and a lithotripsy without hemorrhagic events,

another patient had a very slight decrease in factor VIII and IX levels, and suffered a catastrophic hemorrhage during a pleural tumor resection due to cava vein laceration, while the fourth patient had slightly low levels of factor VIII, and underwent a knee arthroscopy without complications.

Conclusions: In our experience, none of the patients with TTPA inhibition after a mixing study suffered a hemorrhagic complication during surgery. All the patients with an abnormal mixing study who were tested for LAC had a positive result.

PB0703 | Algorithm for Detection of Procoagulant Samples with Elevated Prothrombin and Reduced Antithrombin by their Ability to Deplete Fluorogenic Thrombin Substrate

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Background: Thrombin generation (TG) test is a global hemostasis assay potentially suitable for detection of procoagulant conditions caused by elevated coagulation factor activity. However, commercially available TG assays may underestimate elevated TG in cases when fluorogenic substrate for thrombin is depleted or fluorescence is attenuated by the inner filter effect. An artifact correction approach called Calibrated Automated Thrombogram (CAT) is available but it remains unknown whether correction is either needed or sufficient to detect elevated TG.

Aims: We analyzed substrate depletion and its effect of TG parameters in plasma samples with elevated coagulation factor levels or reduced antithrombin, tested by commercial CAT platform.

Methods: CAT experiments and in-house software capable of internal thrombin calibration with or without substrate artifact correction.

Results: An increase in the Thrombin Peak Height (TPH) and Endogenous Thrombin Potential (ETP) after 2x- or 4x-fold increase in factors V, VIII, IX, XI, or prothrombin was detected in the presence and absence of artifact correction. CAT algorithm applied correction to every sample regardless of their procoagulant potential, decreasing TPH in low procoagulant samples by up to 10% and increasing TPH in all factor supplemented samples (except prothrombin) by up to 18%. Consistently, substrate was not consumed except in samples with elevated prothrombin or decreased antithrombin. For these highly procoagulant samples, uncorrected TPH or AUC values were underestimated and, in some cases, comparable to normal range, but application of CAT correction algorithm produced inconsistent or missing TG curves because of the failure of correction algorithm. Each sample that was poorly resolved by uncorrected or CAT correction analyses was characterized by the substrate depletion algorithm proposed in this work.

Conclusions: Our data suggest that substrate consumption correction algorithms are neither necessary nor sufficient for detection of extremely procoagulant samples, and can be replaced with a much simpler screening for substrate depletion.

PB0704 | Batroxobin Based Method to Measure Fibrinogen to Overcome Interfering Effects of New Anticoagulant Agents Targeting Thrombin

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Background: The interfering effects of new anticoagulant agents targeting thrombin on fibrinogen assay has not been completely resolved. Reptilase time screens for fibrinogen abnormality similar to the thrombin time, but uses batroxobin to form fibrin clot and is not affected by most thrombin inhibitors, including heparin derivatives, dabigatran, and hirudin derivatives.

Aims: We designed a batroxobin based method to measure fibrinogen and evaluated basic aspects of assay performance.

Methods: We conducted reptilase time assays on standard plasma calibrators. We fitted Gompertz function functions to replicate each clotting curve of reptilase time. From each clotting curve function, we took alpha, calculated maximum of first and second derivatives (max1 and max2) and reptilase time to prepare the calibration curve and formula. We named the fibrinogen values derived from those parameters and Clauss method as $FIB_{rept\text{-}\alpha}$, $FIB_{rept\text{-}max1}$, $FIB_{rept\text{-}max2}$, and FIB_{Clauss}. Then, we compared $FIB_{rept\text{-}\alpha}$, $FIB_{rept\text{-}max1}$, $FIB_{rept\text{-}max2}$ with FIB_{Clauss}. We found $FIB_{rept\text{-}\alpha}$ values were comparable to FIB_{Clauss}.

Results: The within-run coefficient variations of $FIB_{rept\text{-}\alpha}$ at the fibrinogen levels of 216 and 307 mg/dL were 1.6% and 1.2%. The linearity of $FIB_{rept\text{-}\alpha}$ was kept from the high level of 400.7 mg/dL to the low level of 75.7 mg/dL. A comparison study was performed with 182 blood samples by measuring $FIB_{rept\text{-}\alpha}$ and FIB_{Clauss}. In this, were included blood samples from 30 patients on heparin therapy, 72 on low molecular heparin (36 on dalteparin and 36 on enoxaparin. $FIB_{rept\text{-}\alpha}$ and FIB_{Clauss} showed good correlation (correlation coefficient = 0.96). We determined the effect of bivalirudin, heparin, low molecular heparin and dabigatran on $FIB_{rept\text{-}\alpha}$ and FIB_{Clauss}. FIB_{Clauss} falsely decreased at low level of bivalirudin (1.6 mg/dL). $FIB_{rept\text{-}\alpha}$showed no interfence of bivalirudin, heparin, low molecular heparin and dabigatran.

Conclusions: Our reptilase time based fibrinogen assay was well correlated with Clauss method and not interfered by thrombin targeting anticoagulants.

PB0705 | Thrombus Formation Study of Upstream Activation and Downstream Adhesion of Platelets in a Highly Integrated Microfluidic System

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Background: Activation of platelets tends to cause adhesion and aggregation of platelets to the vascular wall. In circulatory environment, activation and adhesion may not occur at the same site since platelet activation requires time.
Aims: Thus, we hypothesize that platelet activation at upstream and adhesion at downstream would be relevant in assessing the increased thrombotic risk associated with hyper-responsive vulnerable blood.
Methods: In this study, we designed a highly integrated microfluidic flow system to mimic the hemodynamic environment of vasculature with an upstream activation of platelets and downstream adhesion of platelets. Platelets can be activated by either agonists or shear stress generated with a rotational disk. Degree platelet adhesion was monitored by the migration distance (MD) of blood through the microchannel until it is blocked.
Results: Degree of pre-activation was widely varied with incubation time, agonist concentration and shear stress levels, which directly affected downstream adhesion of pre-activated platelets. There were linear relation between degree of activation and that of adhesion. However, excessive activation, rather, degraded downstream adhesion of platelets due to upstream aggregation and platelet lysis.
Conclusions: The results suggested that platelet adhesion was strongly dependent on the general conditions of upstream activation of platelets. This study implicates that downstream thrombus formation may be caused by upstream platelet activation rather than on-site activation.

NANOTECHNOLOGY AND NOVEL BIOMOLECULES

PB0706 | In vitro Studies of the Blood Compatibility of Water-Soluble Pristine C60 Fullerenes

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Background: Pristine C_{60} fullerenes and its derivatives are being explored for a variety of diagnostic and therapeutic applications. For further usage of these nanoparticles, the systematic toxicological investigations are required. Blood compatibility should be investigated for C_{60} fullerenes due to the potential blood contact. Currently, available data is not systematic and has not provided insights into the possible side effects of C_{60} fullerenes on circulatory components, including constituents of the hemostatic cascade.
Aims: In this study water-soluble pristine C_{60} fullerenes were tested *in vitro* to assess their hemocompatibility. Hemocompatibility has been evaluated looking at the impact of C_{60} fullerenes on erythrocytes integrity, platelet aggregation, and some coagulation factors.
Methods: ADP-induced aggregation was measured by a photo-optical aggregometer. The plasma coagulation potential was evaluated in clotting tests by a coagulation analyzer. The activities of exogenous thrombin and factor Xa were determined by means of the chromogenic substrates (S-2238/S-2222). Plasmin and protein C activators were used to generate the appropriate endogenous active enzymes followed the assessment of their activity by using the chromogenic substrates (S-2251/S-2366). The hemolytic activity was detected spectrophotometrically by the measurement of hemoglobin released from erythrocytes in response to different fullerene concentrations.
Results: Our results have revealed that C_{60} fullerenes can not provoke hemolysis at studied concentrations and did not show any effect on coagulation process. C_{60} fullerenes in concentration-dependent manner increased ADP-dependent platelet aggregation and significantly changed the kinetic parameters of this process. C_{60} fullerenes inhibited the amidolytic activity of thrombin but did not affect the activities of other studied coagulation factors.
Conclusions: The prothrombotic property of C_{60} fullerenes could be the potential risk factor lead to enhancement of vascular thrombosis. On the other hand, the ability of fullerenes inhibit thrombin activity is important for the pharmacological use of these carbon nanoparticles as anticoagulant agents.

PB0707 | Effects of Squalene-Adenosine Nanoparticles on Platelet Aggregation, Thrombin Generation and Endothelial Cells

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Background: Adenosine receptors represent key drug targets in many human pathologies, including cardiovascular, neurologic, and inflammatory diseases. The use of adenosine is inefficient upon systemic administration because of its fast metabolization and rapid clearance from the bloodstream. The conjugation of adenosine to squalene to form squalene-adenosine nanoparticles (SQAd NPs) improves the pharmacokinetic properties of adenosine and its potential clinical use. In view of the potential impact of adenosine on platelet function, hemostasis and endothelium, the assessment of the effects of SQAd NPs on platelet aggregation, thrombin generation and primary endothelial cells is needed.

Aims: To study the effects SQAd NPs on endothelial cells, platelet aggregation and thrombin generation.

Methods: Platelet aggregation was performed with optical aggregometry using different inducers (ADP, arachidonic acid and collagen) on platelet-rich plasma from 3 healthy donors.

A calibrated thrombin generation assay has investigated the impact of SQAd NPs on the three activation pathways of coagulation, using a normal pool plasma from 60 healthy donors.

Toxicological tests such as LDH activity (cell lysis), MTS (cell proliferation and activity) and ROS (cell inflammation) assays were performed on primary endothelial cells (HUVEC, HAEC) exposed during 24 h in presence of SQAd NPs ranging from 1 ng/ml to 100 µg/ml.

Results: An anti-aggregatory effect was observed with SQAd NPs at concentrations higher than 10 µg/mL, whatever the used inducer. No significant effect of SQAd NPs was observed on blood coagulation for the tested concentrations. Impact of SQAd NPs was observed at concentrations higher than 10 µg/ml on viability and mortality of HUVEC and on HAEC, without effect on inflammation.

Conclusions: At plasma concentrations lower than 10 µg/mL, SQAd NPs do not impact primary or secondary hemostasis and appears safe for endothelial cells. Additional studies are needed to further assess the hemocompatibility and toxicity of SQAd NPs and to establish the suitable dosage for their promising clinical uses.

PB0708 | Blood Compatibility of Injectables Liposomes Encapsulating Estrogen Estetrol to Prevent Cerebral Ischemia of Prematurity

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Background: Neonatal hypoxic-ischemic encephalopathy is one of the primary causes of severe injury among newborns. Nowadays, no effective treatment is available. Estrogens (estradiol (E2) and estetrol (E4)) play an important role in the brain development and protection. New injectable liposomes encapsulating E2 or E4 were developed in order to enhance its crossing through the blood-brain barrier and limit systemic hormone-receptor interactions. The impact of such new formulations on platelet aggregation, coagulation and endothelial cells is not known.

Aims: To assess the hemocompatibility including platelet aggregation, coagulation and endothelial cells of new injectable liposomes encapsulating E2 or E4.

Methods: The impact of E2 and E4, alone or encapsulated into liposomes on the coagulation has been assessed using the calibrated automated thrombogram using normal pool plasma. Platelet aggregation analyses were performed with optical aggregometry using different inducers (adenosine diphosphate, arachidonic acid and collagen) on platelet-rich plasma. Viability and mortality assays were performed on endothelial cell lines (EAhy926) exposed during 24 hours to E2 and E4, alone or encapsulated into liposomes at doses ranging from 1 ng/ml to 100 µg/ml.

Results: No toxicity was observed on endothelial cells, coagulation and platelet aggregation with liposomes encapsulating E4 and E4 alone. For liposomes encapsulating E2, an antiaggregating effect is observed when collagen is used as inducer while E2 alone had no impact on platelets. E2 encapsulated in liposomes and E2 alone had not effect on coagulation. On endothelial cells, E2 alone induced a decrease of cell viability from 10 µg/ml while encapsulated E2 has not.

Conclusions: Estetrol (E4) alone or encapsulated has no effects on platelet aggregation and thrombin generation and on endothelial cells confirming the hemocompatibility of this formulations for intravenous administration. The use of injectable liposome formulations encapsulating E4 is a promising strategy in newborns at risk of neonatal hypoxic-ischemic encephalopathy.

PB0710 | Thermostable Inhibitor of Contact Activation (TICA) Fully Blocks Contact Activation when Added to Blood Withdrawal Tubes

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Background: Thrombin generation and other clotting assays suffer from a wide variation of pre-analytical variables. One of those pre-analytical variables is contact activation through blood withdrawal methods, different syringes, differences in blood coagulation tubes, blood transport and sample handling. It has been shown that the addition of contact activation inhibitors in low tissue factor activated thrombin generation leads to a correction of the, in these circumstances significant, increase in thrombin generation due to contact activation. We compare thermostable inhibitor of contact activation (TICA) to the currently used corn trypsin inhibitor (CTI).

Aims: To compare the effectiveness of novel contact activation inhibitor TICA to CTI in low tissue factor-induced thrombin generation and recalcification in sodium citrate anticoagulated platelet poor plasma (PPP) and platelet rich plasma (PRP).

Methods: We compared TICA, CTI and plasma without contact activation inhibitors in low tissue factor PPP thrombin generation and in PRP recalcification thrombin generation, the latter the most sensitive condition for contact activation. In addition, off-target effects were investigated using factor XII-deficient plasma. Thermostability - as a measure of shelf life - was measured and compared to CTI.

Effectivity of contact activation inhibition before and after blood withdrawal was tested using thrombin generation.

Results: TICA is able to fully block contact activation in recalcified PRP and is comparable to CTI in doing so. TICA significantly lowers low tissue factor-induced thrombin generation by blocking contact activation. Pre-loading vacuum blood collection tubes with contact activation inhibitors is superior in inhibiting contact activation compared to addition of the inhibitor during the thrombin generation assay itself. TICA did not alter coagulation activity when added to FXIIa deficient plasma in thrombin generation. TICA, in contrast to CTI, is heat-stable improving processing and shelf life of pre-loaded blood drawing tubes.

Conclusions: TICA is able to fully block contact activation and has several advantages over CTI.

FIBRINOLYSIS AND PROTEOLYSIS

FIBRINOGEN AND FACTOR XIII

PB0711 | A Case Report of Spontaneous and Recurrent Arterial and Venous Thrombotic Events in a Child with FGA c.1372C>T Mutation

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Background: Dysfibrinogenemia is a rare qualitative fibrinogen disorder, characterized by a discrepancy between the activity and the antigenic levels of fibrinogen, that can lead to a wide spectrum of clinical manifestations, including asymptomatic, hemorrhage and thrombosis. Congenital dysfibrinogenemia (CD) mostly viewed as an autosomal dominant disease and caused by heterozygous missense mutations in one of the three FG. Diagnosis of dysfibrinogenemia can be misleading, since patients can be asymptomatic and present with bleeding or thrombosis. And there is no specific coagulation test available up to now, which posing a clinical challenge.

Aims: Describe a recurrent thrombosis in children with CD that has not be described in the literature.

Methods: Whole exome sequencing showed a missense mutation in exon 5 of FGA gene at the position 1372C →T, causing the Arg→Cys substitution at the α,chain, which came from his mother whereas there was a hybrid variation of this site of his mother. This mutation has been reported associated with VTE in the Human Gene Mutation Database and is predicted to be a deleterious mutation by SIFT software.

Results: Our patient, an 11-year-old boy with a history of CD with PE, right ventricle embolus, presented with acute abdominal pain and was subsequently diagnosed with superior mesenteric artery embolus. He was treated with enterectomy, subcutaneous Nadroparin during perioperative period and then transitioned lifelong anticoagulation with warfarin therapy. The general condition of the boy after surgery was good, radiological progress was good and he has experienced no more thrombotic problems since.

Conclusions: The heterozygous missense mutation of FGA c.1372C>G in patients with CD is associated with spontaneous thrombotic events at young age with normal fibrinogen level and prothrombin time. A familial screening and additional functional assays should be carried out if possible. A lifelong anticoagulation therapy may beneficial.

PB0712 | Assessment of in vitro Effects of Direct Thrombin Inhibitors on Activation of Coagulation Factor XIII through Enzyme Kinetics in the Synthetic Substrate-Based Assay

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Background: Factor XIII (FXIII) is activated by thrombin to catalyze covalent cross-linking formation between fibrin molecules, making clot stable and resistant to fibrinolysis. While FXIII is expected to be a novel target of therapeutic anticoagulation, FXIII reportedly prevents pulmonary emboli in mice by stabilizing deep vein thrombi. It remains to be elucidated how suppression of FXIII-mediated mechanisms influences thrombosis. Basic aspects of inhibition by direct thrombin inhibitors (DTIs) of FXIII activation are expected to provide insights into the concern.

Aims: The present study aims to characterize effects of DTIs on FXIII activation from the perspectives of enzyme kinetics.

Methods: The synthetic substrate-based assay for FXIII activity quantification was applied to analyses based on enzyme kinetics. The Lineweaver-Burk plot and the Dixon plot were used to assess inhibition types. The Hill plot using drug concentrations was subjected to calculation of Hill coefficients measuring inhibition cooperativity as well as half maximal inhibitory concentrations (IC_{50}). The Hill plot using FXIII concentrations was subjected to calculation of Hill coefficients measuring substrate cooperativity.

Results: According to combination of observations from the Lineweaver-Buk plot with observations about how FXIII concentrations impact the IC_{50} of each DTI, dabigatran and argatroban exhibited non-competitive inhibition or mixed inhibition mimicking non-competitive binding while hirudin and bivalirudin exhibited mixed inhibition mimicking uncompetitive binding. Dabigatran and argatroban exhibited positive inhibition cooperativity while bivalirudin exhibited negative inhibition cooperativity. The Hill plot analysis was inapplicable to the dose-response curve of hirudin putatively because of its irreversibility. Hill coefficients calculated using FXIII concentrations were around 1 with or without DTIs, proving that DTIs do not induce positive or negative substrate cooperativity of FXIII.

Conclusions: The results revealed the properties of effects of DTIs on FXIII activation from the perspective of enzyme kinetics, providing insights into the relevance of FXIII-mediated mechanisms to thrombosis.

PB0713 | Fibrinogen Concentrate Replacement Guided by Thromboelastometry Can Improve Clot Strenght in Adult Dengue with Severe Thrombocytopenia

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Background: Severe thrombocytopenia is a common manifestation in adult dengue. It could be associated with higher risk of life-threatening bleeding. Prophylactic platelet transfusion to prevent bleeding is a traditional practice, but It still has remained strongly questionable. Blood component transfusion has been known as potential risk for the patients. It can complicate with pulmonary injury or infection. Both fibrinogen and platelets are the main determinants of clot firmness, and fibrinogen has been considered a potential hemostatic factor that can compensate thrombocytopenia.
Aims: We reported a succesfull case of an adult dengue patient admitted in the intensive care unit with severe thrombocytopenia, treated with fibrinogen concentrate replacement instead of prophylactic platelet transfusion, guided by thromboelastometry.
Methods: This is a case report.
Results: A 28-year-old, white, male, with a history of liver transplantation due to primary cholangitis was admitted in the ICU with the diagnosis of dengue and severe thrombocytopenia. His physical examination revealed a GCS 15, HR: 80 bpm,BP:120/80 mm/Hg, spontaneous breathing, normal abdominal examination, preserved diuresis, disseminated rash throughout the body. Lab tests at the admission showed Hb 11.5 g/dl, Ht 35.2%, leucocytes 900 m/l, platelets 16.000/mm^3, fibrinogen 174 mg/dl, INR 1.1, creatinine 0.92 mg/dl, urea 31 mg/dl, ALT 917 u/l, ASP 699 u/l, bilirubin 0.3 mg/dl, CRP 3.13 mg/dl. NS 1 was positive for dengue. Thromboelastometry identified hypocoagulable state due to lower platelet count and fibrinogen concentration (Figure 1 and 2). 8 grams of fibrinogen concentrate was replaced to increase the clot firmness, instead of platelet concentrate transfusion, to avoid spontaneous bleeding. This patient was discharged from the ICU with an improvement in his leucopenia, thrombocytopenia and liver function only with fibrinogen concentrate replacement. No bleeding no further transfusion.

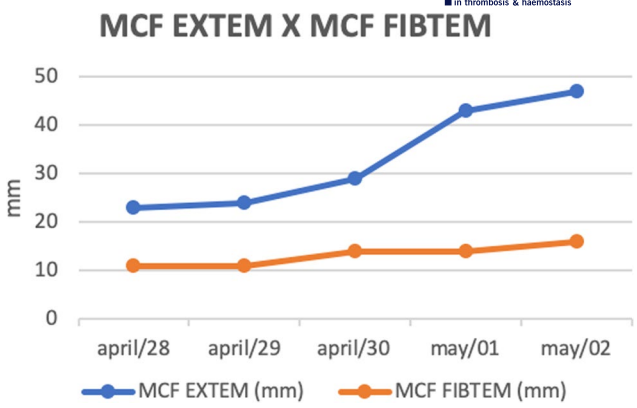

FIGURE 1 Relationship between MCF in EXTEM and FIBTEM

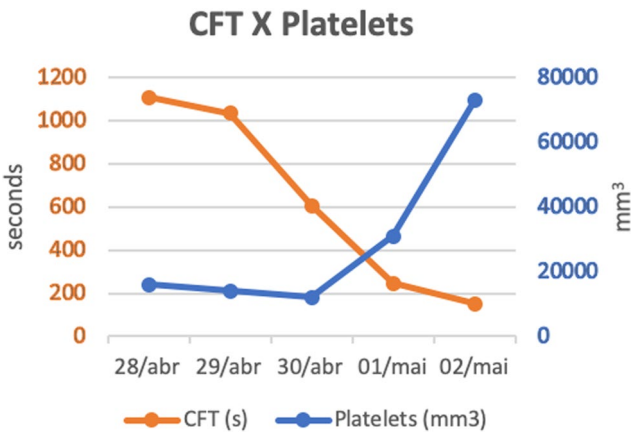

FIGURE 2 Relationship between CFT and platelets

Conclusions: In this case, fibrinogen replacement guided by thromboelastometry prevented spontaneous bleeding due to dengue related-severe thrombocytopenia, with no need of platelet transfusion.

PB0715 | Factor XIIIa-Mediated Red Blood Cell Retention in the Contracting Clot Is Impaired in Hereditary Dysfibrinogenemia

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Background: Clot contraction is a key event of hemostasis, inducing compression of erythrocytes into tightly packed polyhedra and redistribution of platelets and fibrin leading to the formation of an impermeable barrier. Red blood cell (RBC) retention in the clot is modulated by factor XIIIa crosslinking of α-chains. Patients with fibrinogen disorders have an abnormal clot structure and a heterogeneous clinical phenotype.

Aims: To assess if factor XIIIa-mediated RBC retention during clot contraction is altered in patients with hereditary fibrinogen.

Methods: Plasma rich plasma and whole blood clot contraction in the absence or presence of FXIIIa inhibitor (T101) was studied in hypofibrinogenemic and dysfibrinogenemic patients with either a bleeding and/or thrombotic phenotype, or asymptomatic. Contracted clots were weighed at 120 minutes and prepared for scanning electron microscopy.

Results: Overall, 4 dysfibrinogenemic and 6 hypofibrinogenemic patients were included (Table 1). The kinetics of clot contraction were similar between controls and patients, but clots formed from hypofibrinogenemic patients were significantly smaller and lighter (0.012 ± 0.06 vs 0.029 ± 0.09 mg/µl; p = 0.001). Clot contraction was almost complete within the first hour in all samples. The contraction slope significantly varied among patients and was correlated to rate of polymerisation as measured by turbidity. There was no correlation between the clot contraction parameters and the clinical phenotype nor with the causative mutation. Inhibition of FXIII resulted in lighter and smaller clots in controls (0.477 ± 0.33) and hypofibrinogenemia (0.314 ± 0.27) but not in dysfibrinogenemia samples with both *FGA* and *FGG* variants (0.933 ± 0.62; p = 0.01). Scanning electron microscopy confirmed the presence of polyhedrocytes inside the contracted clots.

Conclusions: Clot contraction was slower in hypofibrinogenemic patients compared to controls, highlighting the influence of fibrinogen concentration in this process. FXIII-induced RBC retention in the clot was altered in all tested dysfibrinogenemia samples. Further investigations will allow to determine whether these observations could be correlated to patients' clinical phenotype.

TABLE 1 Patients' biological and clinical data

FGA Arg35His	Dysfibrinogenemia	Asymptomatic	Fg:Act 0.7 g/L	Fg:Ag 2.8 g/L
FGB Arg294Ser	Hypofibrinogenemia	Asymptomatic	1.7	1.8
FGB Arg294Ser	Hypofibrinogenemia	Bleeding	1.8	2
FGB Trp470X	Hypofibrinogenemia	Asymptomatic	1	1.1
FGG Arg301His	Dysfibrinogenemia	Bleeding	0.8	2.6
FGA Arg38Gly	Dysfibrinogenemia	Asymptomatic	0.6	3.1
FGG Trp217Cys	Hypofibrinogenemia	Thrombosis	1.2	1.6
FGB Trp 470X	Hypofibrinogenemia	Bleeding	1.3	1.6
FGG Arg301His	Dysfibrinogenemia	Bleeding	0.9	2.3
FGG Tyr237His	Hypofibrinogenemia	Bleeding	1.4	1.6

PB0716 | Effects of Lysine-Containing Peptides Treatment on FXIII Activity and Fibrinogen Level of Metabolic Disorders

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Background: The disease, known as metabolic syndrome (MS), is often accompanied by thrombotic conditions caused by hyperproduction of the coagulation factor FXIII and the acute phase protein fibrinogen. The increase in fibrinogen levels, leading to increased clot density, and increased FXIII activity, enhancing the clot mechanical strength, the result lead to the slowing of fibrinolysis. Previously, the ability of short glyproline family peptides to influence the coagulation factors activity in health rats was established.

Aims: To evaluate the treatment effects of glyproline peptides RKKRPGP and KRKPGP on the fibrinogen levels and the FXIII activity in animals with experimental MS.

Methods: The experimental MS was reproduced on adult male Wistar rats, which were kept on high-calorie diet (130% of the

normal caloric content) for 6 weeks. At the 43rd day of diet, MS rats were treated with peptides either RKKRPGP or KRKPGP (0.1 mg/kg, intranasally, once daily, 7 days). Control (untreated MC rats) and healthy animals received 0.85% saline. In blood samples obtained on the 50th and 57th days, the fibrinogen level and FXIII activity were determined. All procedures were carried out in accordance with international bioethics principles.

Results: RKKRPGP and KRKPGP-treatment led to a decrease in the FXIII activity (by 41 and 55% vs. control, respectively). The reduced FXIII activity (75 and 79% of the MS control), corresponding to the values of healthy animals, was maintained a week after treatment. At the 50th day of the experiment, a significant decrease in the fibrinogen concentration was found only after KRKPGP-treatment (71% vs. control), but not a week after treatment with both peptides (57th day).

Conclusions: So, the peptides RKKRPGP and KRKPGP affect the fibrinolysis processes in metabolic disorders due to their ability to reduce the FXIII activity and fibrinogen levels (only KRKPGP), which can be useful in the fight against MS complications.

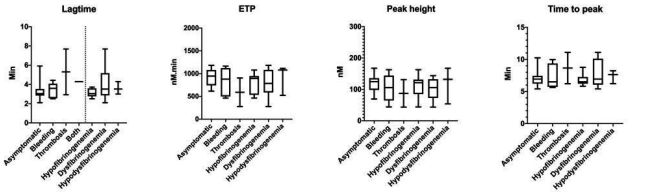

FIGURE 1 Mean TG variables with STG-BLS Genesia according to clinical and laboratory phenotypes

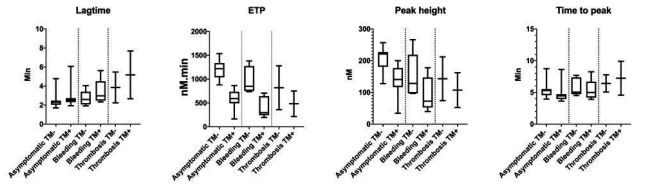

FIGURE 2 Mean TG variables with STG-TS Genesia according to clinical and laboratory phenotypes

PB0717 | High Individual Variation of Thrombin Generation by Genesia and Correlation of Fibrin-Associated ROTEM with Fibrinogen Activity in Patients with Congenital Fibrinogen Defects

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Background: The clinical course of hereditary hypo- or dysfibrinogenemia is often unpredictable. Asymptomatic individuals at the time of diagnosis may carry a lifetime risk of adverse outcomes. Standard coagulation assays fail to predict the patient's clinical phenotype (CP).

Aims: To assess the clinical outcome by studying thrombin generation (TG) and ROTEM in relation to various phenotypes.

Methods: 24 patients, 15 from Finland (11 unrelated families) and 9 unrelated from Switzerland were studied. Six experienced bleeding, 5 thrombotic complications, and 13 were asymptomatic with a familiar history of bleeds in 3 and thrombosis in 6. Fibrinogen (Fg) activity and antigen, and TG by Genesia with both Bleed- (STG-BLS) and ThrombosSreen (STG-TS) in the presence or absence of intrinsic pathway influence (corn-trypsin inhibitor, CTI) were studied in all. ROTEM was available for 11 patients. Genotyping was performed for 15 patients.

Results: Overall, 12 patients had hypo-, 10 dys- and 2 hypodysfibrinogenemia. Of the 15 different genetic defects, 6 were in the Fg gamma-chain, 4 in Fg Bbeta-chain and 3 in Fg Aalpha-chain. High inter-individual variation (up to 7-fold) in all TG variables with both STG-BLS and STG-TS was observed among the different groups, irrespectively of CP or Fg levels, use of TM (Figures 1 and 2) or of CTI. No correlation between Genesia and CP could be detected. FIBTEM picked up hypofibrinogenemia (MCF < 5 mm) in all patients, while being normal in dysfibrinogenemia. MCF by FIBTEM correlated with Fg antigen, unlike activity.

Conclusions: CP failed to associate with Rotem or TG in Genesia. FIBTEM detected hypofibrinogenemia but was unable to depict dysfunctional fibrinogen. The accordance of CP, Fg levels, fibrin clot structure and Genesia with genotypes will be explored as the next step. The impact of fibrin polymerization at the level of TG potential and FXIIIa activities may offer an underlying mechanism for these differences.

PB0718 | The Glycan Structure Associated to Factor XIII Subunit B and its Functional Importance

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Background: Coagulation factor XIII consists of two potentially active A subunits and two protective/inhibitory B subunits (FXIII-A2B2). In plasma FXIII-B2 is in a two-fold excess over FXIII-A2. FXIII-B is a ~80 kDa glycoproteincontaining 8.5% carbohydrate on two N-glycosylation sites. Neither the structure nor the functional role of the glycans on FXIII-B has been explored.
Aims: To reveal the glycan structures linked to FXIII-B, to deglycosylate the native protein and use it for functional studies.
Methods: The asparagine linked carbohydrates were released from human FXIIII-B by peptide-N4-(N-acetyl-beta-glucosaminyl)asparagine amidase (PNGase F) digestion under both denaturing and native conditions. The released N-linked oligosaccharides were fluorophore labeled and analyzed by capillary electrophoresis. Structural identification utilized glycan database search and exoglycosidase digestion based sequencing. The monomeric/dimeric structure of deglycosylated FXIII-B (dFXIII-B) was investigated by gel filtration. The association of native and dFXIII-B2 to FXIII-A2 were compared by surface plasmon resonance. Deglycosylated and native FXIII-B were injected into FXIII-B knock out mice and their clearance was monitored.
Results: Deglycosylation of the native protein was achieved by repeated PNGase F digestion. The total N-glycan profile of FXIII-B featured 9 individual structures, The core structure contained two N-acetyl glucosamines and three mannoses, four of them were fucosylated. Each structure contained at least one sialic acid. The elution profile of native and deglycosylated FXIII-B were similar, i.e, the absence of glycan structure did not change the dimeric structure. The equilibrium dissociation constant for the interaction of FXIII-A2 and dFXIII-B2 was one magnitude higher (2.35x10-8 M) than with the native protein (6.80x10-9 M). Deglycosylation highly accelerated the clearance of FXIIII-B2 from the circulation of FXIII-B knock out mice.
Conclusions: Characterization of the glycan moieties attached to FXIII-B is reported for the first time. The attached glycan very likely plays an important role in prolonging the persistence of FXIII-B2 and FXIII-A2B2 in the circulation.

PB0719 | Congenital Qualitative Fibrinogen Disorders: Case Series from Argentina; Multicentric Collaborative Study

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Background: Congenital qualitative fibrinogen disorders include dysfibrinogenemia (DF) with decreased functional and normal antigenic fibrinogen levels, and hypodysfibrinogenemia (HDF) with discrepant decrease of functional and antigenic fibrinogen levels. Most of patients are asymptomatic, but a bleeding, generally mild, and thrombosis could be present. Its prevalence is low and underestimate.
Aims: To describe a case series of qualitative fibrinogen disorders with their mutations found in Argentina.
Methods: Fibrinogen (FBG) were measured by Clauss Method (FBG-C). Immunologic FBG by radial-immunodiffusion technique (FBG-RID). Prothrombin Time derived FBG (FBGPTd). Thrombin Time (TT) and Reptilase Time (RT) were performed. Thromboelastometry ROTEM® Delta: FIBTEM MCF and EXTEM was recorded. Genetic studies: PCR Amplification and Sanger Sequences for the three FBG genes were also performed. We present the results found in 8 patients from 4 different centers in Argentina and 6 of their relatives.
Results: Patients characteristics, phenotypic expression, clinical manifestations and laboratory results are included in table 1. Two patients were asymptomatic, and the diagnosis was a laboratory finding. Three patients presented mild bleeding, one thrombosis and severe bleeding, one aortic valve thrombosis and one acute myocardial infarction. Three unrelated patients presented the same mutation (AαGly33(14)del), previously reported in China. Two patients presented "hotspot" mutations, and two unidentified mutations were found (one with thrombotic HDF, and the other with DF and mild bleeding). Only in one of the relatives studied we found the same mutation of the father.
Conclusions: Genetic defects found in the FBG genes could explain the discrepancy between levels of antigenic FBG and FBG-C. Two

TABLE 1 Patients investigated with the mutations found. DF: Dysfibrinogenemia, HDF: Hypodysfibrinogenemia, AMI: Acute myocardial infarction

Patient (Sex, Age)	Phenotype Clinical Manifestations	FBG-C mg/dL	FBG-RID mg/dL	FBGPTd mg/dL	TT sec	RT sec	ROTEM MCF (mm)	Genetic studies	Relatives studied
BOB MAT (M, 24)	DF Asymptomatic	52	388	285	30	30	Normal EXTEM	Heterozygous for AαGly33(14)del	-
DEL CRIS (F, 36)	DF Asymptomatic	67	251	363	32	23	Normal EXTEM	Heterozygous for AαGly33(14)del	-
PAS CLA (M, 63)	DF, Arterial thrombosis and severe bleeding	109	546	609	29	30	FIBTEM 61	Heterozygous for AαGly33(14)del	-
BUL ALI (F, 66)	DF, Mild bleeding	72	388	430	24	54	FIBTEM 15	Heterozygous for a previously unidentified mutation in FGA exon2:c. 106G>C Gly36Arg (Gly17Arg without the signal peptide)	Daughter: normal FGA Gly36
REI FAC (M, 38)	HDF, Aortic valve thrombosis and stroke	59	114	224	27	37	FIBTEM 4	Heterozygous for a previously unreported missense mutation in FGG exon 8: Tyr375Cys (Tyr349Cys in the mature gamma chain lacking the signal peptide)	Parents and sister: all normal FGG Tyr375
LUN PAB (M, 48)	DF, Mild bleeding post-surgery	56	190	214	30	24	FIBTEM 11	Heterozygous for FGA exon 2 c.96_98 del AGG, del Gly 33 (del Gly 14 in the mature chain without the signal peptide).	Daughter: Heterozygous for FGA exon 2 c.96_98 del AGG, del Gly 33 (del Gly 14 in the mature chain without the signal peptide).
RAM LAM (M, 79)	DF, Mild bleeding	45	373	240	35	>120	FIBTEM 8	Heterozygous for "hotspot" in FGA exon 2 c.103C>T, Arg35Cys	-
GER OMA (M, 76)	DF, AMI	90	No result	No result	21	22	FIBTEM 4	Heterozygous for a common "hotspot" dysfibrinogenemia mutation in FGG exon 8, Arg301Cys (Arg275Cys without the signal peptide)	Grand daughter: normal FGG Arg301
Reference range	-	180-400	150-400	200-400	14-18	15	FIBTEM 9-25	-	-

novels mutations and only few "hotspot" mutations were found in our case series. Most patients present normal or even increased viscoelastic fibrinogen assessment. Few patients with mild bleeding manifestations (only one severe bleeding), two asymptomatic and some patients with thrombotic manifestations were found, reflecting the phenotypic variability interpatient described in the literature.

PB0721 | Fibrin Deposition in Mouse Transfusion-Related Acute Lung Injury Via Coagulation or Fibrinolysis Dysfunction

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Background: Transfusion-related acute lung injury (TRALI) is a serious post-transfusion respiratory syndrome. However, the underlying pathophysiology of TRALI remains obscure. Fibrin deposition in lung is not only the pathological feature of lung inflammation but

also plays a pathogenic role in destroying the vascular endothelial structure and inhibiting the alveolar surfactant. Nevertheless, the role of the fibrin in TRALI has never been reported.

Aims: To determine whether fibrin deposition in lung participates in the progress of TRALI.

Methods: Eight-to-ten weeks male Balb/c mice were primed with lipopolysaccharide(LPS)18 h prior to challenge with an anti-MHCI mAb or isotype control antibody administrated into the tail vein.The characteristics of TRALI were assessed by the ratio of pulmonary wet to dry (W/D) and lung tissue histology score. Immunofluorescent staining assay and corresponding mean optic density (MOD) were used to evaluate fibrin deposition in lung.

Results: Compared with control mice, TRALI group mice displayed a significant increase in lung W/D ratio($P < 0.05$). In addition, increased alveolar septal thickening, exudated indicating edema intra-alveolar polymorphonuclear leucocyte infiltration and high lung tissue histology score($P < 0.05$)were observed in TRALI mice. Moreover, we found that upon TRALI induction,the fibrin deposition in lung was significant and the MOD obviously upregulated in mice($P < 0.01$).

Conclusions: Our results suggest that the presence of fibrin deposition in lung is involved in TRALI pathological responses, possibly resulting from excess activation of the coagulation cascade or decreased fibrinolysis. Further studies are needed to prove the hypothesis.

PB0722 | Cellular Factor XIII Externalization in Platelets and Platelet Microparticles Induced by Receptor Mediated and Non-Receptor Mediated Platelet Activation

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Background: Plasma factor XIII consists of two potentially active A subunits (FXIII-A) and two carrier/inhibitory B subunits. Its cellular form (cFXIII), a dimer of FXIII-A, is present in platelets in high concentration. cFXIII is of cytoplasmic localization, not released by the usual secretion mechanisms. It is present in microparticles (MPs), sub-micron seize extracellular vesicles, shed from cells upon activation by strong stimuli. Platelets activated by convulxin (CVX) and thrombin externalize cFXIII (Mitchell et al. Blood 2014;124:3982-90) and also induce the transposition of phosphatidylserine (PS) to the surface of activated platelets.

Aims: Monitoring the surface exposure of FXIII-A and PS on platelets undergoing receptor-, and non-receptor mediated activation and on the formed microparticles.

Methods: To generate MPs, gel-filtered platelets were stimulated by CVX, thrombin, CVX+thrombin or Ca^{2+}-ionophore (A23187). Platelets and platelet derived MPs were identified by anti CD41a antibodies. For flow cytometry monoclonal FITC-labeled anti-human FXIII-A antibody and phycoerythrin-conjugated annexin V were used. In immunfluorescent studies FXIII-A was labeled by rabbit polyclonal anti-human FXIII-A and DyLight 488-labeled horse anti-rabbit IgG, annexin V was conjugated to Alexa Fluor 568. Platelets and MPs were incubated with the labeled antibodies, then analyzed by flow cytometry and by immunfluorescent microscopy.

Results: After receptor-mediated activation of platelets with CVX+thrombin 35% of platelets and more than half of MPs became annexin V-positive and cFXIII-A became transposed to the outer membrane surface in 66% of platelets and 65% of MPs. The overwhelming majority of PS-positive platelets and MPs also showed FXIII-A positivity. Non-receptor mediated activation triggered by Ca^{2+}-ionophore resulted annexin V positive platelets and MPs, however neither the cells nor the formed MPs expressed FXIII-A on their surface.

Conclusions: Receptor-mediated platelet activation induced the transposition of FXIII-A to the surface of activated cells and the formed microparticles, while non-receptor mediated activation failed to externalize cFXIII.

PB0723 | Fibrinogen Supplementation in Patients with Acquired Hypofibrinogenaemia: Meta-Analysis and Systematic Review

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Background: Hypofibrinogenaemia is associated with poor patient outcomes in various clinical settings including trauma, cardiac surgery and obstetric haemorrhage; however, no study to date has combined data from multiple bleeding indications.

Aims: To systematically assess the evidence for the efficacy and safety of fibrinogen supplementation in coagulopathic bleeding patients with low fibrinogen levels, and analyse whether fibrinogen supplementation leads to improved outcomes in these patients.

Methods: EMBASE and PubMed/Medline databases were searched for parallel-group randomised controlled trials with patients receiving fibrinogen supplementation to manage severe haemorrhage due to acquired hypofibrinogenemia (until June 2018). Trials comparing treatment with fibrinogen concentrate (FCH) or cryoprecipitate with any another treatment were eligible; trials involving prophylactic

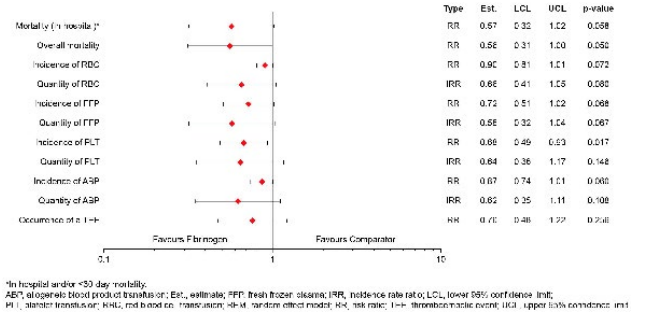

FIGURE 1 Summary of efficacy analyses

fibrinogen supplementation were excluded. The primary endpoint was patient mortality.

Results: Fifteen studies were identified, including 1195 patients (n = 586 received fibrinogen supplementation; n = 609 received a comparator). Baseline fibrinogen levels were comparable, and 14 studies used FCH. Bleeding indications included cardiac surgery (n = 563; 47.1%), obstetric haemorrhage (n = 299; 25.0%) and trauma (n = 270; 22.6%). The risk ratio (RR) for in-hospital and/or ≤ 30-day mortality was 0.57 (14 studies; 95% confidence interval [CI] 0.32-1.02); this result was not significant (p = 0.058) but trended towards lower mortality with fibrinogen supplementation versus comparator. A similar pattern was shown for other efficacy endpoints (Figure), with incidence of platelet transfusion associated with significant benefit versus comparator (12 studies; RR 0.68; 95% CI 0.49-0.93; p = 0.017). No significant difference was identified in thromboembolic event occurrence between groups (p = 0.256). Overall risk of bias was high in all studies.

Conclusions: Results for all endpoints trended in favour of fibrinogen supplementation versus comparator, with no safety signals identified. The significantly reduced incidence of platelet transfusion with fibrinogen supplementation suggests reduced blood loss versus comparator.

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PB0724 | Transcriptome Profiling of Coagulation Factor XIII Genes: Implications for Pleiotropic Pathways and Inter-Subunit Regulation

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Background: The plasma circulating pro-transglutaminase coagulation factor XIII is a heterotetrameric protein complex, composed of dimeric FXIII-A_2 and FXIII-B_2 subunits both of which are expressed in different cell types. While the FXIII- A_2 subunit is expressed in a variety of cell types which include platelets, monocytes and macrophages, although recently it has been demonstrated that the major contributor to plasma FXIII-A_2 are resident macrophages. The FXIII-B_2 subunit on the other hand is exclusively expressed in hepatocytic cell lineage. Little is known of FXIII gene's promoter regions in the context of their transcriptional regulation.

Aims: To investigate the transcriptomic profile of coagulation FXIII genes.

Methods: Our current study presents a bioinformatic analyses of publicly available selective microarray expression datasets from cells expressing FXIII subunits i.e. monocyte derived macrophages and hepatocytes/ hepatic-progenitor cells.

Results: The MXDI and HES2 transcription factors binding to the same EBOX binding site showed positive and negative significant correlation to FXIII-A_2 expression respectively in monocyte derived macrophages. Similarly, HES6 binding to the EBOX binding site was observed to be negatively correlated with FXIII-B_2 expression, whereas RFX5 was found to upregulate FXIII-B_2 in hepatocytes and hepatic progenitor cells. Transcriptional levels of the two subunits also show an inverse correlation within time specific array data sets raising the possibility that the two subunits could regulate each other's levels through indirect mechanisms. Gene ontology analysis of differentially expressed genes in time specific array data sets suggests the participation of the two FXIII subunits in newer (sterol biosynthesis) and also previously suggested (immune response/wound healing/membrane invagination) biological pathways outside or indirectly related to the coagulation pathway.

Conclusions: Our investigation reveals that both FXIII-A_2 and FXIII-B_2 subunits are regulated by common transcription factors. Enrichment of correlated genes with FXIII-subunits suggests pleiotropic roles.

PB0725 | Comparison Natural History and Genotype of 31 Patients with Inherited Dysfibrinogenemia

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Background: Inherited dysfibrinogenemia (ID) is a rare disorder of hemostasis. The majority of cases are caused by heterozygous missense mutations in one of the three fibrinogen genes. Patients with ID are associated with bleeding, thrombosis, but many are asymptomatic.

Aims: Better describe and compare the clinical, laboratory and genotypic picture of ID.

Methods: We evaluated 31 patients with ID from 7 unrelated families using standard coagulation tests (fibrinogen activity and antigen, activated partial thromboplastin, prothrombin, thrombin and reptilase time), rotational thromboelastometry (EXTEM,INTEM,FIBTEM) and genetic analysis.

Results: The clinical phenotype consisted of bleeding in 13/31 (42%) patients (bleeding score > 1 at any site), additional subjects 18/31 (58%) were asymptomatic. Among patients with bleeding, symptoms were mostly mild, at one or more anatomical sites. There were not observed thrombotic complications. Results of rotational thromboelastometry showed that median of selected parameters of CD were in the most of cases in the normal range. Paradoxically, the asymptomatic patients had a lower median of maximum clot firmness compared to symptomatic subjects. In the study there were identified three mutations in the FGA (c.95G>A, c.104G>A) and FGB (c.586C>T) genes.

Conclusions: The variants of ID identified in this cross-sectional study were either asymptomatic or had bleeding manifestations and show similar laboratory features irrespective of genotype. However, results from genetic, and clinical studies will continue to yield valuable information on the structure and function of the fibrinogen molecule as well as on the development and course of ID.

PB0726 | Factor XIII Cross-Links Fibrin(ogen) Independent of Fibrin Polymerization in Experimental Acute Liver Injury

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Background: Intravascular fibrin clot formation proceeds in a well-ordered series of reactions catalyzed by thrombin cleavage of fibrinogen leading to fibrin polymer formation and cross-linking by the transglutaminase factor XIIIa (FXIIIa). Extravascular fibrin(ogen) deposition is also a common feature in injured tissues, however, the mechanisms regulating fibrino(ogen) polymerization and cross-linking in this setting are poorly understood.

Aims: The objective of this study was to determine the mechanisms of fibrin polymerization and cross-linking in an established setting of acute liver injury induced by acetaminophen (APAP) overdose.

Methods: Mice were challenged with a hepatotoxic dose of APAP (300 mg/kg) or saline. Hepatic fibrin(ogen) deposition and cross-linking was measured following APAP overdose in wildtype mice, mice lacking the catalytic subunit of FXIII (FXIII$^{-/-}$), and in Fib^{AEK} mice, which express a mutant fibrinogen insensitive to thrombin-mediated fibrin polymer formation.

Results: Hepatic fibrin(ogen) deposition was similar in APAP-challenged wild-type and FXIII$^{-/-}$ mice, yet cross-linking of hepatic fibrin(ogen) was dramatically reduced (>90%) by FXIII deficiency. Hepatic fibrin(ogen) deposition was partially reduced (~50%) in APAP-challenged Fib^{AEK} mice, but most of the residual fibrin(ogen) deposits were still cross-linked. This result in Fib^{AEK} mice suggests that cross-linked fibrin(ogen) deposits in the APAP-injured liver may not all be in the form of polymerized fibrin. We then tested the hypothesis that the oxidative environment in the injured liver, which contains high levels of reactive mediators (e.g., peroxynitrite), modifies fibrinogen such that fibrin polymerization is impaired without impacting FXIII-mediated cross-linking. Notably, fibrin(ogen) modified with 3-nitrotyrosine adducts was identified in the APAP-injured liver. In biochemical assays, peroxynitrite inhibited thrombin-mediated fibrin polymerization in a concentration-dependent manner but did not block fibrin(ogen) cross-linking over time.

Conclusions: Collectively, these studies depict a unique disease state wherein defective thrombin-catalyzed fibrin polymerization is circumvented to allow tissue deposition and FXIII-dependent cross-linking of fibrin(ogen).

PB0727 | The Effects of (Patho)Physiological Fibrinogen γ' Levels on Static and Dynamic Clot Formation

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Background: Fibrinogen-γ′ accounts for 3-40% of plasmatic fibrinogen. In-vitro studies found γ′ influences clot structure, and clinically γ′ levels have been associated with arterial and venous thrombosis. However, the effects of γ′ at (patho)physiological levels and under flow are poorly understood.

Aims: Investigate the effects of (patho)physiological γ' levels on clot structure in static and flow conditions.

Methods: Fibrinogen and γ' were measured by Clauss and ELISA, respectively, in 1164 individuals (LEADS Study). Patient samples with high (n = 33, 30%), normal (n = 45, 10%) and low (n = 41, 3%) γ' were compared to purified fibrinogen samples with increasing γ' (3%, 10%, 30%). Clots were analysed by confocal microscopy, permeation, turbidity and lysis assays. Whole blood ROTEM was performed for 78 patients with a range of γ' levels. In a microfluidics model, fibrinogen-deficient plasma repleted with increasing γ' (3%, 10%, 30%), and plasma with high (n = 5, 30%) and low (n = 5, 3%) γ' were flowed over preformed platelet aggregates at venous (150s^{-1}) and arterial (500s^{-1}) shear rates.

Results: In purified and plasma static clots, higher levels of γ' (3-30%) caused a higher fibrin clot density (purified + 104.9%, patient + 14.1%),

thinner fibres (purified-24.6%, patient-9.4%) and slower lysis (purified + 31.1%, patient + 19.0%). In ROTEM, higher γ' correlated with reduced clotting lag-time (r = -0.254, p = 0.025), increased clotting rate (r = 0.248, p = 0.028), but not clot firmness (r = 0.125, p = 0.277). Under flow at $150s^{-1}$, higher γ' (3-30%) enhanced fibrin formation in repleted (lag time-94.6%, Max fibrin + 314.6%) and patient plasma (lag time-85.4%, Max fibrin + 167.1%), with clots of increased volume (repleted + 655%, patient + 252.7%) and height (repleted + 130%, patient + 146.2%). These effects were reproduced at $500s^{-1}$.

Conclusions: Fibrinogen-γ' at (patho)physiological levels affects clotting by increasing fibrin fibres, thickness and lysis resistance. Elevation of γ' enhanced clot formation under stasis or flow, without affecting viscoelastic properties. The changes in clot kinetics and structure may explain how γ' influences thrombosis.

PB0728 | Performance of a New Automated FXIII Activity Assay

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Background: Plasma transglutaminase FXIII confers mechanical and biochemical stability to blood clots. Congenital or acquired FXIII deficiency occurs rarely but activity levels ≤ 30% may be associated with severe bleeding, therefore careful patient monitoring is recommended. There are several assays for the manual determination of FXIII, however there is still an unmet diagnostic need for a reliable high-throughput assay.

Aims: The aim of the study was to demonstrate the performance characteristics of an automated FXIII activity assay which is suitable for use in routine laboratories.

Methods: The assay principle is based on the use of a highly sensitive fluorogenic substrate in combination with a thrombin reagent. FXIII is activated by thrombin. FXIIIa cleaves a dark quenching molecule from the side chain of a modified peptide incorporating glycine methyl ester. Subsequently, the fluorescence intensity of an N-terminal coupled dye increases and is detected by the automated coagulation analyser.

Results: Linearity was shown using SSCLOT5 reference plasma diluted in FXIII depleted plasma ranging from 0.026 IU/mL to 0.77 IU/mL with a correlation of $R^2 \geq 0.95$. Recovery of samples in this range was between 95% und 105%. Analytical sensitivity of the assay showed a LoD of 0.004 IU/mL and a LoQ at 0.009 IU/mL of FXIII activity. Inter assay variation was determined with samples of different activity levels on various time points and with multiple reagent lots. Observed precision for normal plasma (0.77 IU/mL) was ≤ 3% and for plasma in the medical decision range (≤0.3 IU/mL) was ≤ 5%. Comparison with a manual chromogenic assay for FXIII activity using samples covering the whole assay range gave both a regression (Passing and Bablok) and correlation of ≥ 0.95.

Conclusions: We have demonstrated in our performance study that this reliable and robust automated assay fulfils the current diagnostic need for the determination of FXIII deficiency.

PB0729 | Plasma Fibrin Clots of Acute Pulmonary Embolism Patients Present Reduced Amounts of Coagulation Factor XIII and α2-antiplasmin Compared to Clots Generated after 3-Month Anticoagulation

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Background: Fibrin cross-linking by coagulation factor (F)XIII leads to clot stabilization. Reduced plasma FXIII levels have been reported in acute pulmonary embolism (PE) patients.

Aims: We investigated associations of clot-bound amounts of FXIII and α2-antiplasmin with fibrin clot properties in patients with acute PE.

Methods: Clots generated from plasma of 18 PE patients on admission and after withdrawal of 3-month anticoagulation were assessed using mass spectrometry with a multiple enzyme digestion filter aided sample preparation (MED-FASP) along with measurement of fibrin clot permeability (K_s), clot lysis time (CLT), endogenous thrombin potential (ETP) together with plasma FXIII activity and α2-antiplasmin antigen levels, thrombin generation markers, and fibrinolytic proteins.

Results: In clots prepared from plasma obtained on admission, FXIII constituted 0.31%, while α2-antiplasmin 0.99% of the clot mass and their amounts increased after 3 months of anticoagulation by 36.3% and 14.4%, respectively. Clot-bound FXIII correlated with plasma F1 + 2 prothrombin fragments levels (r = 0.51, p = 0.03) and clot-bound α2-antiplasmin (r = 0.63, p = 0.0056), but not with fibrinogen levels, fibrin clot properties or ETP. Moreover, CLT, but not K_s or thrombin generation, was associated with clot-bound α2-antiplasmin (r = 0.43, p = 0.036) and tended to correlate with clot-bound thrombin activatable fibrinolysis inhibitor (r = 0.44, p = 0.08) on admission. Plasma FXIII activity on admission was associated with both clot-bound FXIII (r = 0.35, p = 0.043) and clot-bound α2-antiplasmin amounts (r = 0.47, p = 0.048) solely on admission, while plasma α2-antiplasmin showed no association with clot-bound α2-antiplasmin at both time points. The PE severity index correlated inversely with plasma FXIII activity (r = -0.5, p = 0.039) and tended to correlate with clot-bound FXIII amounts (r = -0.51, p = 0.056) but not with other variables tested. Following anticoagulation associations of clot-bound FXIII and α2-antiplasmin were not observed.

Conclusions: Our preliminary data suggest that decreased clot-bound amounts of FXIII with the subsequent reduction in α2-antiplasmin

can be detected in acute PE and show associations with reduced fibrinolytic capacity.

PB0730 | Eight Succesfull Pregnancies in Patients with Congenital Fibrinogen Disorders - Single Centre Study

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Background: Congenital fibrinogen disorders are rare and challenging disorders in pregnancy with no firm treatment guidelines and only case reports are published. The clinical course of these disorders varies from no manifestation, to bleeding or thrombotic events. Pregnant women with congenital fibrinogen disorders suffer from recurrent pregnancy loss. Patients were reffered to our centre after repeating miscarriages, unsuccesfull IVFs or through their hypo/dysfibrinogenemia family history.
Aims: We present 8 succesfull pregnancies and their management in 7 patients.
Methods: Seven patients and 8 pregnancies were treated between 2008-2019: 6x dysfibrinogenemia and 1x hypofibrinogenemia. Average age was 32 years (22-41) at delivery. Standard laboratory before pregnancy was: Clauss fibrinogen level, fibrinogen antigen level, additional functional tests (RT, TT, fibrin polymerization, TEG) and trombofilia (1x FVL heterozygot). Every pregnancy was laboratory monitored by fibrinogen and DD levels in each trimester. In all patients was proven fibrinogen heterozygous missense mutation. Our mutation findings were correlated with international registry (Human fibrinogen database) and its clinical manifestations.
Results: When sampling this unique patient cohort, we discovered one new mutation called „Fibrinogen Praha III". The fibrinogen substitution was used as prophylaxis in 6/8 deliveries and 1 amniocenthesis. Therapeutic fibrinogen substitution was in bleeding complications. Five pregnancies were covered by LMWH in prophylactic/therapeutic dose concerning laboratory and clinical presentation. We have treated 3 serious complications in 8 succesfull pregnancies: 1 patient with pulmonary embolism in the 1th trimester despite LMWH prohylaxis, 2. patient with hypofibrinogenemia presented with severe bleeding in the 2nd and preeklampsia in the 3rd trimester. Fibrinogen treatment led to no trombotic complications and vice versa LMWH had no bleeding complication.
Conclusions: Treatment of patients with so rare diagnosis is recommended in specialized centre. Further follow-up of patiens, international registry collaboration and effort to establish treatment guidelines is warranted.

PB0731 | Factor XIII-A Is Present within Organelles Known to Mediate Unconventional Protein Secretion

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Background: Factor (F)XIII-A is secreted into plasma by macrophages and may be released locally into injured tissues. However, FXIII-A lacks an ER signal sequence. Unconventional secretion of certain proteins, including fatty acid binding protein (FABP)-4, is induced by amino acid starvation (AAS) in cultured cells and this may activate various pathways including trafficking via endosomal/lysosomal membranes marked by Rab7 and LAMP-1..
Aims: To determine whether FXIII-A follows an endo-lysosomal route of secretion.
Methods: Transfected HeLa cells or human bone marrow derived macrophages (BMDM) were subjected to AAS. Protein secretion was determined by immunoblotting of the media and cell lysates. Marker proteins and FXIII-A were visualised by immunofluorescence, following cytosol depletion with saponin.
Results: AAS promoted selective release from transfected HeLa cells of both FXIII-A and FLAG-tagged FABP-4, relative to LDH (lactate dehydrogenase, 4 to 6 fold enrichment) and to tubulin (minimal release). Membrane-associated FXIII-A was essentially absent from HeLa cells under standard culture conditions, but became apparent following AAS. Transfected FXIII-A showed partial co-localisation

TABLE 1 Pregnant patients with congenital fibrinogen disorders - dys(D) or hypo(H)fibrinogenemia

Patient	Mutation	D/H	Phenotyp in register(B/T)	Previous miscarriages	Bleeding(B)	TEN (T)	Delivery
P1	Aa19Arg/Gly	D	Y/Y	0	0	0	2017
P2	Aa16Arg/His	D	Y/Y	2	0	0	2016
P3	Aa16Arg/His	D	Y/Y	1+unsucc. IVF	0	0	2019
P4A	Aa16Arg/His	D	Y/Y	0	1th trimester	0	2008
P4B					0	0	2011
P5	Aa13Gly/Glu	D	Y/N	3+unsucc. IVF	1th trimester	0	2011
P6	g363Tyr/Asn	D	new	0	0	recid. PE	2010
P7	g82Ala/Gly	H	Y/N	1	1th+2nd trimester	preeclampsia	2017(gemini)

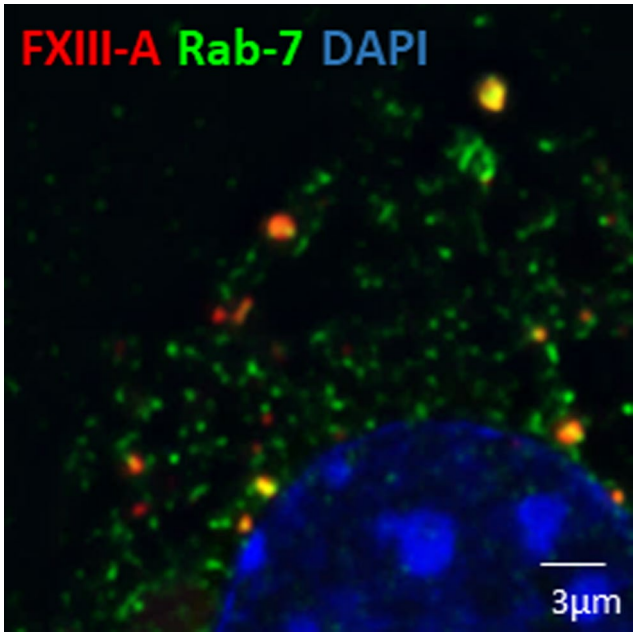

FIGURE 1 Colocalisation of FXIII-A and Rab-7

with Rab7 positive membranes in HeLa cells following AAS, and also with lysosome associated membrane protein (LAMP-1) positive structures. FXIII-A also co-localised with FLAG-FABP-4 in endo-lysosomes of co-transfected HeLa cells. A proportion of transfected FXIII-A co-sedimented with Rab7 in the membrane fraction of HeLa cell homogenates, while STED microscopy confirmed that FXIII-A was present within the lumen of Rab7 positive organelles. A proportion of endogenous FXIII-A was detected within Rab7 positive vesicles of BMDM following AAS (Figure below). Under these conditions, FXIII-A is also selectively released from BMDM although the enrichment of FXIII-A (2-4 fold) was lower than in transfected HeLa cells.

Conclusions: FXIII-A is sequestered to endo-lysosomes that are known to mediate unconventional secretion, and these organelles may mediate the delivery of FXIII-A into the plasma and/or into injured tissue.

PB0732 | A Genetic Modifier of Venous Thrombosis in Zebrafish Reveals a Functional Role for Fibrinogen AαE in Early Hemostasis

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Background: A fibrinogen isoform with C-terminally-extended Aα chains (AαE) represents ≈ 1% of circulating fibrinogen molecules. Larval zebrafish produce predominantly AαE-containing fibrinogen, with unknown functional significance. Distinct zebrafish strains differ in their ability to produce fibrinogen AαE, which correlates with inter-strain variability in experimental venous thrombosis. A causal role for altered AαE expression in thrombosis has not been demonstrated.

Aims: To determine if fibrinogen AαE expression is the basis of inter-strain variability in larval zebrafish venous thrombosis.

Methods: Time-to-occlusion and thrombocyte activity were monitored in larval zebrafish after venous laser injury. Aα or AαE chain overexpression and fibrinogen knockdowns were made using transgenesis and an antisense morpholino, respectively. Inter-strain sequence variation was assessed by Sanger and whole-genome sequencing.

Results: We measured the time-to-occlusion (TTO) of laser-induced venous thrombosis in AB and TU zebrafish strains. The AB strain gave a longer mean TTO than TU, and mating AB with TU produced larvae with the TU phenotype. AB larvae failed to produce fibrinogen AαE due to a mutation in the AαE-specific *fga* exon 6: c.1428delG (p.Ser477LeufsX4). The link between delayed TTO and the mutation was confirmed in larvae through mapping crosses of AB with the TL strain, followed by whole genome sequencing. We investigated whether the lack of AαE explained the delayed AB strain TTO. Transgenic expression of AαE shortened the AB TTO to that of TU and rescued venous occlusion in fibrinogen null mutants or larvae with morpholino-directed fibrinogen knockdown, but Aα expression was less effective. In 5-day-old larvae, when thrombocytes begin to contribute to hemostasis, laser-induced thrombocyte adhesion and aggregation was reduced in fibrinogen mutants. Expression of Aα or AαE restored thrombocyte accumulation similarly.

Conclusions: We define a genetic modifier of venous thrombosis, a role for fibrinogen AαE in early hemostasis, and suggest a link between differentially expressed fibrinogen isoforms and cell types available for clotting.

PB0733 | Multi-ethnic Whole Genome Sequence Analysis of Plasma Fibrinogen in over 150,000 Samples

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Background: Compared with array-based, imputation-based, and exome-focused analyses, whole genome sequencing (WGS) data provides better coverage of the genome and better representation of non-European variants.

Aims: To use WGS data to better understand the genetics underlying plasma levels of fibrinogen.

Methods: To better understand the genetics underlying plasma levels of fibrinogen we utilized data from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium

and the NHLBI's Trans-Omics for Precision Medicine (TOPMed) program. TOPMed phenotypes were centrally harmonized across up to 12 studies of European, African, Asian, or Hispanic ancestry then association analyses were conducted using Freeze 6 deep WGS data (N = 32,575). Inverse normalized and rescaled residuals adjusting for age, sex, study, TOPMed phase, study-specific parameters, self-reported ancestry, 11 ancestry informative principal components, and a kinship matrix. Analyses were conducted on the Analysis Commons using the SMMAT function implemented in GENESIS and included all variants with a minor allele count ≥ 40. Each CHARGE cohort undertook the same analyses using genotypic dosages imputed to the TOPMed backbone (N = 75,882). Inverse-variance weighted meta-analysis was undertaken using GWAMA for a final sample size of 108,454.

Results: 16 regions were significantly associated with plasma levels of fibrinogen. Most were in loci previously associated with these phenotypes, and the majority were common variants in high linkage disequilibrium with previously reported variants. The most significant association for fibrinogen was a rare missense mutation (rs148685782, $p = 5.85 \times 10^{-161}$, MAF = 0.003, *FGG*) located within the fibrinogen structural genes region.

Conclusions: WGS data provided better resolution of existing and discovery of new genetic regions associated with plasma fibrinogen levels. Additional data is anticipated from several more CHARGE cohorts, therefore update results from > 150,000 samples will be presented.

PB0734 | The Fibrinogen αC Region Is a Major Contributor to the Mechanical Strength of Fibrin Fibers

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Background: Fibrinogen is a key mechanical protein in blood coagulation since it is the building block of fibrin fibers, and these 100 nm thick fibers provide mechanical and structural stability to blood clots as they stem the flow of blood. Fibrinogen has two large, intrinsically unfolded regions, termed alphaC regions, which comprise 27% of its molecular weight. The role of these unfolded regions has long puzzled scientists.

Aims: We aim to determine if the αC region contributes significantly to the mechanical properties of fibrin fibers, and if so, which part of the αC region it is.

Methods: We used a nano-mechanical manipulation method, based on a combined atomic force and optical microscope, to determine the mechanical properties of native fibrin fibers, and of fibers formed from fibrinogen variant AαΔ1-10. In this variant, amino acids α264-391 in the aC connector region, which consists of ten, 13-amino acid repeat segments, are deleted.

Results: Compared to native fibrin fibers, fibers formed from the variant showed dramatically different mechanical properties. The extensibility (fracture strain) was reduced by a factor of 1.6 (from 1.96 to 1.26); the fracture stress was reduced by a factor of 22 (from 13.7 MPa to 0.61 MPa); and the modulus (stiffness) decreased by a factor of 5.8 (from 6.11 MPa to 1.05 MPa).

Conclusions: The large, intrinsically unfolded αC region of fibrinogen (specifically α264-391) is a major contributor to the mechanical strength of fibrin fibers, and thus to the strength of blood clots. It suggests that the αC region has a major role in fibrin fiber assembly.

PB0735 | A New Genetic Alteration in Fibrinogen Gene with a Direful Phenotype: A Tie-in in a Newborn

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Background: The inborn absence of fibrinogen protein in plasma is an uncommon hereditary disorder with low prevalence of approximately one in a million. The clinical symptoms ranges from mild to moderate bleeding with umbilical stump bleeding as a first sign to appear at birth. Severe clinical presentation may vary from bleeding diathesis including intracranial Hemorrhage and splenic ruptures to musculoskeletal deformities.

Aims: The study aimed the exploration of gene variants in an infrequently affected fibrinogen encoding gamma gene.

Methods: The observational study was carried with approval of Institutional Ethical Board. The fibrinogen quantitative assay (Clauss Assay: Diagnostic Stago) was performed for the confirmation of diagnosis. Gene sequencing was performed on ABI PRISM® 3100 (Applied Biosystems). The results were sought on HGMD (Human Gene Mutation Database) http://www.hgmd.cf.ac.uk/ac/index.php & www.geht.org) and Seattle SNPs (http://pga.gs.washington.edu/). ISTH-BAT was utilized for the assessment of bleeding symptoms in scores.

Results: The prolonged prothrombin Time (PT) and Activated Partial Thromboplastin time (APTT) along with negligible levels of fibrinogen. The bleeding scores were shown to have higher values. The novel gene variant identified as nonsense change, produced a truncated nonfunctional protein most likely due to skipping of NMD pathway.

Conclusions: Inherited Afibrinogenemia; a long remained unheard disease, now surfacing with an increasing incidence especially in high blood kinship regions. The Diversified pattern of symptoms in patients makes this disease more challenging with necessitation of large data contribution to design a standardized document of clinical pattern in correlation with genetic modifications.

FIBRINOLYTIC FACTORS AND INHIBITORS

PB0736 | Fibrinolytic Response Plays a Central Role in Trauma Induced Coagulopathy

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Background: Mortality and morbidity from severe trauma constitute a major global burden. Major causes of early deaths are massive hemorrhage and brain injury. Both hemostatic and fibrinolytic abnormalities are present. The use of antifibrinolytic agents is controversial as the pathophysiology of trauma induced coagulopathy (TIC) remains unclear.Though there was benefit of tranexamic acid in the CRASH-2 study, not all trauma patients have excessive fibrinolysis. In fact, tranexamic acid may be harmful for the non-fibrinolytic cohort. There is thus a need to find out the incidence of hyperfibrinolysis, and their relationship to the outcome.
Aims: Identify fibrinolytic phenotypes in major trauma.
Characterize the fibrinolytic response and its temporal relationship to trauma.
Provide guidance for antifibrinolytic therapy.
Methods: Data identification: An English-language literature search using PubMed and Medline, 2009 -2019, for articles with search terms “trauma”, “coagulopathy” and “fibrinolysis”.
Study Selection: 284 articles that addressed the stated purpose.
Results: In addition to activation of coagulation and impaired platelet function, changes of fibrinolytic activity were present in all patients with major trauma.
The incidence and state of fibrinolytic activity and the relationship to outcome were identified.
Fibrinolytic activity undergoes temporal change during the early stage of trauma (1-2 days).
Fibrinolytic response includes vascular release of fibrinolytic activator, tPA, as a result of endothelial injury leading to hemorrhage, hypotension and catecholamine release; but only few investigations have been made into the role of inhibitors of fibrinolysis.
The antifibinolytic agent tranexamic acid is of benefit only to patients with excessive fibrinolysis, but harmful in those with hypofibrinolysis.
Conclusions: Among the elements of trauma induced coagulopathy, changes in the fibrinolytics factors have been observed to play a central role. The mechanism by which the fibrinolytic system respond to trauma remains unclear. There is an unmet need for guidance on use of antifibrinolytic agents.

PB0737 | Carboxypeptidase U (CPU, TAFIa, CPB2) Generation in Acute Ischemic Stroke Patients Undergoing Thrombolysis and Endovascular Thrombectomy

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Background: Procarboxypeptidase U is activated by thrombin(-thrombomodulin) or plasmin into carboxypeptidase U (CPU, TAFIa, CPB2), a potent antifibrinolytic enzyme and an appealing target for the treatment of acute ischemic stroke (AIS). The kinetics of the total active and inactivated CPU (CPU+CPUi) have been studied in AIS; however, the kinetics of CPU generation and the influence of endovascular thrombectomy (EVT) on the CPU system are unknown.
Aims: Studying CPU generation in AIS patients receiving thrombolysis with or without EVT.

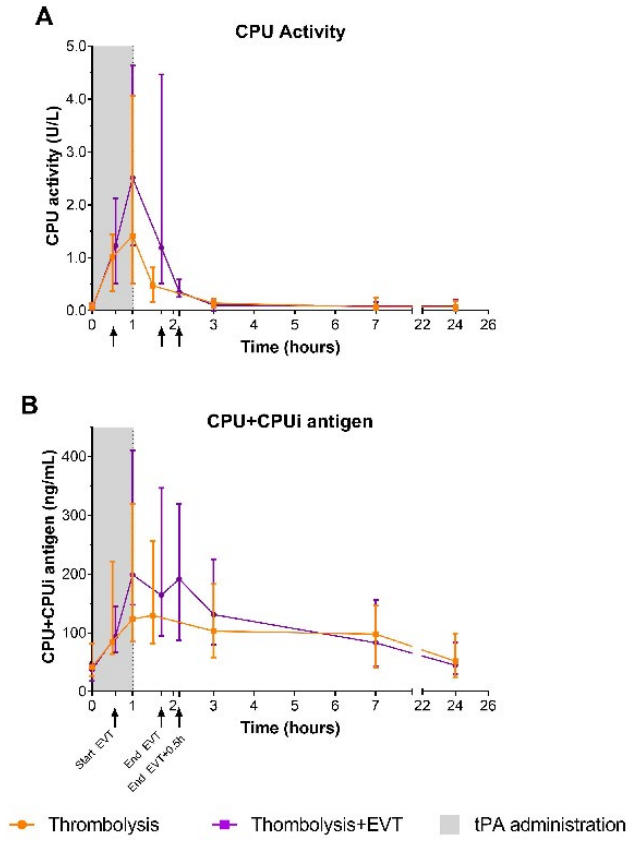

FIGURE 1 Median (interquartile Range, IQR) CPU activity and CPU+CPUi antigen in plasma of AIS patients undergoing thrombolysis +/-EVT

Methods: 37 AIS patients with documented cerebral artery occlusion and treated with rtPA (N = 20) or rtPA+EVT (N = 17) were included in this study which was conducted according to the tenets of the declaration of Helsinki revised in Fortaleza. CPU generation was studied by measurement of CPU activity and CPU+CPUi antigen levels. Blood samples were collected at baseline and at several time points over 24 h after thrombolysis initiation.

Results: Demographic characteristics were similar in both groups (mean age: 77.8 ± 17 y.o.). Occlusions were mainly distal in the rtPA group and proximal in the rtPA+EVT group. CPU and CPU+CPUi levels were highly variable in both groups. Levels increased upon rtPA administration (Figure 1A-B) and reached peak values at the end of thrombolysis. Then, CPU activity decreased rapidly to baseline level within 3 h, while CPU+CPUi levels remained elevated until 7 h after thrombolysis induction. CPU activity and CPU+CPUi levels tended to be higher in the rtPA+EVT cohort compared to the rtPA cohort.

Conclusions: CPU was rapidly generated during thrombolysis and decreased immediately after the end of rtPA infusion. Conversely, CPU+CPUi levels remained elevated for several hours. This underlines the complementarity of both measurements to assess the dynamics of the CPU system during AIS. Whether the trend towards higher CPU levels during EVT is due to larger thrombus size or to the procedure itself remains to be investigated.

PB0738 | Dipicolinic Acid Produced by Fermentation (*Bacillus subtilis natto*)

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Background: Natto greatly contributes to the fibrinolytic system of human, and dipicolinic acid (2,6-pyridinedicarboxylic acid:DPA) produced by fermentation easily produces tissue plasminogen activator (t-PA) from human endothelial cells. In vivo, DPA also increases t-PA in plasma and has various chelating effects on heavy metals.

Aims: This time, we succeeded in significantly increasing the production of these physiologically active substances from *Bacillus subtilis natto* (natto) using Shochu lees-glycerol.

Methods: Nattokinase (NK) was measured by the fibrin plate method (37°C, 4 h), and DPA was measured by HPLC.

Results: A liquid culture of Bacillus natto using 4% polepepton was used as a control, and 0.4% by weight of potato Shochu lees or buckwheat Shochu lees and 1 to 5% glycerol were added. It was measured. Glycerol 5% showed the highest value, and NK activity increased more than 5 times (673.3/130.6 mm^2) in stationary culture for 7 days, and at the same time, DPA production increased more than 7 times (673.4/89.2 ppm). Shochu lees was diluted with distilled water so that the initial nitrogen concentration was 0.4% by weight, and glycerol was added to this so that the final concentration was 6.0% by weight to obtain a culture medium. A jar fermenter containing 1500 ml of culture medium was inoculated with the precultured culture solution, and cultured for 72 hours at 37°C under aeration conditions of 1.0vvm and stirring at 600 rpm while controlling the pH at 7.0. As a result, it was found that the DPA production increased remarkably from 0.01 g/L to 4.4 g/L culture solution.

Conclusions: NK was found to be produced by fermentation in the presence of Shochu lees and glycerol. Under these optimal conditions, the yield of DPA in jar fermenter culture strongly increased (4.4 g/L). DPA is food-derived, tasteless, odorless, and highly safe, and is considered to be useful in many future administration studies.

PB0739 | Nattokinase - Inhibition of Melanin Synthesis (Whitening Effect)

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Background: We have reported on the properties of nattokinase (NK) produced by fermentation of *Bacillus subtilis natto*. In clinical practice, it has been found that it is effective as an antihypertensive and, more recently, has the potential to prevent dementia.

Aims: This time, it was revealed that NK has kinin production from kininogen and melanin aggregation inhibitory activity.

Methods: NK is a high-purity (423 IU/g) substance provided by (Honda Trading) Organo Food Teck Co. Ltd., Japan. The activity was measured by a well plate method using a synthetic substrate (Bz-Ile-Glu-(OR)-Gly-Arg-*p*NA:S-2222). The kinin-forming ability of NK was tested by the Magnus method using rat uterus as usual. B16 cells, a mouse melanoma cell line, were cultured in D-MEM supplemented with 10% fetal calf serum, 0.05% L-glutamine, and 0.2% sodium bicarbonate at 37°C and 5%CO_2 for 25 cm^2. Propagation and subculture were performed using a culture flask. Total RNA was extracted using the AGPC (Acid-Guanidium-Phenol-Chloroform) method.

Results: When NK was applied to plasma kininogen, strong kinin activity was observed even at 1 ng/ml or less. In addition, tyrosinase was added to a mixed solution of NK and DOPA (0.05%), and incubation revealed that the melanin aggregation reaction was inhibited. NK was found to efficiently inhibit tyrosinase synthesis using L-tyrosine as a substrate (Ki = 1.17×10^{-6}M). It also acted on mouse melanoma B16 producing cells, which are melanin-synthesizing cells, and the inhibition was confirmed by Western blotting or tyrosinase mRNA expression.

Conclusions: NK is a fibrinolytic enzyme, which has been found to have a direct kinin-producing effect. Kallikrein was thought to work to improve circulation. In addition, we have confirmed for the first time that NK has an effect of suppressing melanin synthesis.

PB0740 | Transition Patterns in the Fibrinolytic Response are Superior to Admission ROTEM Values in Stratifying Risk for Late Clinical Outcomes after Major Trauma

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Background: ROTEM/TEG hypofibrinolysis after injury and empiric use of tranexamic acid (TXA) have both been implicated as potential causes of multi-organ dysfunction syndrome (MODS) in major trauma patients.

Aims: Examine the transition patterns in the ROTEM detectable fibrinolytic response that occurs after injury and the impact of TXA in determining late clinical outcomes.

Methods: Prospective cohort study of adult trauma patients at a single UK major trauma centre. Individuals were grouped according to ROTEM Maximum Lysis (ML) values (ML-Hypo, ML-Normal, ML-Hyper) at admission and Hr24, and transition between groups over time. Clinical outcomes (MODS and mortality) were analysed according to ML patterns and sub-divided by TXA use and requirement for a major haemorrhage protocol (MHP).

Results: 740 patients were included and in non-TXA patients one third transitioned between ML groups. Patients who reverted from ML-Normal to ML-Hypo compared to those who remained ML-Normal had no difference in injury severity, coagulopathy or degree of shock but had a more than two-fold increase in MODS (56% vs 22%, $p < 0.001$). Persistent ML-Low in contrast to ML-Low that normalised at Hr24 was associated with significantly different clinical outcomes: MODS (67% vs 32%) and late mortality (15% vs 4%), despite similar admission physiology and blood transfusions. In MHP patients, empiric TXA administration abolished ML-Hyper on admission (2% vs 25%, $p < 0.001$) and improved early mortality (15% vs 30%, $p = 0.008$). TXA use was associated with increased ML-Hypo at Hr24 (70% vs 40%, $p < 0.001$), but in patients without ML-Hyper we found no evidence of increased MODS or late deaths.

Conclusions: ML Hypo at Hr24 is associated with increased risk of MODS and these patients cannot be stratified on admission by either ROTEM or clinical characteristics. Empiric TXA is associated with reduced early mortality and alters the fibrinolysis transition patterns but does not worsen late outcomes regardless of ML values.

PB0741 | Modulation of Thrombin Generation or Thrombin Activity on Activated Platelets Surface Largely Affected TAFI-Dependent Regulation of Fibrinolysis

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Background: Activated platelets play crucial roles on hemostatic responses. They play diverse roles in the fibrinolysis and express of both pro- and anti-fibrinolytic activities. Employing real-time imaging analysis of the platelet-containing plasma clot formation and dissolution, we demonstrated that

1) activated platelets initiated not only coagulation but also fibrinolysis via triggering accumulation of plasminogen and tissue-type plasminogen activator (tPA) on their surface, and that

2) activated platelets attenuated fibrinolysis largely through the activation of thrombin-activatable fibrinolysis inhibitor (TAFI).

Aims: We evaluated how inhibition of coagulation cascade and/or thrombin activity modify TAFI-dependent inhibition of fibrinolysis, using anticoagulants including direct oral anticoagulants; DOACs.

Methods: Employing human platelet-containing plasma, tissue factor-triggering clotting time (CT) and tPA-initiated clot lysis time (CLT) were measured by turbidimetric assay, and these processes together with the accumulation of plasminogen were also visualized by confocal laser scanning microscopy. Thrombin generation during platelet-containing plasma clot formation was determined using fluorogenic substrate of thrombin.

Results: 1) In the presence of platelets, CLT was prolonged due to the activation of TAFI, which seemed dependent on the augmented thrombin generation.

2) Both rivaroxaban (activated factor X [FXa] inhibitor) and dabigatran (thrombin inhibitor) at the pharmacological concentration which effectively prolonged CT, similarly shortened CLT, and which were not shortened further by activated TAFI (TAFIa) inhibitor.

3) These two anticoagulants, however, differently modified platelet-associated fibrin network structure and localization of florescent-labeled plasminogen accumulation.

Conclusions: Modulation of the activated platelet-associated thrombin activity largely affected TAFI-dependent regulation of fibrinolysis.

PB0742 | HISTORY, an International Retrospective and Prospective Registry of Patients with Plasminogen Deficiency

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Background: Plasminogen deficiency (PLGD), an ultra-rare, life-long, multi-system disorder affecting ~1.6 per million population, is characterized by uncontrolled growth of fibrin-rich pseudomembranes on mucous membranes. The most common manifestation is ligneous conjunctivitis (81% of patients) which can result in vision impairment/loss; other affected systems may include the oral cavity, respiratory tract, ears, female genitourinary tract, kidneys and CNS. Persons with PLGD (PwPLGD) have delayed/misdiagnosis and can suffer significant morbidity, reduction in quality of life, and mortality. No specific commercial therapies are available, current non-specific treatments are inadequate, and surgery results in pseudomembrane regrowth.

Aims: HISTORY (*H*ypoplasminogenemia: an *I*nternational retro*S*pec*T*ive and pr*O*spective coho*R*t stud*Y*) collects data from an international group of PwPLGD and their first-degree family members; its goal is to comprehensively investigate PLGD natural history and ultimately develop severity categories and treatment guidelines.

Methods: Up to 100 probands and 500 total subjects will be enrolled in the 4-year observational study (Figure 1) with IRB approval and informed consent. Clinical, genetic, and laboratory data are collected at baseline with additional data obtained at scheduled/unscheduled visits; specimens are collected to support planned and future research. Markers that assist in disease course prediction, triggers of disease manifestations and utility of diagnostic assays will be evaluated. Therapeutic interventions will be recorded along with efficacy and safety observations. HISTORY (NCT03797495) expands upon the database framework of the Prospective Rare Bleeding Disorders Database (PRO-RBDD).

Results: To date, 26 centers in 12 countries have agreed to participate; 51 subjects are currently enrolled (Table 1). Initial results have identified asymptomatic siblings with PLGD; this affords a unique opportunity to investigate triggers of initial symptom development.

Conclusions: HISTORY is the first comprehensive retrospective/prospective international registry to investigate the current knowledge gaps in PLGD. Due to the rarity of PLGD, all interested parties are strongly encouraged to participate. Additional information is available at www.plgdeficiency.com.

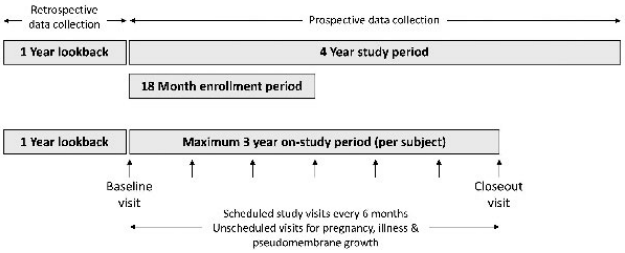

FIGURE 1 Outline of the 4-year clinical study. Subjects will spend a maximum of 3 years on-study

PB0743 | Polyphosphate Nanoparticles Are More Potent Stabilizers of Fibrin than Linear Polyphosphates and Histones Enhance their Effect

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Background: Activated platelets secrete polyphosphates in two forms: linear (PolyP-lin) and as aggregates, nanoparticles (PolyP-NP). In pathological thrombi the fibrin meshwork is tightly intertwined with a secondary matrix, neutrophil extracellular traps (NETs)

TABLE 1 Demographics of the initial 51 subjects; n/a, not applicable; *The subject with 42% PLG received antibiotics prior to diagnostic sample

Demographics		Subjects with PLGD	Unaffected family members
Gender	Male	9	11
	Female	15	16
Age	<18 years	14	8
	≥18 years	10	19
Endogenous PLG activity	Median (%)	18.5	n/a
	Range (%)	1-42*	n/a
Endogenous PLG antigen	Median (mg/dl)	3.4	n/a
	Range (mg/dl)	1-12	n/a

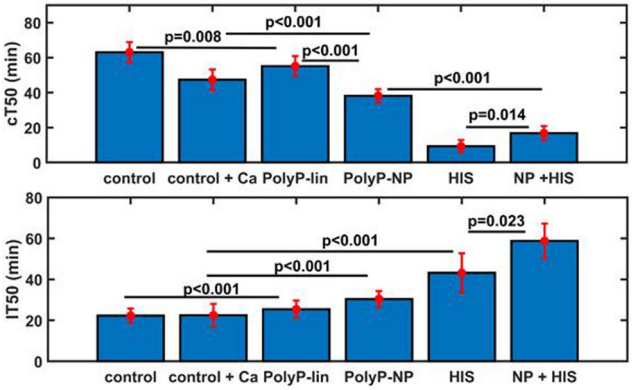

Fig1: Parameters of kinetics of fibrin formation and lysis in the presence of PolyP-lin, PolyP-NPs, histone and PolyP-NP + histone combination.

Fibrin clots were generated by adding thrombin to mixtures of fibrinogen and plasminogen in the presence of PolyP-lin, PolyP-NP, histone or PolyP-NP + histone combination. Turbidity of the forming fibrin clots was monitored by measuring absorbance at 340 nm and when absorbance plateaued, lysis was initiated by adding tissue plasminogen activator. The time to reach half maximal turbidity was used as a measure of reaction kinetics both by clotting and lysis. The maintenance of the linear form of PolyP required a Ca^{2+}-free medium with a separate control.

FIGURE 1

composed primarily of DNA and histones. The interplay of the two polyphosphate forms with the NET components in the formation and resolution of fibrin clots are largely unexplored.

Aims: Firstly, to investigate and compare the effects of PolyP-lin and PolyP-NPs on fibrin formation, structure and lysis. Secondly, to examine the possible interaction of these two types of polyphosphates with NET components (DNA, histones).

Methods: PolyP-lin was precipitated with $CaCl_2$ in aqueous milieu to generate PolyP-NPs followed by extrusion through a 220 nm filter. Fibrin formation and tissue plasminogen activator-induced lysis were monitored by turbidimetry and the time to reach half-maximal turbidity in the ascending and descending phase of the turbidity signal was used as a measure of clotting kinetics (cT50) and lysis (lT50). Fibrin structure was examined by scanning electron microscopy (SEM). Histone-fibrinogen aggregation was investigated by turbidimetry, digestion of fibrinogen by plasmin was monitored by SDS gel electrophoresis.

Results: Histones at 3-20 μM formed aggregates with fibrinogen and inhibited fibrinogen digestion by plasmin. PolyP-NP and PolyP-lin at 330 μM decreased cT50 by 20% and 13%, while histone (20 μM) and PolyP-NP + histone shortened cT50 by 80% and 61%. PolyP-NP and PolyP-lin raised lT50 by 36% and 13%, while lT50 was prolonged 1.7-fold and 2.5-fold by histone alone or in combination with PolyP-NP (Fig. 1). Similarly to plyphosphates, DNA accelerated fibrinogen clotting, but only in the absence of Ca^{2+}. The slower clotting kinetics resulted in thicker fibrin fibers in the presence of PolyP-NP, PolyP-lin and DNA according to the SEM measurements.

Conclusions: PolyP-NPs enhance fibrin formation and lytic resistance more effectively than PolyP-lin and PolyP-NPs act in synergy with histones in fibrin stabilization.

PB0744 | Hypercoagulability State in Iranian Patients with β-Thalassemia

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Background: Thalassemia is an inherited blood disorder that is requiring lifelong transfusion treatment and is associated with a high incidence of thrombosis.

Aims: To investigate changes in the activity of natural anticoagulants in β-thalassemia patients.

Methods: A total of 127 patients with β-thalassemia major (β-TM) and β-thalassemia intermedia (β-TI) were studied for clinical evidence of thrombosis and were compared with 110 age and sex-matched healthy control. Protein C, S and antithrombin III (AT III) along with routine laboratory tests, serum levels of D-dimer and ferritin were measured. Patients with liver disease especially chronic hepatitis were excluded. The statistical analysis was performed using SPSS Software Version 23.0, chi-square, test-t and Pearson tests with a 95% confidence interval.

Results: The patient and control participant characteristics are summarized in Table 1. Of the 127 patients, 114 patients (89.5%) were splenectomized. 7 patients (5.5%) had a history of thrombosis and all were splenectomized. This group in comparison with Patients without thrombosis had lower Pro C, Pro S ($p < 0.005$). Concomitant reductions in Pro C, Pro S and AT III were observed in β-TM and β-TI than in controls ($p < 0.005$). Protein C decreased in 61% and 62% and protein S in 63% and 71% of β-TM and β-TI respectively. However, AT III decreased 13.4% and 32.7% of β-TM and β-TI (Table 2). When we compared Protein C, S and AT III between β-TM and β-TI only AT III showed a significant difference ($P = 0.001$). PC and PS were reduced ($p = 0.001$ and $p = 0.006$) in nonsplenectomized patients compared with splenectomized patients.

Conclusions: Protein C, S and AT III significantly decreased in thalassemia patients compared to the control. These deficiencies of anticoagulant proteins might be contributing to chronic hypercoagulability in these patients. Thrombosis was seen in 7 (5.5%) patients and they showed lower protein C and S than patients without thrombosis.

TABLE 1 Baseline characteristics in patients and controls

Subjects	N (%)	Age Median (Range)	Sex M/F	Splenectomy	Thrombophilia n(%)	Median Serum ferritin (µg/L)
β-TM	64 (50.4%)	24 (13-42)	31/33	57	2 (3.1%)	1140 (96-9760)
β-TI	63 (49.6%)	34 (16-67)	30/33	57	5 (7.9%)	798 (28-5300)
Healthy control	110	28 (15-55)	61/65	-	-	NA

TABLE 2 Level of Coagulation Inhibitors in Patients and Controls, Data are shown as median (range)

Variable (%)	β-TM (N = 64)	β-TI (N = 63)	Healthy control (N = 110)	Patients With Thrombosis (N = 7)
Pro C	70 (17-149)	69 (31-133)	96 (71-147)	42 (33-79)
Pro S	54 (19-92)	54 (32-79)	86 (55-123)	38 (19-45)
AT III	93 (62-142)	80 (50-113)	104 (77-145)	83 (62-128)

PB0745 | Downregulation of the Procarboxypeptidase U (proCPU, TAFI, proCPB2) System in ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ Mice Is a Non-Lipid Related Pleiotropic Effect of Statin Therapy

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Background: In a proof-of-concept observational study it was demonstrated that statins (hydroxymethyl-glutaryl-CoA-reductase inhibitors) lower procarboxypeptidase U (proCPU, TAFI, proCPB2). However, it is challenging to prove whether this is a lipid- or non-lipid related pleiotropic effect, since statin treatment is lipid lowering in humans. Discriminating between cholesterol-dependent and -independent effects is thus difficult.

In apolipoprotein E-deficient mice with a heterozygous mutation in the fibrillin-1 gene (*ApoE$^{-/-}$Fbn1$^{C1039G+/-}$*), a model of advanced atherosclerosis, statins do not lower cholesterol. Consequently, studying cholesterol-independent effects of statins can be done more straightforwardly in these mice.

Aims: Study the influence of atorvastatin on proCPU-biology in *ApoE$^{-/-}$Fbn1$^{C1039G+/-}$*mice.

Methods: Female *ApoE$^{-/-}$Fbn1$^{C1039G+/-}$*mice were fed a Western diet (WD). At 10 weeks of WD, mice were divided in a control (WD) and atorvastatin (10 mg/kg/day+WD) group. After 15 weeks, blood was collected from the retro-orbital plexus and mice were sacrificed. Total plasma cholesterol and C-reactive protein (CRP) were measured with commercially available kits. Plasma proCPU-levels were determined with an in-house activity based assay. Atherosclerotic plaque size in the proximal ascending aorta (AoPA) was analyzed on haematoxyllin-eosin stained sections. All animal procedures were approved by the local ethics committee.

Results: Total plasma cholesterol levels were not significantly different between controls (504 ± 122 mg/dL; n = 12) and mice receiving atorvastatin (447 ± 103 mg/dL; n = 16). ProCPU-levels were 238 ± 101 U/L and 159 ± 63 U/L in control and atorvastatin-treated mice respectively, indicating a significant downregulation of the proCPU-system (33.2%;p = 0.017) under influence of atorvastatin. ProCPU-levels correlated with plasma CRP (Pearson r = 0.70;p = 0.001) and plaque size in the AoAP (Pearson r = 0.49;p = 0.016).

Conclusions: ProCPU-levels were significantly lower under influence of atorvastatin therapy even though no lipid reduction was observed in *ApoE$^{-/-}$Fbn1$^{C1039G+/-}$*mice. This suggests that the downregulation of the proCPU-system is - at least partly - a non-lipid related pleiotropic effect of statins. Furthermore, the association between high proCPU-levels and larger atherosclerotic plaques is an interesting finding and requires further research.

PB0746 | To Evaluate the Effect of Hydroxyurea on Blood Coagulation and Endothelial Activation in Sickle Cell Disease

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Background: Sickle cell disease (SCD) is frequently referred to as a hypercoagulable state. Hydroxyurea (HU) is known to decrease the frequency of vaso-occlusive complications and need for blood transfusions in severely affected individuals.

Aims: Our aim wasto assess the effect of HU on markers of fibrinolysis (D-dimer) and endothelial activation (soluble vascular cell adhesion molecule-1 [soluble VCAM-1]) in patients with SCD in their non-crisis, "steady state."

Methods: Patient's ≤15 years of age, with documented HbSS or HbSβ-thalassemia, eligible for treatment with HU were studied in this prospective, observational study.

Results: Seventy-five patients with SCD were enrolled (females: 45 [60%]), with a median age of 13 years (IQR: 3). Following 6 months of HU, median values for WBC count (9.02 x 109/L vs. 7.4 x 109/L,

p < 0.001), APTT (33 sec vs. 24 sec p < 0.000), D-dimer (1243.2 ng/mL vs. 802.9 ng/mL, p < 0.000), lactate dehydrogenase (744 U/L vs. 562 U/L, p < 0.002), soluble VCAM-1 (544.9 ng/mL vs. 492 ng/mL, p < 0.003), platelet count (250 109/L vs. 196 109/L, p < 0.002), and retic count (11.7% vs. 5.4%, p < 0.000) were significantly lower than baseline values, while the mean corpuscular volume (74 fL vs. 76 fL, p < 0.000) were higher than the baseline value. No significant differences from baseline was observed in the median values for hemoglobin (9.1 g/dl vs. 10.5 g/dl, p < 0.72) following 6 months of HU therapy.

Conclusions: In this study of children from Pakistan, we find that treatment with HU for up to 6 months resulted in decreased levels of D-dimer and VCAM-1. With the increasing evidence that coagulation activation contributes to SCD pathophysiology, the clinical benefit of HU may be due, in part, to decreased coagulation activation.

PB0747 | Online Apps Improve Haemostasis Assay Reproducibility and Facilitate Open Data and Code Sharing

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Background: Scientists are widely concerned about the 'crisis of reproducibility' and proposed solutions include standardised, open and freely shared analytical software. Many methods used in haemostasis involve specialist equipment and inaccessible software for data collection and analysis, while in-house methods may lack traceability or be difficult to transfer.

Aims: To develop open source software tools that are freely available to use online to enhance reproducibility of analysis within and between haemostasis laboratories.

Methods: Online apps were developed using the statistical programming language R, in combination with the 'Shiny' add-on package that can be run through a computer browser to help analysis of user data from haemostasis assays.

Results: Spectrophotometric time courses of clotting and lysis using clots made from purified components or plasma are common in haemostasis work but extracting parameters from these curves is not trivial. The 'Clotlysis' app streamlines curve fitting and calculation of times to % clotting or lysis, absorbance values and first derivatives (Fig. 1). Results can also be readily explored using heatmaps or scatter plots (e.g. Fig 2). All critical settings for baselines and interpolation are recorded to promote reproducible analysis. Similar apps are available to analyse chromogenic assays (e.g. zymogen activation with automated time squared calculations), fibrinolytic halo assays, clotting assays, D-dimer test positive and negative predictive values, and thrombin generation assays. More details and links are available at https://drclongstaff.github.io/shiny-clots/. Code can be downloaded from Github.

Conclusions: NIBSC organises global collaborative studies on behalf of WHO and the SSC/ISTH. We observe that analysis of the same data by participating labs or centrally by NIBSC can produce different results. Our recent experience making reference preparations for ancrod, batroxobin, streptokinase and thrombin shows that this source of variability can be reduced by using shared, standardised software. Data analysis is speeded up and simplified, while reproducibility is facilitated.

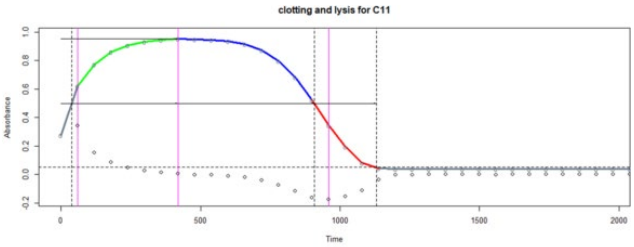

Fig 1. Representative analysis of a single 'Clotlysis' curve including first derivative plot (◯) with indication of calculated time and absorbance parameters that are stored in a table for further processing.

FIGURE 1

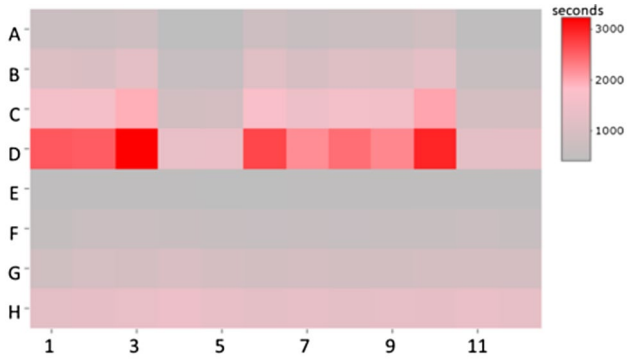

Fig 2. Rapid analysis using the 'Clotlysis' app showing a heatmap for time between clotting and lysis over a range of plasminogen activator concentrations in a microtitre plate format.

FIGURE 2

PB0748 | Deep Mutational Scanning and High-Throughput Kinetics to Quantitatively Assess Plasminogen Activator Inhibitor-1 Variants

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Background: The SERPIN plasminogen activator inhibitor-1 (PAI-1) regulates the fibrinolytic process as the primary inhibitor of tissue- and urokinase-type plasminogen activators (tPA and uPA). In contrast to other SERPINs, PAI-1 exhibits a relatively short functional half-life (~2 h) before it loses inhibitory activity.

Aims: The goal of this study is to apply deep mutational scanning by coupling phage display with high-throughput DNA sequencing (HTS) to quantitatively map the mutational landscape of PAI-1 with respect to both its functional half-life, as well as the rate of target protease inhibition.

Methods: Fully functional PAI-1 was expressed as a fusion protein on the surface of M13 bacteriophage and mutagenized using error prone PCR. Phage-displayed PAI-1 was reacted with uPA, the phage-displayed PAI-1:uPA complex isolated by immunoprecipitation, and the corresponding phage genomes subjected to high-throughput DNA sequencing.

Results: A mutational library of ~10^7 independent phage-displayed PAI-1 clones was generated, with Sanger sequence analysis identifying ~3-4 mutations per clone, and HTS demonstrating coverage of ~74% of all possible single amino acid substitutions. uPA reactive phage were collected at multiple time points and either the functional half-life or second order rate constants for uPA inhibition calculated simultaneously from the fractional representation of each variant as determined by HTS. Comparison of these massively parallel determined half-lives and rate constants to those measured for a set of recombinantly-expressed PAI-1 variants each carrying a single amino acid substitution, demonstrated a high degree of correlation confirming the validity of this approach.

Conclusions: The quantitative fitness landscapes developed through this analysis serve as a useful tool for understanding the evolution and diversity of the SERPIN superfamily, as well as, for cataloging the potential clinically significance for the large set of human mutations that will be identified by the application of whole genome sequencing to large and rapidly expanding human cohorts.

PB0749 | Changes in Fibrinolytic Factors in a Simulated Extracorporeal Membrane Oxygenator Circuit

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Background: Thrombosis and haemorrhage are common complications of extracorporeal membrane oxygenation (ECMO). Haemostatic changes that occur during ECMO and their contribution to these complications have been poorly studied, especially fibrinolysis.

Aims: To assess the changes in fibrinolytic factors over a 24-hour period in an *ex vivo* model of ECMO.

Methods: A simulated adult *ex vivo* circuit with a centrifugal pump was designed for 24-hours using whole blood (donors had hereditary haemochromatosis) diluted with 3.2% sodium citrate and PlasmaLyte 148 solution. Six circuits were analysed. Samples were taken with time-matched controls at 6 time points (0, 1, 4, 8, 12 and 24 hours). Clauss fibrinogen levels and ELISA were performed for D-dimer, plasminogen activator inhibitor (PAI)-1, tissue plasminogen activator (t-PA) antigen, prothrombin fragments (PF) 1 + 2, thrombin-activated fibrinolysis inhibitor (TAFI) antigen and plasmin-antiplasmin (PAP) complexes. Statistical analysis was measured by Mann-Whitney U test. Haemostatic results were expressed as % of the value at initiation of the experiment.

Results: Haemodilution occurred with a haematocrit fall from a median 0.40 (range 0.37 - 0.54) to 0.19 (0.18-0.22) after addition of PlasmaLyte, but there were no further significant changes. There were no changes over time in D-dimers, PF1 + 2 and fibrinogen. tPA levels decreased over 24-hours with a larger decrease in samples from the circuit than control (56% vs 12%, p = 0.025). PAP complexes, TAFI antigen and PAI-1 levels increased over time (91%, 20% and 48% increases from baseline respectively, non-significant). There was an increase in PAP complexes and PAI-1 levels (91% vs 32% and 48% vs 27% at 24-hours) from the circuit compared to controls although similar TAFI levels (20% vs 18% at 24-hours).

Conclusions: This *ex vivo* simulated circuit showed an elevation in PAP complexes during ECMO. PAI-1 increased and tPA decreased over 24-hours. Further analysis is required in patients during ECMO and correlated with haemostatic phenotype.

TABLE 1 Mean % changes in fibrinolytic factors at 24 hours compared to initiation of ex vivo circuit

Fibrinolytic Factor	Fibrinogen	D-dimer	PF1 + 2	PAP Complex	tPA Antigen	PAI-1	TAFI
Circuit after 24 hours	−5.5%	+4.1%	+9.8%	+90.7%	−56.2%	+48.2%	+19.7
Control after 24 hours	+2.6%	+0.5%	−5.7%	+31.6%	−12.4%	+26.6%	+17.6%

PB0751 | Down-regulation of Plasminogen Activator Inhibitor-1 (PAI-1) by *in vivo* Thrombin Generation and Subsequent Formation of Activated Protein C (APC) in Carriers of the Factor V Leiden (FVL) and Prothrombin 20210G>A Mutation (PTM)

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Background: Proteolytic degradation of PAI-1 by APC has been suggested to contribute to a hyperfibrinolytic state in acquired coagulopathies in sepsis or trauma. However, *in vivo* evidence remains inconclusive. Recently, we have shown increased APC generation in FVL and PTM carriers in response to *in vivo* thrombin formation

triggered by low-dose administration of recombinant activated factor VIIa (rFVIIa).

Aims: To investigate the effects of this approach of stimulated hemostasis activity pattern evaluation (SHAPE) on fibrinolysis.

Methods: Blood samples were collected from FVL/PTM carriers (30 each) and 29 healthy controls immediately before and over 8 hours following injection of 15 μg/kg rFVIIa. APC plasma levels were measured using an oligonucleotide-based enzyme capture assay (OECA). Other monitored parameters included prothrombin activation fragment 1 + 2 (F1 + 2), thrombin-antithrombin complexes (TAT), tissue-type plasminogen (t-PA), PAI-1, α2-antiplasmin, plasmin-α2-antiplasmin complexes (PAP), soluble fibrin monomers, d-dimer, and thrombin-activatable fibrinolysis inhibitor (TAFI) antigen and activity levels.

Results: PAI-1 levels were higher in FVL (29.8 ng/mL, $P < 10^{-4}$) and PTM carriers (27.7 ng/ml, $P = 0.002$) than in controls (9.1 ng/mL). Other baseline parameters did not differ significantly. In all cohorts a comparable increase of F1 + 2 and TAT was observed after administration of rFVIIa, whereas the APC increase was greater in FVL (by 7.79 pmol/L, $P < 10^{-4}$) and PTM carriers (by 4.71 pmol/L, $P < 10^{-4}$) than in controls (by 2.38 pmol/L) (Fig. 1). Concurrently, PAI-1 levels decreased more in FVL (by 19.9 ng/mL, $P = 2 \cdot 10^{-4}$) and PTM carriers (by 21.0 ng/mL, $P = 0.009$) than in controls (by 5.5 pmol/L) (Fig. 2). TAFI antigen, PAP, and d-dimer increased at comparable extents within reference ranges in all cohorts. Other parameters remained unchanged.

Conclusions: Increased APC formation rates in thrombophilic mutation carriers were associated with a greater decline of PAI-1 levels in the absence of interfering changes in t-PA levels. These data provide further *in vivo* evidence that APC down-regulates PAI-1.

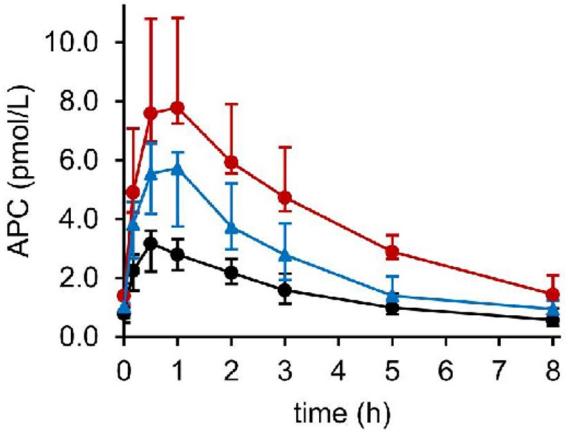

Fig. 1. In-vivo plasma levels of activated protein C (APC). After t=0 recombinant activated factor VII (15 μg/kg) was administered to carriers of the factor V Leiden mutation (red symbols, n=30), prothrombin 20210G>A mutation (blue symbols, n=30) and to healthy controls (black symbols, n=29). Data are shown as median and interquartile range.

FIGURE 1

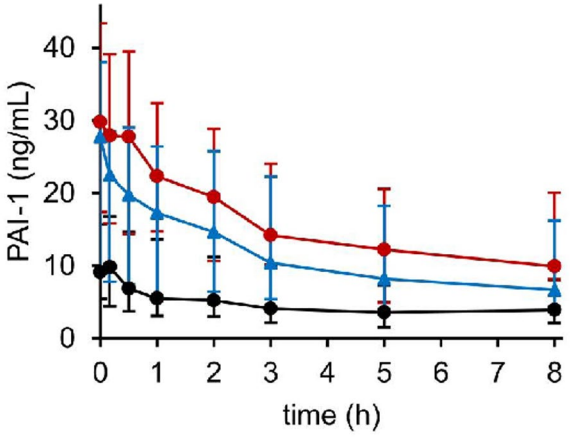

Fig. 2. In-vivo plasma levels of plasminogen activator inhibitor-1 (PAI-1). After t=0 recombinant activated factor VII (15 μg/kg) was administered to carriers of the factor V Leiden mutation (red symbols, n=30), prothrombin 20210G>A mutation (blue symbols, n=30) and to healthy controls (black symbols, n=29). Data are shown as median and interquartile range.

FIGURE 2

PB0752 | Total and C-terminally Truncated Alpha2-plasmin Inhibitor Levels in Venous Thrombosis

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Background: Alpha2-plasmin inhibitor (A2-PI) in the plasma undergoes N- and C-terminal cleavages. The N-terminally cleaved form is cross-linked to fibrin more efficiently by activated factor XIII. The C-terminally truncated form of A2-PI lacks the plasminogen-binding site, but remains an active plasmin inhibitor, although reacts more slowly with plasmin.

Aims: In this case-control study we investigated whether total A2-PI antigen level and A2-PI C-terminal cleavage are associated with venous thrombosis.

Methods: Citrated plasma samples were obtained from 300 healthy controls and 300 patients with venous thrombosis at least three month after the acute event. Total and C-terminally intact A2-PI antigen levels were measured by in house sandwich ELISA methods. Other laboratory parameters were measured by routine methods.

Results: BMI, fibrinogen and cholesterol levels showed independent positive, while age showed negative association with total A2-PI antigen levels in the control group. Total A2-PI antigen levels adjusted for the above parameters were significantly elevated in the VTE group comparing to controls (adjusted mean (95% CI) 71.4

(70.4-72.4) mg/L vs. 65.3 (64.1-66.5) mg/L; p < 0.001). C-terminally truncated A2-PI was associated with gender and fibrinogen level and was significantly elevated in the VTE group (28.8 (27.9-29.8) mg/L vs. controls (21.6 (20.9-22.4) mg/L; p < 0.001). The ratio of C-terminally truncated A2-PI in patients was higher than in controls (41.6 ± 6.3% vs. 33.6 ± 9.1%). Total A2-PI in the upper quartile (>70 mg/L) significantly increased the risk of VTE (OR (95% CI): 4.554 (3.12-6.65); p < 0.001). C-terminally truncated A2-PI in the upper quartile (>26.5 mg/L) also significantly increased the risk of VTE (OR (95% CI): 5.58 (3.86-8.05); p < 0.001) and the association remained significant after adjustment for BMI, age, gender, fibrinogen and high total A2-PI level (OR (95% PI): 5.03 (2.75-9.22; p < 0.001).

Conclusions: Elevation of total and C-terminally truncated A2-PI concentration represents a significant risk of VTE.

Funding: GINOP-2.3.2-15-2016-00043, NKFI-K120633:

PB0753 | Different Incorporation of Apha2-plasmin Inhibitor C-terminal Forms into the Fibrin Clot

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Background: Apha2-plasmin inhibitor (A2-PI) is the main inhibitor of plasmin. In the circulation A2-PI undergoes proteolytic cleavages. The C-terminally cleaved form of A2-PI lacks the plasminogen-binding site, which is located in the C-terminal 26 amino acids of the molecule. It remains an active plasmin inhibitor, but reacts more slowly with plasmin.

Aims: To investigate the incorporation of C-terminally intact and truncated A2-PI into the fibrin clot and the effect of FXIII on this process.

Methods: Citrated plasma samples from 42 healthy individuals were clotted by thrombin and calcium. Total A2-PI and C-terminally intact A2-PI and FXIII antigen levels were measured by sandwich ELISAs from the plasma and serum samples, and the concentration of the truncated form was calculated from these values. Washed and dissolved plasma clots were analyzed by western blotting using antibodies to total and C-terminally intact A2-PI.

Results: The ratio of intact:truncated A2-PI in the plasma samples was 66.3%:33.7%. 49.2 ± 4.8% of the plasma total A2-PI incorporated into fibrin clot based on ELISA measurements. The amount of incorporated C-terminally intact A2-PI was significantly higher than the amount of the truncated form (32.2 ± 5.1 mg/L vs. 18.7 ± 3.4 mg/L, p < 0.0001) that was 43.3 ± 5.7% and 60.9 ± 8.5% of the original plasma values, respectively. Plasma FXIII significantly correlated with incorporation of the intact form (r = 0.819, p < 0.001), however did not show correlation with incorporation of the truncated form (r = 0.079, p = 0.618). Western blotting results showed complete covalent cross-linking of the C-terminally intact form to α-chain monomer and α-chain polymers of fibrin, while the truncated form presented both in non-crosslinked and crosslinked bands.

Conclusions: Strong correlation of FXIII concentration with the incorporation of C-terminally intact A2-PI into fibrin clot suggests that C-terminally intact A2-PI is the primary substrate of FXIIIa for the cross-linking to fibrin.

Funding: GINOP-2.3.2-15-2016-00043, NKFI-K120633

PB0754 | Production of Nanobodies Targeting Plasminogen and Plasmin

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Background: Plasmin (Plm) which is generated from its zymogen plasminogen (Plg) is a serine protease which plays an important role in fibrin degradation and is a potent modulator of immunological processes and cancer progression. A heavy chain only antibody (VHH), nanobody, which specifically binds to Plg or Plm might render diagnostic and clinical applications.

Aims: To generate VHH(s) against Plg/Plm with high affinity and specificity.

Methods: Plg was isolated and purified from citrated pool plasma using lysine sepharose affinity chromatography (AC) followed by size-exclusion chromatography (SEC). The Plg activation process was optimized in terms of incubation time and temperature, and the activator type. The purified active Plm (200 μg) was injected 6 times subcutaneously to two llamas and peripheral blood lymphocytes were collected and subjected to mRNA extraction and cDNA reverse transcription. The cDNA was used for phage display, panning and ELISA-affinity assays. Selected VHHs were characterized in terms of binding affinity and specificity to both Plg and Plm using bio-layer interferometry (BLI).

Results: Using AC and SEC, 2 mg Plg was isolated from 250 ml citrated pool plasma having 90% purity determined by activity testing and Bradford protein determination. The optimum activation of Plg to Plm was achieved by incubation of Plg with urokinase-type plasminogen activator for 72 h at RT. Based on ELISA-affinity results and BLI, 46 hit compounds were found. Among them, seven VHHs bind Plg and Plm with K_Ds ranging from 1 to 20 nM. One VHH showed selective binding to Plm.

Conclusions: Using purified Plm to immunize llamas, a group of affine nanobodies were isolated against Plg and/or Plm. Further comprehensive functional characterization will reveal the potential of VHHs for the future development of possible drug candidates.

PB0756 | The Influence of Prothrombin Complex Concentrates on Clot Stability

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Background: Prothrombin complex concentrates (PCCs) are used in clinical practice to reverse major bleeding while on anticoagulant therapy and for the treatment and prophylaxis of inherited and acquired bleeding disorders. Thrombin generation essential for clot strength is enhanced when PCC is administered.

Aims: To assess clot stability in plasma spiked with PCC using clot lysis time assay.

Methods: The clot lysis time assay incorporating phospholipid 10 μM, tissue factor 5pM, and calcium 17 mM (final concentrations) was performed in a 96 well plate. Clot lysis was achieved with the addition of tissue plasminogen activator 50 ng/mL (final). Normal pooled plasma (NPP) obtained from ten healthy volunteers was tested at baseline and after addition of three concentrations of PCC: 0.15 IU/mL, 0.3 IU/mL and 0.6 IU/mL. Clotting factors (Factor II, VII, IX, X and Fibrinogen) and anticoagulants (Protein S and Protein C) were measured in NPP pre and post PCC addition.

Changes in optical density (OD) as a result of clot formation and lysis were measured kinetically using a spectrophotometer (37°C, 405 nm). Clot lysis times (CLTs) were calculated using *Excel*.

Results: Addition of PCC to NPP resulted in a dose dependent increase in maximum OD when compared to NPP alone. Similarly, we observed a progressive prolongation of CLTs with corresponding increments in PCC concentration. PCC 0.15 IU/ml, 0.30 IU/ml and 0.60 IU/ml resulted in CLTs of 68.5 min, 69.5 min and 73.5 min respectively. From the NPP CLT of 64.5 min to the highest PCC concentration there was an increase of 12.2%.

Conclusions: Increasing the concentration of pro-coagulant factors in plasma by adding PCC results in clots that are more resistant to fibrinolysis. Further research is needed to investigate CLT in parallel to thrombin generation to better understand the impact of PCC in correcting coagulopathies.

PB0757 | Low Fibrinolytic Activity Following Ischemia Reperfusion Is Not a Single Etiology: Fibrinolysis Shutdown versus Hypofibrinolysis

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Background: Fibrinolytic activation with subsequent inhibition is termed fibrinolysis shutdown (SD). SD has recently been interchanged with hypofibrinolysis, which is inaccurate. Hypofibrinolysis represents a lack of fibrinolytic activation. When the native liver is removed from the patient(anhepatic) during liver transplantation tissue plasminogen activator cannot be systemically cleared and provides the ideal human model to differentiate these phenotypes. We hypothesize that a population of liver transplant patients fail to generate a fibrinolytic response during the anheptic phase of surgery and represent a hypofibrinolytic phenotype, which is distinct from patients that generate a fibrinolytic response with subsequent inhibition following graft reperfusion representing SD.

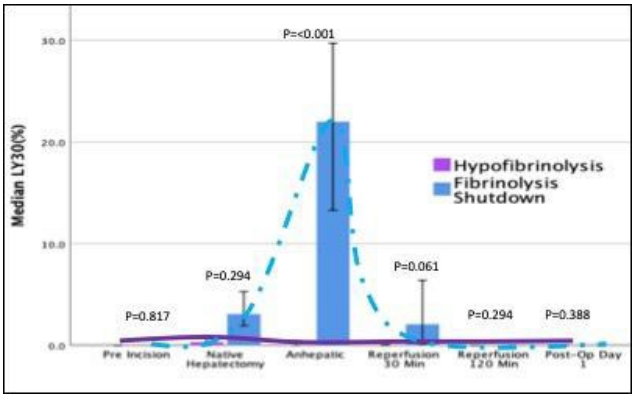

FIGURE 1 Fibrinolytic Changes in Liver Transplant Recipients

Aims: Define the fibrinolytic trends in liver transplant patients that develop fibrinolytic shutdown versus hypofibrinolysis and contrast blood product utilization.

Methods: Liver transplant patients had serial blood draws before surgical incision through post-operative day-1. Samples were analyzed with thrombelastography. Patients were dichotomized based on the lysis at 30 minutes (LY30). LY30 > 3% defined fibrinolytic activation (based on the existing literature) vs hypofibrinolysis (LY30 < 3%). Groups were contrasted on temporal changes in LY30 and transfusion requirements.

Results: Hypofibrinolysis was identified in 43% of 98 transplant patients analyzed. Hypofibrinolytic patients had a stable LY30 for the duration of surgery, whereas all of the patients with fibrinolytic activation (figure) had subsequent shutdown of fibrinolytic activity following graft reperfusion, which persisting through post-operative day-1. Blood product utilization was similar with the exception of the anhepatic phase, in which the SD group were transfused more red blood cell units(3 vs 2 units P = 0.030).

Conclusions: Despite liver transplant patients having low fibrinolytic activity at the start of surgery and the following day, two distinct fibrinolytic changes occur during surgery. These data demonstrate that fibrinolysis shutdown and hypofibrinolysis are not interchangeable terms and their impact on patient outcomes warrants ongoing investigation.

THROMBOLYTIC THERAPY

PB0761 | B- Mode Ultrasound-Guided Photomechanic-Shock Wave Combination Thrombolytic Therapy of Embolic Artery Accompanied by Simultaneously Streptokinase - Loaded Microbubbles Administration

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Background: A plaque may rupture with high risk of subsequent thrombus mediated acute clinical events such as myocardial infarction and stroke. Catheter-based lasers and shock wave generator systems have been extensively studied as effective methods to treat thrombosis.

Aims: The aim of this study was to generate a rabbit model of femoral artery thromboembolism occlusion and the subsequent investigating the feasibility of B- mode ultrasound- guided photomechanic- shock wave combination thrombolytic therapy accompanied by simultaneously intravenous streptokinase- loaded microbubbles administration in this model

Methods: Briefly, New Zealand White rabbits were submitted to thromboembolism occlusion by injecting autologous blood clots through femoral artery. Then treatment group underwent catheter-based Q-switched Nd: YAG laser (F = 532 nm, P = 30 W, PD = 5 ns) and low- level electrohydraulic pulsed- focused shock wave (V = 10 Kv, F = 0.5 Hz, Impulses = 200) thrombolytic therapy accompanied by simultaneously intravenous streptokinase (25000 Units/kg)- loaded PESDA (Perfluorocarbon- Exposed Sonicated Dextrose Albumin) microbubbles (100 ml/kg, 2-5 × 105 bubbles/ml) administration, wherein diagnostic B- mode ultrasound is combined with therapy system, with a goal of increased safety.

Results: Results from B-mode ultrasonography and histopathology, showed a significant reduction in the mean value for thrombus content at the embolic region in the treatment group compared with the other groups ($P < 0.05$).

Conclusions: Enhanced thrombolytic effect of photomechanic-shock wave combination therapy, induced by inertial cavitation effect of microbubbles accompanied by enhanced thrombolytic effect of streptokinase, induced by sonoporation therapy effect of shock waves can cause to reduce the thrombus content and significantly dilate the luminal cross-sectional area at the embolic region and lower treatment time.

PB0762 | Effect of B-Mode Ultrasound-Guided Pulsed - High Intensity Focused Ultrasound-Mediated Inertial Cavitation Thrombolytic Therapy Accompanied by Air-Contained Sonicated Dextrose Albumin Microbubbles Administration on Embolic Artery Occlusion

H. Mehrad[1]; M. Farhoudi[2]
[1]*Mehrad Research Lab, Division of Noninvasive Therapy, Tabriz, Iran, Republic of Islamic;* [2]*Tabriz University of Medical Sciences, Neurosciences Research Center, Tabriz, Iran, Republic of Islamic*

Background: A plaque may rupture with high risk of subsequent thrombus mediated acute clinical events such as myocardial infarction and stroke. Pulsed- high intensity focused ultrasound have been extensively studied as an effective method to treat thrombosis.

Aims: The aim of this study was to generate a rabbit model of carotid artery thromboembolic occlusion and the subsequent investigating the feasibility of extracorporeal B- mode ultrasound- guided pulsed- high intensity focused ultrasound- mediated inertial cavitation thrombolytic therapy accompanied by simultaneously intravenous air-contained sonicated dextrose albumin administration in this model.

Methods: Briefly, New Zealand White rabbits were submitted to thromboembolic occlusion by injecting autologous blood clots through carotid artery. Then treatment group underwent extracorporeal pulsed- high intensity focused ultrasound (F = 150 KHz, P = 25 W, PD = 250 ms) inertial cavitation therapy accompanied by simultaneously intravenous air-contained sonicated dextrose albumin (ASDA) (200 ml/kg, 2-5 × 105 bubbles/ml) administration, wherein diagnostic B- mode ultrasound is combined with therapy system, with a goal of increased safety.

Results: Results from B-mode ultrasound imaging concurrent with extracorporeal pulsed- high intensity focused ultrasound therombolytic therapy, showed the generation of inertial cavitation in the carotid artey. Also, histopathology results, showed a significant reduction in the mean value for thrombus content at the embolic region in the treatment group compared with the other groups ($P < 0.05$).

Conclusions: Enhanced thrombolytic effect of extracorporeal pulsed- high intensity focused ultrasound, induced by inertial cavitation effect of ASDA microbubbles can cause to significantly reduce the thrombus content and dilate the luminal cross-sectional area at the embolic region and lower treatment time and reduce total costs of treatment.

PB0763 | Effect of Streptokinase-Loaded Microbubbles-Mediated B-Mode Ultrasound-Guided Sono-Photomechanic Combination Thrombolytic Therapy on Atherothrombotic Stenosis

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[1]*Medical Biology Research Center, Kermanshah University of Medical Sciences, Division of Noninvasive Therapy, Tabriz, Iran, Republic of Islamic;* [2]*Tabriz University of Medical Sciences, Neurosciences Research Center, Tabriz, Iran, Republic of Islamic*

Background: Thrombus formation on a disrupted atherosclerotic plaque is a key event that leads to atherothrombosis.
Aims: The aim of this study was to generate a hamster model of abdominal aorta atherothrombotic stenosis with morphological similarities to the human disease and the subsequent assessment of the reliability of B- mode ultrasound- guided sono- photomechanic combination thrombolytic therapy accompanied by streptokinase-loaded microbubbles administration in this model.
Methods: Briefly, Golden Syrian hamsters were submitted to abdominal aorta atherothrombotic stenosis by primary balloon injury followed by a 1.5% cholesterol- rich diet injury for 12 weeks and finally perivascularly severe cold injury. Then treatment group underwent extracorporeal pulsed- high intensity focused ultrasound (F = 150 KHz, P = 25 W, PD = 250 ms)- mediated inertial cavitation therapy and catheter- based Q-switched Nd: YAG laser (F = 532 nm, P = 25 W, PD = 15 ns)- mediated inertial cavitation therapy accompanied by simultaneously intravenous streptokinase (25000 Units/kg)- loaded PESDA (Perfluorocarbon- Exposed Sonicated Dextrose Albumin) microbubbles (100 ml/kg, 2-5 × 105 bubbles/ml) administration, wherein diagnostic B- mode ultrasound is combined with therapy system, with a goal of increased safety.
Results: Results from color Doppler and B-mode ultrasonography and histopathology, showed a significant reduction in the mean value for blood mean velocity and the percentage of luminal cross-sectional area of stenosis and a significant increase in the mean value for blood volume flow at the stenotic region in the treatment group compared with the other groups ($p < 0.05$).
Conclusions: Enhanced thrombolytic effect of sono- photomechanic combination therapy, induced by inertial cavitation effect of microbubbles can cause to enhance the anti-thrombotic effect of streptokinase and significantly dilate the luminal cross- sectional area of stenosis.

PB0765 | The Effect of the Fibrino(geno)lytic Enzymes from Tissues of Antarctic Hydrobionts on Hemostasis

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Background: Considering the continuing increase of morbidity and mortality rates associated with cardiovascular diseases, the search for novel compounds, which are able to affect the hemostasis system, are among the current trends of modern science and pharmacology.
Aims: To study the effects of fibrino(geno)lytic enzymes from the Antarctic hydrobionts (*Odontaster validus*, *Sterechinus neumayeri*) on hemostasis system.
Methods: The fibrino(geno)lytic enzymes were isolated by a complex approach which included affinity chromatography and size-exclusion chromatography. The influence of fibrino(geno)lytic enzymes on platelets aggregation was assessed using a aggregometer AT-02 (Medtech, RF). Fbrin(ogen)olytic activity was assessed by examination a cleavage pattern of the fibrinogen in SDS-PAGE after incubation with the enzymes. Activated partial thromboplastin time (aPTT), prothrombin time (PT), and thrombin time (TT) were measured using coagulation analyzer (Rayto, Shenzhen, China). The substrate specificity of enzymes was measured using chromogenic substrates H-D-Phe-Pip-Arg-*p*NA, pyroGlu-Pro-Arg-*p*NA, H-D-Val-Leu-Lys-*p*NA, and Bz-Ile-Glu(γ-OR)-Gly-Arg-*p*NA (Sigma, USA).
Results: The fibrino(geno)lytic enzyme from *S. neumayeri* cleaved preferentially the Aα-chain and more slowly the Bβ-chain of fibrinogen. The action of the enzyme from *O. validus* was similar, but lower. None of the proteases degraded the γ-chain of fibrinogen. Both fibrino(geno)lytic enzymes mediated the prolongation of clotting time in all chronometric tests. The degree of platelet aggregation was 20% and 31%, respectively after incubation with the fibrino(geno)lytic enzyme from *S. neumayeri* and *O. validus*. Enzymes also exhibit activity against chromogenic substrates more expressed in case of pyroGlu-Pro-Arg-pNA, which is a specific synthetic substrate for activated protein C and factor XIa.
Conclusions: In this study, we provide evidence that enzymes isolated from the tissues of Antarctic marine hydrobionts act as a fibrin(ogen)olytic enzymes. Further investigations of these enzymes are interesting as they can be of medical interest as therapeutic agents in the treatment and prevention of thrombotic disorders.

PB0766 | Microlyse: A VWF-Targeting Thrombolytic Agent for Thrombotic Thrombocytopenic Purpura

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Background: Patients with thrombotic thrombocytopenic purpura (TTP) experience attacks of microvascular thrombosis, when platelets complex to von Willebrand Factor (VWF). This is caused by ADAMTS13 deficiency, which normally regulates VWF thrombogenicity. Unfortunately, ADAMTS13 re-supplemental therapy can be hampered by persistent autoantibodies. We previously reported that systemic plasminogen activation (with streptokinase) was therapeutic in a TTP mouse model, suggesting that plasmin can act as

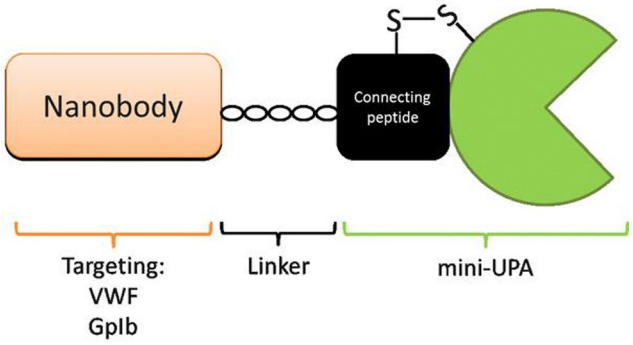

FIGURE 1 Microlyse: a nanobody-UPA fusion protein

an alternative to ADAMTS13. For optimal therapeutic efficacy and safety, it is wishful to localize plasminogen activation to sites of microvascular occlusion. While plasmin(ogen) can directly bind to VWF in microthrombi, its natural activators (tissue- and urokinase type plasminogen activator; tPA, uPA) cannot.

Aims: Development of fusion proteins (microlyse; fig. 1) for targeted delivery of plasminogen activators to platelet-VWF complexes.

Methods: We fused the catalytic domain of uPA to nanobodies against VWF or a non-targeting control nanobody.

Results: Microlyse variants are well expressed in HEK293 cells, show no spontaneous activity, but develop enzymatic activity upon incubation with plasmin(ogen). In the presence of open VWF, plasminogen activation is accelerated 6-fold for the anti-VWF microlyse variants. In VWF-dependent platelet agglutination experiments, both anti-VWF and -GP1b microlyse variants accelerate destruction of microthrombi, as compared its non-targeting control. At equimolar concentrations, targeting microlyse variants degrade microthrombi 7-fold more rapidly than the bivalent humanized nanobody Cablivi. In a flow model for TTP, targeting fusion proteins accelerate the removal of VWF-platelet strings from the endothelial surface in ADAMTS13 deficient plasma. Finally, in a ADAMTS13(-/-) mice in a model of thrombotic thrombocytopenic purpura, anti-VWF but not the non-targeted microlyse variant, attenuate thrombocytopenia within 24 hours with an efficacy that is comparable to that of cablivi. However, where we find evidence for circulating microthrombi after cablivi treatment, these are not present after microlyse treatment.

Conclusions: Targeted delivery of plasminogen activators to microthrombi is a promising approach for the treatment of TTP.

PB0767 | The Mechanism of Antifibrinolytic Action of Anionic Polyamidoamine Dendrimers

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Background: Polyamidoamine (PAMAM) dendrimers are a new class of monodisperse polymers used for systemic drug delivery. A key enzyme in the fibrinolysis system, plasmin, dissolving clot fibrin, is formed upon plasminogen activation by tissue plasminogen activator (tPA) and urokinase (uPA). Native Glu-plasminogen (Glu-Pg) adopts closed conformation in the presence of Cl^-. Glu-Pg without Cl^- and Lys-Pg adopt relaxed conformation. We recently discovered that anionic PAMAM dendrimers inhibit Glu-Pg activation by tPA.

Aims: Clarify the mechanism of the inhibitory effect of PAMAM-COOH dendrimers on the fibrinolysis system activity.

Methods: The concentration effects of dendrimers on intrinsic activities of plasmin, tPA and uPA, on fluorescent spectra of Glu-Pg and Lys-Pg, on the plasminogen activation rate with tPA and uPA, and on plasma clots lysis induced by two activators were studied.

Results: With increasing generation (G1.5-G3.5) and concentration of dendrimers, amidolytic activity of plasmin practically did not change, while tPA and uPA lost intrinsic activity by a maximum of 13 and 30%, respectively, at 300 μM dendrimer G3.5. The fluorescence spectrum peak (340 nm) of Glu-Pg and Lys-Pg (λ_{exc} 280 nm) decreased with increasing dendrimers concentration of both in the absence and in the presence of Cl^-. Consequently, dendrimers and Cl^-, binding to different centers of Glu-Pg, independently change the conformation of open and relaxed forms of zymogen. Sharp decrease in the Glu-Pg activation rate with tPA and uPA was caused by G2.5 and G3.5 dendrimers (by 95-99% at 300 μM derdrimers), and the additing 60 μM soluble fibrin weakened the inhibitory effect of dendrimers. Plasma clot lysis induced by tPA and uPA was inhibited by dendrimers to a lesser extent than Glu-Pg activation.

Conclusions: Mechanism of antifibrinolytic action of PAMAM-COOH dendrimers consists mainly in changing the Glu-Pg conformation and, partially, in reducing the tPA and uPA activity after their binding to dendrimers.

PB0768 | Covalent Prourokinase-dendrimer Complexes with Increased Plasma Stability and High Thrombolytic Activity *in vitro*

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Background: Single-chain prourokinase (scuPA) is an effective plasminogen activator used as thrombolytic agent. However, it is quickly

cleared from the bloodstream. In addition, with the formation of plasmin (Pm), scuPA loses its fibrin specificity owing to rapid conversion to two-chain urokinase (tcuPA). Nanoscale polyamidoamine (PAMAM) dendrimers are widely used as drug carriers due to their unique structure and polyvalent surface.

Aims: Increasing the scuPA stability in human plasma by its modification with anionic PAMAM dendrimer G2.5.

Methods: COOH-groups of PAMAM dendrimer G2.5 was activated the mixture of EDC and NHS (pH 2.0) and purified. ScuPA was conjugated with activated dendrimer in ratios of protein:polymer 1:1, 1:5, 1:10 and 1:20 (M:M) during 2 h at pH 8.5. Methods for studying the plasma stability of fibrinolytic activity, plasminogen-activating, and thrombolytic activities of scuPA and conjugates of scuPA-dendrimer were based on measuring the fibrin plate lysis area, Glu-plasminogen activation rate and plasma clot lysis rate *in vitro*, respectively.

Results: The conversion rate of scuPA-dendrimer to tcuPA-dendrimer by plasmin slowed somewhat with increasing the modification degree of the scuPA positively charged groups by PAMAM-COOH dendrimer (from 1:1 to 1:20), and the resulting tcuPA-dendrimer conjugates retained 92-85% of amidase activity. Plasminogen-activating activity of scuPA-dendrimer conjugates was comparable or slightly higher than free scuPA. Thrombolytic activity of scuPA-dendrimer conjugates was 90-95% of the scuPA activity measured by clot lysis in 4 h. Conjugation with dendrimer increased the plasma stability of scuPA fibrinolytic activity ($\tau_{1/2}$ 48 min): the $\tau_{1/2}$ of conjugates increased from 60 to 130 min with at increasing the ratio of scuPA: dendrimer from 1:1 to 1:20.

Conclusions: Supposedly, due to the modification of the PAI-1-binding Arg-Arg-Arg-Arg-cluster in scuPA by anionic dendrimer, the scuPA-dendrimer conjugates are significantly more stable in plasma than scuPA, but retain high plasminogen-activating and thrombolytic activities.

PB0771 | Elevated Levels of Circulating Markers of Neutrophil Extracellular Traps (NETs) Are Associated with Unfavorable Outcomes in Acute Ischemic Stroke Patients Undergoing Intravenous Thrombolysis

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Background: The outcome of intravenous thrombolysis using recombinant tissue plasminogen activator (rtPA) is favorable in only approximately 35% of acute ischemic stroke (AIS) patients. Neutrophil extracellular traps (NETs), composed of extracellular networks of DNA, histones, and neutrophil granular proteins are important constituents of cerebral thrombi. NETs intercalate to fibrin and create a dense network that impairs fibrinolysis.

Aims: To investigate whether levels of circulating markers of NETs, as assessed in AIS patients before thrombolysis, are associated with therapy failure.

Methods: In this prospective observational study, blood samples of 287 consecutive AIS patients, all undergoing i.v. thrombolysis by rtPA within 4.5 h of their symptom onset, were taken on admission. Levels of human neutrophil elastase-α1 antitrypsin (HNE-AT) complex, cell-free DNA (cfDNA) and histone-complexed-DNA-fragments (DNA-histone complexes) were measured from stored platelet-poor-plasma samples. Detailed clinical, laboratory investigations and imaging data (CT angiography) were obtained from patients on admission. Stroke severity was determined by NIHSS. Therapy-associated intracerebral hemorrhage (ICH) was classified according to ECASSII criteria. Short- and long-term outcomes were defined at 7 days and 3 months post-event according to the change in NIHSS and by the modified Rankin Scale (mRS), respectively. All patients or their relatives provided written informed consent.

Results: Levels of cfDNA on admission showed significant, stepwise elevation in case of more severe stroke. Patients with favorable long-term outcomes (mRS 0-1) had significantly lower cfDNA levels (median:65.8 [IQR:54.7-79.3] ng/mL) as compared to patients with poor outcomes (mRS 2-6: median:74.3 [IQR:59.4-88.7] ng/mL, p = 0.027). Similar results were obtained for HNE-AT (mRS 0-1: median:0.49 [IQR:0.34-0.72] ng/mL vs. mRS 6: 0.79 [IQR:0.46-1.60] ng/mL, p = 0.002). In patients who suffered therapy-related ICH (n = 24), HNE-AT levels were significantly higher as compared to those without hemorrhagic complication (median:0.53 [IQR:0.35-0.84] vs. 0.82 [IQR:0.42-1.62] ng/mL, respectively, p = 0.016).

Conclusions: Circulating markers of NETs, particularly cfDNA and HNE-AT might be useful to predict thrombolysis outcomes in AIS patients.

HEMOPHILIA AND RARE BLEEDING DISORDERS

ACQUIRED HEMORRHAGIC COAGULATION DISORDERS

PB0772 | The VWF/ADAMTS13 Unbalance, but Not Global Coagulation or Fibrinolytic Status, Is Associated with Outcome and Bleeding in Patients with Acute Liver Failure

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Background: Although patients with acute liver failure (ALF) were considered to have a bleeding tendency, they rare bleed and have

in fact rebalanced hemostasis with hypercoagulable features. This includes an unbalance between Von Willebrand Factor (VWF) and ADAMTS13 and a hypofibrinolytic status. Rodent models have shown a link between VWF, hepatic platelet accumulation, and ALF-progression.

Aims: Evaluation of the hemostatic state of ALF patients in relation to bleeding and clinical outcome.

Methods: Patients with ALF (INR (international normalized ratio) ≥ 1.5) or acute liver injury (ALI; INR ≥ 2.0) were recruited from the US ALF Study Group Registry between 2011 and 2018. Citrated plasma samples were taken on admission and VWF-levels, ADAMTS13-activity, thrombomodulin-modified thrombin generation, and clot lysis time (CLT) were determined, and compared to 40 healthy controls.

Results: We studied 676 patients, of whom 308 patients (45.6%) had acetaminophen-induced ALF. Bleeding occurred in 50 patients (7.4%), and 483 patients (71.4%) survived without liver transplantation. ALF patients had 5-fold increased VWF-levels, 5-fold decreased ADAMTS13-activity, similar thrombin generation, and 2.4-fold increased CLT, compared to controls. Patients with bleeding complications had higher VWF-levels (523% vs. 441%; $p = 0.0122$), and lower ADAMTS13-activity (15% vs. 22%; $p = 0.005$) compared to patients without bleeding. Thrombin generation and CLT did not differ significantly between bleeding and non-bleeding patients. Non-transplant-free survivors had higher VWF-levels (559% vs. 404%; $p < 0.0001$), lower ADAMTS13-activity (18% vs. 22%; $p = 0.0111$), similar total thrombin generation, and shorter CLT (136 min vs. 146 min; $p = 0.04$), compared to transplant-free survivors.

Conclusions: Hemostasis in a large cohort of ALF patients is characterized by VWF/ADAMTS13 unbalance, normocoagulability, and hypofibrinolysis, features which do not suggest a bleeding diathesis. The VWF/ADAMTS13 unbalance is associated with poor outcome, consistent with studies suggesting a detrimental effect of VWF in ALF in mice. The association between VWF/ADAMTS13 unbalance and bleeding complications suggests systemic inflammation rather than coagulopathy in ALF patients.

PB0773 | Acquired Deficiency of Dependent Coagulation Factors of Vitamin K, at the Teaching Center: Experience In 10 Years

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Background: Vitamin K (VK) is a fat-soluble vitamin that acts as a cofactor in the coagulation process, and factor (F)II, FVII, FIX, FX, and proteins anticoagulants (S, C, and Z) are functional. The acquired deficiency of VK-dependent factors is a heterogeneous entity in its causes and clinical hemorrhagic manifestations. The medical challenge is to approach the patient with hemorrhagic or asymptomatic clinic, with prolongation of PT without clear cause.

Aims: Describe the demographic and clinical characteristics of patients with acquired deficiency of VK-dependent coagulation factors.

Methods: Descriptive study, serial type of cases, patients over 18 years of age, with acquired deficiency of VK-dependent factors, January 2008 to December 2018. Patients with other specific deficiencies of coagulation factors were excluded.

Qualitative variables were described by absolute and relative frequencies and quantitative variables by central trend and dispersion measures by distribution.

Results: We describe 7 patients with acquired impairment of dependent factors of VK. The median age was 41 years. The average hospital stay value was 15 days with 11-day IQR.

The predominant clinical manifestation was mucosal bleeding in 42.8% of cases. The median PT was 26.9 seconds and the median INR was 2.4 with IQR of 17.7.

Etiology in 3 cases due to administration of auto formulated warfarin. 1 for Multivitamin intake (in this case without the conditions of registration by the country's health agency), 2 for prolonged fasting and concomitant use of antibiotics and one patient with bariatric surgery who suspended micronutrients. Treatment was in 4 cases vitamin K, one patient with plasma transfusion and one patient received recombinant factor VII A.

Conclusions: The acquired deficiency of VK-dependent coagulation factors is a difficult diagnosis due to its low frequency. Clinical presentation varies from asymptomatic to severe hemorrhagic complications. The cause must always be sought; the search for psychiatric disorders, or unlawful actions must be deepened.

PB0774 | A Rare Case of Acquired Haemophilia A with Platelet Dysfunction Associated with Non Hodgkin Indolent Lymphoma

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Background: Acquired haemophilia A (AHA) is a bleeding disorder characterized by autoantibodies against factor VIII. Half of patients present an underlying disease such as autoimmune disorders or cancer, particularly lymphoproliferative malignancies

Aims: To describe a patient with non Hodgkin indolent lymphoma who developed acquired AHA and platelet dysfunction.

Methods: FVIII:C was measured by one-stage clotting assay and inhibitor titer by Nijmegen-modified Bethesda assay. Platelet aggregation and ATP secretion were tested by lumi-light transmission aggregometry.

Results: A 67-year-old man was referred to our Internal Medicine Department for spontaneous bilateral gluteus hematomas and

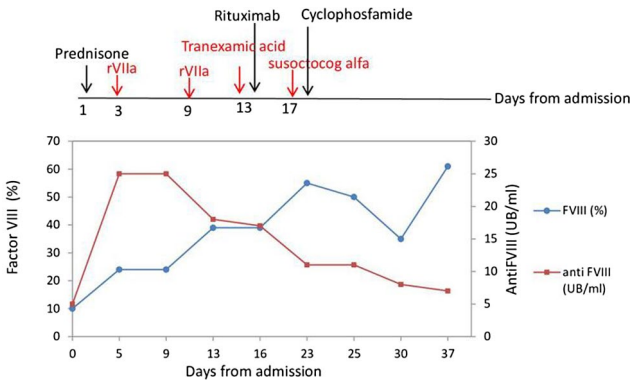

FIGURE 1 The clinical course of patient: therapy and FVIII-related analysis

bruising. Laboratory data showed mild Coombs negative hemolytic anemia and prolonged activated partial thromboplastin time (ratio:1.8). Factor VIII:C resulted 10% and inhibitor titer 5 Bethesda units confirming diagnosis of AHA. Prednisone (1 mg/kg) was started. Because of severe bleeding tendency platelet aggregation and secretion were investigated and they were severely impaired. Platelet levels of ADP were reduced with high ATP/ADP ratio, supporting the diagnosis of acquired platelet δ-storage pool defect.

Computed tomography scan documented splenomegaly and bone marrow biopsy revealed a not further characterizable lymphoproliferative B cell disorder CD20 + CD5-CD10-.

For bleeding symptoms factor rVIIa was introduced (100 U/Kg every 8 hours) together with rituximab 375 mg/m^2 weekly x 4 weeks to treat both AHA and the associated lymphoma. Cyclophosphamide (2 mg/Kg) and tranexamic acid were added. Susoctocog alfa (a recombinant B-domain deleted porcine FVIII) was successfully administered (37 UI/Kg) to treat an episode of severe acute anemization. (Fig. 1)

After four months AHA is in remission with progressive reduction of steroid and platelet function has also improved.

Conclusions: This rare and complex case underlines the need of multiple diagnostic and therapeutical approaches to AHA, due to the combination of coagulation and platelet dysfunction. Our case, suggest also the usefulness of Rituximab when used early in AHA secondary to lymphoproliferative disorders.

PB0775 | *In vitro* Correction of Thrombin Generation by Recombinant Porcine Factor VIII (rpFVIII) in Plasma Containing Anti-Factor VIII Inhibitory Antibodies

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Background: Development of anti-FVIII inhibitors is a major complication of the treatment of patients with hemophilia A. Antihemophilic factor VIII (recombinant), porcine sequence (rpFVIII; susoctocog alfa, OBI-1, BAX 801, TAK-672; Baxalta US Inc., a Takeda company, Lexington, USA) has low cross-reactivity to anti-human FVIII antibodies, and can result in therapeutic levels of functional FVIII activity despite the presence of antibodies.

Aims: To evaluate the hemostatic efficacy of rpFVIII *in vitro* using blood samples from patients with acquired hemophilia A and FVIII inhibitors, and commercially available FVIII-depleted plasma spiked with monoclonal antibodies to FVIII as determined by thrombin generation assay and thromboelastography.

Methods: Blood samples collected from patients attending routine hemophilia center visits (ethical approval and patient informed consent obtained) were assessed for FVIII inhibitors using the Nijmegen-modified Bethesda assay. Thrombin generation time course was determined at low tissue factor (1pM) concentration. Variables measured were lag time, peak height, and endogenous thrombin potential (ETP). Samples were classified by Bethesda titer as low (< 5 BU/mL) or high (≥5 BU/mL) for both human and porcine inhibitors, and stratified into subgroups: 1) low anti-human/low anti-porcine; 2) high anti-human/low anti-porcine; 3) high anti-human/high anti-porcine.

Results: Data from 19 patients were analyzed. Mean (range) anti-human FVIII inhibitor titer was 14 (1-427) BU/mL (14 high, 6 low), anti-porcine FVIII inhibitor titer was 12 (0-886) BU/mL (11 high, 9 low). A modest-to-moderate inverse correlation was found between anti-porcine inhibitor titer and thrombin generation response using ETP by addition of rpFVIII for concentrations of 2.7-5.4 U/mL (r: -0.520 to -0.573; $P < 0.03$; Table 1). Anti-human inhibitor titer did not affect thrombin generation (0.87; $P = 0.00006$).

Conclusions: rpFVIII correction of thrombin generation appears to be dependent on the anti-porcine inhibitory antibody titer and not on the anti-human inhibitory titer. Consistent with real-world observations, presence of pre-existing FVIII inhibitors does not exclude a positive response to rpFVIII.

ETP, endogenous thrombin potential; rpFVIII, recombinant porcine factor VIII.

TABLE 1 Spearman rank correlation between anti-porcine titer and thrombin generation response by addition of rpFVIII

rpFVIII concentration (U/mL)	*n*	Correlation: anti-porcine inhibitor and ETP*	P value	Correlation: anti-porcine inhibitor titer and peak height*	P value
0	19	−0.003	0.9903	−0.084	0.7412
2.7	19	−0.520	0.0270	−0.644	0.0027
5.4	19	−0.573	0.0130	−0.717	0.0008
10.8	19	−0.426	0.0781	−0.590	0.0099

*Anti-porcine inhibitor values as measured by local laboratories and ETP and peak height as measured by central laboratory.

PB0776 | The FARES Study: A Multicenter, Randomized, Active-Control, Pragmatic, Phase 2 Pilot Study Comparing Prothrombin Complex Concentrate versus Frozen Plasma in Bleeding Adult Cardiac Surgical Patients

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Background: Coagulopathic bleeding is a frequent complication of cardiac surgery and can lead to excessive blood loss, blood product transfusion and bleeding-related complications. Hemostatic management in bleeding surgical patients is evolving towards targeted therapy with purified products undergoing pathogen reduction technology. Unlike frozen plasma (FP), purified prothrombin complex concentrates (PCC) do not require ABO matching or thawing, are pathogen-reduced, and associated with lower risk of transfusion-associated circulatory overload and transfusion-related acute lung injury.

Aims: The FARES study will compare the efficacy and safety of PCC (*Octaplex*; Octapharma) versus FP in bleeding cardiac surgical patients.

Methods: The randomized, active-control, single-blinded, pragmatic, investigator-initiated, Phase 2 FARES pilot study will enrol 120 bleeding adult cardiac surgery patients requiring coagulation factor replacement from two hospitals. The study outline is shown in Figure 1. Patients will be randomized to PCC or FP, with two weight-based doses, for management of bleeding where coagulation factor deficiency is known or suspected. Required second doses will be given according to randomisation group allocation, but all patients will receive FP for third or subsequent doses. Primary endpoint is hemostatic effectiveness and transfusion requirements. Adverse events will be recorded from the beginning of surgery.

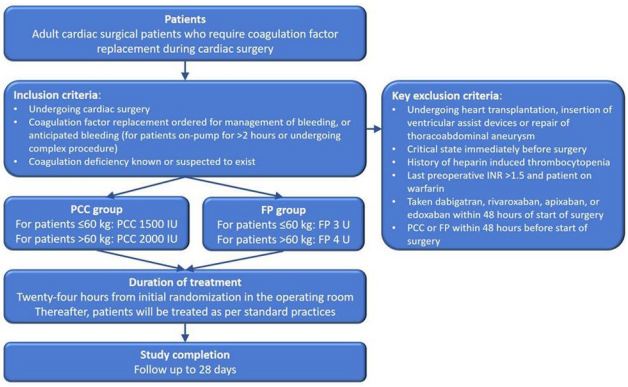

FIGURE 1 Study flow

Results: Results will be monitored and overseen by the IDSMC on an ongoing basis. The study is expected to end Q2 2020. As of January 31 2020, 70 patients have been enrolled in the study.

Conclusions: This study will determine the feasibility, and inform the design and primary outcome parameter, of a definitive Phase 3 trial comparing the efficacy and safety of the PCC *Octaplex* versus FP in bleeding cardiac surgical patients requiring coagulation factor replacement. The pilot study results will also be used in sample size calculations, and to aid detection of any early safety issues.

PB0777 | Postpartum Acquired Hemophilia A Persists as a Diagnostic Challenge

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Background: Acquired Hemophilia A (AHA) is a rare entity, resulting from the production of autoantibodies against factor VIII of the coagulation process. The presence of these autoantibodies, usually idiopathic, may be related to autoimmune conditions, drugs, neoplastic diseases and pregnancy. The diagnosis involves clinical aspects and laboratorial findings, such as prolonged activated partial thromboplastin time (aPTT), decreased levels of factor VIII (FVIII) and the presence of inhibitors against it. Clinical manifestations of AHA are characterized by mucous-cutaneous, intramuscular and/or postpartum bleeding.

Aims: To present a case of AHA associated with pregnancy with several muco-cutaneous hemorrhagic manifestations and intramuscular hematomas with excellent response to the use of immunosuppressants.

Methods: A 34-years-old female underwent cesarean delivery without complications. Forty days later she developed spontaneous hematomas with progressive worsening and intramuscular bleedings. She had no personal/family history of hematologic or autoimmune disease. Referred to hematological investigation at Fundação Hemominas Juiz de Fora-Brazil. The laboratory screening tests for coagulation disorders showed an increase of aPTT by its own, reduction of FVIII levels and the presence of inhibitors against it. Tests for diagnosis of autoimmune diseases and screening for neoplasms were negative. AHA was diagnosed. The patient was treated with prednisone plus tranexamic acid and showed total improvement, normalization of serum levels of Factor VIII, without the presence of inhibitors.

Results: Laboratory study: aPTT 62.5'', FVIII coagulation assay 3.5%, FVIII inhibitors 2.5 Bethesda. Diagnosis was confirmed and the instituted therapy was satisfactory.

Conclusions: Although rare, it is important to consider the diagnosis of AHA for cases of puerperal bleeding in which the treatment based on its most common causes is unsatisfactory. To improve the forecast, an early diagnosis is fundamental and it is essential to disseminate knowledge about this entity among the medical professionals.

PB0778 | Analysis of Patients with Diagnosis of Adquired Haemophilia in a Third Level Hospital

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Background: Acquired hemophilia (AH) is a rare coagulation disorder whose impact is sometimes underestimated by the lack of adequate records and its diagnostic complexity. Its importance lies in the high mortality rate.

Aims: Analyze the clinical profile and analytical parameters of patients diagnosed with AH in a third level hospital.

Methods: Retrospective observational study of patients diagnosed with AH in the Hematology Department of Miguel Servet University Hospital, from 2008 to 2019. Demographic variables, clinical characteristics, laboratory parameters and treatment received have been analyzed.

Results: 8 patients were included, 6 men and 2 women with a middle age of 74,89 years (extremes 47-97; DS 14,45). All patients presented more than one bleeding episode, in total 17, classified according to location (table). About the etiology, 3 patients presented secondary AH (lynfoproliferative syndrome, bullous pemphigus o psoriasis) and 5 had idiopathic HA. With regard to erradication treatment: 4 patients received corticosteroids, 3 patients received corticosteroids and cyclophosphamine and 1 patient received rituximab. Six patients needed by-pass agents, 5 of them with rFVIIa and 1 needed sequential treatment with rFVIIa and aCCP. Besides, 6 patients required transfusion of red blood cell concentrates. Of the 8 registered patients, one patient was hospitalized because of an urinary infection. Two patients died, one in the context of intracranial bleeding and another due to complications of cardiomyopathy. Analytically the hemostasis parameters were: median aPTT of 65.2 seconds (extremes: 53.3-160.9) and a median level of FVIII at diagnose of 3% (extremes: 0.1-25). Finally, the median inhibitory tittle at diagnose was of 8 Bethesda units (BU) (extremes: 8-65).

Conclusions: Acquired hemophilia is a life-threatening clinical situation. Immunosuppressive treatment achieves overall response rates in 70-80% of patients. In our series, the clinical hemorrhagic profile, the observed mortality, as well as the rate of responses to immunosuppressive treatment were similar to those described in the literature.

TABLE 1 Number and location of bleeding episodes

	Cutaneus	Muscular	Genitourinary	Abdominal	Intracranial	Post-surgery
Number of bleeding episodes	8	5	1	1	1	1
%	47.06%	29.41%	5.88%	5.88%	5.88%	5.88%

PB0779 | Real-World Use of Recombinant B-Domain-Deleted, Porcine-Sequence Factor VIII in Patients with Acquired Hemophilia A: An Interim Analysis of the Post-Authorization Safety Study in the European Union (EU)

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Background: Antihemophilic factor VIII (recombinant), porcine sequence (susoctocog alfa, OBI-1, BAX 801, TAK-672; Baxalta US Inc., a Takeda company, Lexington, MA, USA; recombinant porcine FVIII

[rpFVIII]) has low cross-reactivity with anti-human FVIII antibodies, and can be used to treat bleeding episodes (BEs) in adults with acquired hemophilia A (AHA).

Aims: This ongoing post-authorization study assesses the safety (primary objective), utilization, and effectiveness of rpFVIII in clinical practice.

Methods: This is an interim analysis of a multicenter, non-controlled, open-label, single-cohort, observational, non-interventional study in the EU (EUPAS16055; NCT03199794). Patients with AHA aged ≥ 18 years who had received or were receiving rpFVIII for BEs (initial and subsequent events) as per clinical practice were retrospectively or prospectively identified and enrolled. Patients with known anaphylactic reaction to rpFVIII, excipients, or hamster protein were excluded. Adverse events (AEs)/serious AEs were reported up to 180 days after the last dose of rpFVIII. Additional data collected included BEs and rpFVIII treatment, bleeding control, and inhibitor levels.

Results: As of June 3, 2019, 18 patients (12 males; mean±SD age, 75.2 ± 8.8 years) were enrolled (median follow-up [n = 8], 203.5 days). Serious AEs (none related to rpFVIII) were reported in 6/18 patients (cardiac failure, death, multiple organ dysfunction syndrome, sudden cardiac death, atypical pneumonia, syncope, and hemorrhage). No thromboembolic event occurred. Physician-assessed AEs of special interest (drug resistance [lack of effect], n = 1; FVIII inhibition [anti-rpFVIII antibodies], n = 2) were serious. Of the initial BEs (12 spontaneous, 5 traumatic, 1 surgical), 14 resolved (median [range]: 7.5 [1-49] days) and 3 remained unresolved following a mean±SD (median; range) rpFVIII loading dose of 69.8 ± 50.9 (51.5; 32-250 U/kg).

Conclusions: In this interim analysis, these real-world data demonstrate an acceptable safety and effectiveness profile for rpFVIII in controlling bleeds in patients with AHA, and provide insights into how rpFVIII use may be optimized to personalize therapy.

PB0780 | Acquired Hemophilia in Argentina: A Multicentric Study

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Background: Acquired hemophilia (AH) is a rare disease caused by autoantibodies against to factor VIII (FVIII).

Aims: To describe diagnosis, underlying disorders, type of bleeding, treatment, outcome, and factors associated with mortality in patients with AH.

Methods: Multicentric retrospective cohort study. Patients: >18 y.o with AH from 16 centers in Argentina (1999-2019).Inclusion criteria: FVIIIc activity < 50 U/dL due to FVIII inhibitor activity.. Bleeding: classified according to ISTH guidelines. Statistics: Kaplan Meier to assess survival, Chi2 and Kruskal-Wallis for death associated factors.

Results: Patients: n = 61. 43% older than 70 y.o.(table 1). No underlying disorder was detected in 51% of patients. The median(IQR) follow-up was 22(7-48) months.

At diagnosis, 41(67%);14(23%) and 6(10%) presented major, minor and clinically relevant non-major bleeding, respectively. Extensive cutaneous bleeding was reported in 61% of patients.

Thirty-one patients (52%) were treated with bypassing agents. Fifty-seven (93%) received immunosuppressive treatment being corticosteroids plus cyclophosphamide the most used (table 2). Complete remission was obtained in 48(78%) patients but in 8 it was necessary to change to rituximab for primary failure. Relapse was evidenced in 11 patients (23%) with initial complete remission, 9/11 reached new remission during follow-up.

Most frequent treatment complication: infection (n = 11,19%).

Mortality: reported in 24% of the patients, mainly due to bleeding within the first 3 days (table 2).

The 36-months survival was 72%(CI95% 57-82). Patients who died had a significantly higher frequency of major bleeding (93%vs59%,

TABLE 1 Baseline characteristics of the population and diagnostic information

Age in years, median (IQR)	65 (45-78)	**Major bleeding at diagnosis, n (%)**	41 (67)	**FVIII determination method (reported in 47 cases)**	
Female gender, n (%)	38 (62)	Extensive cutaneous hematomas, n (%)	11 (27)	One stage assay, n (%)	45 (95)
Underlying disease		Musculoskeletal hematoma, n (%)	10 (24)	Chromogenic, n (%)	2 (5)
Idiopathic, n (%)	31 (51)	Skin and mucosal hemorrhages, n (%)	5 (12)	**Inhibitor detection method (reported in 45 cases)**	
Pregnancy/puerperium, n (%)	10 (24)	Abnormal uterine bleeding, n (%)	4 (10)	Bethesda, n (%)	34 (75)
Solid tumors, n (%)	5 (12)	Retroperitoneal hematoma, n (%)	3 (7)	Cualitative detection, n (%)	7 (16)
Autoimmune disease, n (%)	4 (10)	Others, n (%)	8 (20)	Nijmegen, n (%)	4 (9)
Hematologic neoplasia, n (%)	3 (7)	**Hemoglobin drop, median (IQR)**	3 (1-5)	**Bethesda Units at diagnosis in UI/mL, median (IQR)**	36 (12-98)
Others, n (%)	8 (20)	**FVIII level (U/dL) at diagnosis, median (IQR)**	4 (1-2)		
Days of active bleeding, median (IQR)	3 (2-11)	**APTT (sec) at diagnosis, median (IQR)**	88 (70-103)		

TABLE 2 Treatment and evolution

Hemostatic therapy		**Immunosuppressive therapy**		**Relapse treatment**	
Recombinant FVIIa, n (%)	25 (42)	Cyclophosphamide + corticosteroids, n (%)	23 (38)	Corticosteroids only, n (%)	4 (40)
FVIII Concentrate, n (%)	6 (10)	Corticosteroids only, n (%)	21 (34)	Rituximab + corticosteroids, n (%)	3 (30)
Recombinant FVIIa after FVIII Concentrate, n (%)	3 (5)	Rituximab after cyclophosphamide failure, n (%)	8 (13)	Cyclophosphamide + corticosteroids, n (%)	3 (30)
Activated Prothrombin complex concentrates, n (%)	3 (5)	Rituximab + cyclophosphamide, n (%)	3 (5)	**Death, n (%)**	15 (24)
Without treatment, n (%)	16 (27)	Rituximab monotherapy, n (%)	2 (3)	**Death cause**	
Others, n (%)	8 (11)	Without therapy, n (%)	4 (7)	Bleeding, n (%)	8 (53)
Start of immunosuppressive therapy <first 24 hours, n (%) (Reported in 49 patients)	38 (78)	**Relapse, n (%)**	11 (23)	Infection, n (%)	2 (13)
Weeks of immunosuppressive therapy, median (IQR) (Reported in 51 cases)	8 (4-12)	**Time to relapse (months), median (IQR)**	3 (2-9)	Thrombosis, n (%)	1 (7)
Weeks until inhibitor negativization, median (IQR) (Reported in 27 cases)	7 (4-16)			Other, n (%)	4 (27)

p = 0.013), no differences on FVIII levels or therapies used were found.

Conclusions: The frequency of idiopathic AH, complete response and mortality were similar to the largest series reported worldwide. Most of the deaths occurred early after diagnosis due to bleeding but only half of the patients received bypassing agents, which highlights difficulties regarding access to prompt and adequate treatment in this life-threatening condition.

PB0782 | Autoimmune Acquired Factor XIII Deficiency: A Case Report

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Background: Autoimmune acquired factor XIII (FXIII) deficiency is a rare disorder characterized by the presence of autoantibodies against FXIII and life-threatening bleedings.
Aims: To empashize the importance of identifying urgent haemorragic conditions.
Methods: We determined FXIII antigen following the ISTH recommendations. Inhibitor was detected by the Bethesda assay and full sequencing FXIII gen was performed by NGS.
Results: We present a 70-year-old patient with repeated muscular haematomas without previous bleeding history. The basic haemostatic study was normal as well as all coagulation factors, but a FXIII antigen of 9% was detected. We diagnosed an autoimmune acquired FXIII deficiency (table 1). No mutations in FXIII gene were found. Immunosuppression with steroids and haemostatic treatment with tranexamic acid were started. We monitored clinical and therapeutic evolution with thrombin generation test (figure 1) and thromboelastography, describing a greater thrombin generation and clot firmness as FXIII levels increased. The body scan and the autoimmunity study were normal. However, due to inhibitor persistence, we changed to azathioprine. The inhibitor titer remained at a low level without clinical bleeding. Three months after discontinuation of immunosuppression, the patient was admitted for thoracic oppressive pain and minimal effort dyspnea. The basic haemostatic study was normal and FXIII levels and inhibitor titer were similar to previous. The scan objectified a mediastinal hematoma. Hemostatic treatment with tranexamic acid and fresh frozen plasma and immunosuppressive therapy with rituximab were administrated. FXIII concentrate was not given, as the patient presented clinical stability. Nowadays, the patient remains asymptomatic. The inhibitor is still detected at low titer.

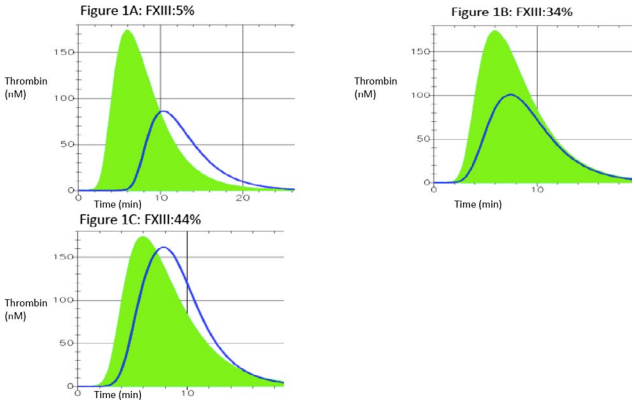

FIGURE 1 Thrombin generation assay (in green certified normal plasma/in blue patient's sample)

Conclusions: Despite its rarity (93 cases in the literature), it is crucial to suspect this disorder if severe clinical bleeding is present and routine coagulation tests are normal. Global assays may help monitor clinical evolution. Appropriate treatment should be early initiated to gain control of bleeding and inhibitor eradication.

PB0783 | Global Coagulation Analysis for Acquired Hemophilia A Patients with the Marked Elevation of Factor VIII Activity during Remission Phase

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Background: Acquired hemophilia A (AHA) is a rare bleeding disorder marked by the development of autoantibodies to factor (F) VIII. Elevation of factor VIII activity (FVIII:C > 150 IU/dl) has been reported in AHA patients who achieved remission during immunosuppression therapy.
Aims: We evaluated the comprehensive coagulation function in AHA patients with elevation of FVIII:C.

TABLE 1 Laboratory follow-up and immunosupressive dose

Date	Baseline FXIII	Mixing test without incubation	Mixing test after 2-hour incubation	Inhibitor titre	Immunosupression
26/02/19	5%	26%	35%	12 UB	Prednisone 60 mg daily
8/03/19	17%	52.8%	61%	9 UB	
20/03/19	34.3%	56.3%	61.8%	3.6 UB	Prednisone 45 mg daily
4/04/2019	44%	58.1%	63%	4.22 UB	
15/04/2019	31%	58%	60%	0.86 UB	Prednisone 30 mg daily
25/04/2019	30%	53%	56.7%	2.1 UB	
16/05/2019	21%	51%	52.7%	1.59 UB	Prednisone 15 mg daily
3/06/2019	35%	52%	53%	1.4 UB	Azathioprine 100 mg daily

Methods: We performed clot wave form analysis (CWA), triggered by tissue factor and ellagic acid, and thrombin generation assay (TGA) by tissue factor and ellagic acid in eight AHA patients. We measured protein S and protein C activities as anticoagulant factors measured by activated partial thromboplastin time (APTT) based assay.

Results: Medians (range) of FVIII:C and von Willebrand factor antigen were 219 (151-344) IU/dl, and 370 (210-433) IU/dl, respectively. CWA parameter, adjusted maximum coagulation velocity (ad|min1|), was used. Two patients (ad|min1|; 6.0, and 6.4) showed below control range (ad|min1|; 7.7 ± 0.5). Five patients (ad|min1|; 7.2-8.2) were within control range. Only one patient (ad|min1|; 9.0) showed the higher value than that of control range. Peak thrombin was used for TGA parameter. Five patients (peak thrombin; 236-391 nM) showed the lower than that of control range (463 ± 21 nM). One patient (peak thrombin; 449 nM) was within control range. Only two patients (peak thrombin; 554, and 577 nM) showed the higher peak thrombin than that of control range. On the other hand, protein S activities in all AHA patients were normal [median (range); 107 (57-118) IU/dl]. Interestingly, protein C activities in six of eight AHA patients were high [median (range); 177 (165-206) IU/dl]. Two AHA patients showed normal protein C activities (112 and 144 IU/dl).

Conclusions: Majority of AHA patients with marked elevation of FVIII:C did not show high coagulation function according to the comprehensive coagulation function assays.

PB0784 | Emicizumab for the Treatment of Acquired Hemophilia A

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Background: Acquired hemophilia A (AHA) is a severe bleeding disorder caused by autoantibodies to FVIII. For hemostatic treatment bypassing agents, human or porcine FVIII are currently used. Emicizumab is a bispecific, FVIII-mimetic therapeutic antibody that reduced the ABR in hemophiliacs.

Aims: Preclinical data and pathophysiology suggest efficacy in AHA.

Methods: 12 patients with AHA, 6male, 6female, age 79 yrs (51-87) (medians and ranges), were treated with emicizumab. FVIII was < 1%, inhibitor 69BU/mL (3-2300). 7 patients had severe bleeding (4 after major surgery). Emicizumab was given at 3 mg/kg sc. weekly for 4 weeks, followed by 1.5 mg/kg weekly. For FVIII monitoring chromogenic assays with human and bovine reagents were used.

Results: APTT normalized after 1-3 days, FVIII exceeded 10% after 13 (7-18) days. Hemostatic efficacy was obtained, bypassing therapy stopped after 4 (0-9). FVIII exceeded 50%, after 24 and 42 days (n = 2), and emicizumab could be stopped after 3 and 6 injections. All patients received immunosuppression with steroids and rituximab. Patients were discharged after 2 weeks (2-30 days) after the first emicizumab injection, and followed and treated every 3 weeks until complete remission. In median, 6 doses of emicizumab (2-8 doses) were given, the last dose after 53 (14-161) days. No breakthrough bleeding was observed.

Conclusions: Emicizumab seems to be effective in AHA, with advantages of sc. therapy every 1-4 weeks, good hemostatic efficacy, outpatient therapy, option to reduce immunosuppressive therapy, and to be more cost effective than bypassing agents. Special attention is needed on the use of appropriate lab assays, the recognition of remission, and to avoid the concomitant use of APCC. Strategies for patients with comorbidities, severe over- or underweight, after, or in need for major surgery, concomitant risk of thromboembolism need to be developed, and a clinical trial comparing emicizumab with the current standards of care is warranted.

PB0785 | Real Life Experienceon 61 Acquired Haemophilia A (AHA) Patients from a Single Center

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Background: AHA is a rare hemorrhagic disease caused by autoantibodies against the FVIII. The response to immunosuppressive therapy (IST) is high (60-80%) but the time to obtain it can be long, exposing the patient at risk of fatal bleeding.

Aims: We describe an AHA cohort in which we evaluated the impact of some parameters on response, time to response (TTR) and overall survival (OS).

Methods: Data were collected from the clinical charts. Response criteria: complete response (CR) FVIII > 50%, undetectable inhibitor, absence of bleeding; partial response (PR) FVIII > 50%, persistent inhibitor; no response (NR) FVIII < 50%, persistent inhibitor.

Results: 61 patients were diagnosed over a period of 41 years (median follow-up 26.6 months). Tables 1-2 show the baseline characteristics of the patients and bleedings. 50/61 patients (82%) underwent hemostatic therapy: rFVIIa (52%); aPCC (38%) with no significant differences in the response rate (RR) (rFVIIa 92.3% vs aPCC 100%). No thromboembolic events occurred. 60/61 patients underwent first-line IST: corticosteroids (76.6%); corticosteroids-cyclophosphamide (13.4%). The RR after 4 weeks was 53%: CR 38%, PR 15%, NR 28%. At the last follow-up: CR 61%, PR 5%, NR 29%. The 1-year OS was 85%. The bleeding mortality rate was 3%. We evaluated: the impact of FVIII < 1% and inhibitor > 5 BU, at diagnosis, on TTR and that of Hb level, FVIII:C, inhibitor titer, age, sex, related disorders, on the response and OS. None of these was statistically significant on TTR and response. Age < 70 years (p = 0.011), achievement of response (p = 0.002) and post-partum (p = 0.001) improved the OS.

Conclusions: Our cohort is the largest as single center with a long follow up, a highest 1-year OS and a lowest mortality described. We highlight obtaining a response improves the OS. It would be useful to identify response predictors to drive therapy and shorten TTR.

TABLE 1 Bleeding at diagnosis

Bleeding as a trigger for diagnosis n. (%)	61 (100)
Time from bleeding event to diagnosis	
Median (days-range)	31 (0-264)
Cause of bleeding episodes n (%)	
Spontaneous	57 (93)
Trauma	4 (7)
Site of bleedings n (%)	
Deep musculoskeletal	52(85)
Genitourinary	8 (13)
Mucosal and skin	3 (5)

TABLE 2 Demographics characteristics and related disorders at diagnosis

Patients n (%)	61 (100)
Median age at diagnosis (range)	71 (18-89)
Male: female n (%)	26/35 (42.6%/57.4%)
Median FVIII activity (U dL- range)	1.5 (<0.25- 42.5)
Median Inhibitor titer (BU- range)	10.95 (1.06-1188)
Median hb at diagnosis (g/dL-range)	11 (4.9-15.2)
Idiophatic (%)	64
Autoimmune (%)	13
Cancer (%)	8
Post-partum (%)	16.5

PB0786 | Diagnosis and Management of Acquired Von Willebrand Disease in Two Italian Centers

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Background: Acquired Von Willebrand Syndrome (AVWS) is a rare acquired coagulopathy, often associated to an underlying disorder. Diagnosis is not easy and relies on a negative familial/personal hemorrhagic history and a late onset in life of bleedings, associated with a laboratory pattern for Von Willebrand Disease (VWD).

Aims: To describe the experience on diagnosis and management of AVWS patients (pts) in two Italian centers.

Methods: Diagnosis was made on the basis of clinical/laboratory features suggestive of AVWS. The screening for VWF:RCo inhibitor was made utilizing mixing studies. Data were collected from clinical charts.

TABLE 1

AVWS type 1, 2 A, 3	3 patients, 7 patients, 4 patients
Median VWF:Ag (n.v. blood group 0: 41-101%; no 0: 50-130%)	15% (range 1.6-51%)
Median VWF:RCo (n.v. blood group 0: 41-97%; no 0: 52-124)	13% (range < 6.25-33%)
Median FVIII:C (n.v.58-130%)	19.2% (range 2.1-53%)
VWF:RCo inhibitor (searched in 4 cases)	3 negative, 1 positive

Results: Between 2004-2019, 14 AVWS pts were diagnosed and managed [8F, 6M; median age 62.45 years (45.4-85.9)]. Reasons for diagnosis were: recent onset of bleeding symptoms in 9 pts, increased aPTT in 5. Patients characteristics are reported in table 1. All the pts did not have either a family or past personal history of hemorrhages. The spontaneous bleeding events presented at diagnosis and during follow up were: GI and gum bleeding, muscle hematomas, hematuria, ecchymoses, epistaxis, menometrorrhagia. Three pts bled after dental extractions/procedures, although they were prophylactically treated with VWF/FVIII concentrate. Desmopressin, Tranexamic Acid, Intravenous Immunoglobulins (IvIg) VWF/FVIII concentrate, and recombinant FVIII were used as prophylaxis or treatment of bleedings in all pts, with variable responses on clinical symptoms. Thirteen/14 cases showed a concomitant disorder: 1 gastric B cell MALT lymphoma, 1 indolent B cell lymphoma, 7 MGUS, 2 Waldenstrom Disease and 2 patient with breast cancer and concomitant MGUS. In one case AVWS was idiopathic. Patients management is reported in table 2.

Conclusions: AVWS is a rare syndrome, probably underdiagnosed because unrecognized. AVWS must be suspected whenever a patient presents a late onset in life of bleeding symptoms associated to laboratory characteristics compatible with VWD. It is mandatory to search for concomitant diseases.

TABLE 2

Type	Treatment	Response
Gastric B cell MALT lymphoma	Rituximab	Complete remission of lymphoma and AVWS
Waldenstrom Disease (1st patient)	R-CVP, Ibrutinib	After 2nd line therapy, stable lymphoproliferative disease and persistent AVWS
Waldenstrom Disease (2nd patient	Rituximab. After two years for disease progression: Rituximab+Bendamustine	Partial response after Rituximab. Persistent AVWS and partial response of lymphoma after Rituximab+Bendamustine
1 Indolent B cell lymphoma + 3 MGUS	No treatment	NA
MGUS 1 patient	Prednisone + cyclophosphamide and then IvIg	No response to either therapies on AVWS
MGUS 3 patients	Infusion of IvIg (2 cases are under chronic treatment with Iv Ig every 6-8 weeks)	Transient response on AVWS
1 patient with breast cancer + MGUS	Breast surgery	No response on AVWS
1 patient with breast cancer + MGUS	Prednisone	No response on AVWS Improvement of bleeding symptoms
Idiopathic AVWS	Prednisone. At relapse Prednisone + Cyclophosphamide	CR on AVWS after first line therapy and at relapse

PB0787 | Advanced Principles of Prevention and Treatment of Preeclampsia in Women with Antiphospholipid Syndrome (APS) and MTHFR C677T Mutation

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Background: Improve the principles of prevention and treatment of preeclampsia in women with APS and genetic forms of thrombophilia.

Aims: Improve the principles of prevention and treatment of preeclampsia in women with APS and genetic forms of thrombophilia.

Methods: 55 women were studied, which were divided into two groups: group I consisted of 23 women - pregnant women who had episodes of preeclampsia in previous pregnancies (in anamnesis), group II included primigravidas with preeclampsia during the current pregnancy. All pregnant women were diagnosed with lupus anticoagulant and high titers of anticardiolipin antibodies (Anticardiolipin IgG).

Results: Pathogenetically based prevention using low-molecular-weight heparin (bemiparin), folic acid, B vitamins and antioxidants in patients with preeclampsia caused by APS and genetic form of thrombophilia from early pregnancy and throughout pregnancy allowed to improve the gestational period and prolong pregnancy, to improve perinatal outcomes in pregnant women. It should be noted that significantly better outcomes occurred in patients who got therapy already at the pregravidar preparation stage and received low-molecular-weight heparin, folic acid, B vitamins and antioxidants throughout pregnancy.

Conclusions: Timely diagnosis, pathogenetic prevention and differentiated therapy allow not only to prevent the development of obstetric complications, but also to reduce maternal and perinatal morbidity and mortality.

PB0788 | Major Bleeding after a Gynecological Operation in Patient with Thrombocytosis and Anemia: Acquired von Willebrand Syndrome?

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Background: Cancer patients with thrombocytosis have an increased risk of bleeding. Thrombocytosis and a bleeding diathesis may be associated with the pathogenesis of acquired von Willebrand syndrome (AWVS). Significant bleeding phenotype may be associated with low von Willebrand factor (VWF).

TABLE 1 Results of laboratory diagnostics performed at different time points

Assay	Day of admission (before surgery)	Postoperative diffuse bleeding within 24 hours after operation	Major bleeding during and after reoperative surgery (transfusion corrections of blood loss)	Reference range	Commercial method
HCT	0.34 L/L	0.24 L/L	0.20 L/L	0.36-0.47	Sysmex XN-1000 hematology analyzer
PLT	654x109/L	614x109/L	495x109/L	158-424	Sysmex XN-1000 hematology analyzer
PT	102%	74%	78%	>=70	Dade Innovin
APTT	29 sec	30 sec	29 sec	23-32	Actin FS
Fibrinogen	3.3 g/L	3.4 g/L	5.5 g/L	1.8-3.5	Multifibren U
FVIII (% activity)	102%	/	/	70-150	One stage coagulation assay (Actin FS and FVIII deficient plasma)
vWF (% activity)	45%	/	/	48-173	Innovance vWF
COL/EPI (PFA); COL/ADP (PFA)	>300 sec, both	/	/	COL/EPI 82-150; COL/ADP 62-100	PFA-100 analyzer, Siemens Healthineers
Platelet aggregation testing: ADP, EPI, COL, AA, RIS	ADP = 48% EPI = 51% COL = 54% AA = 55% RIS = 52%	/	/	For all agonists > 60	Light transsmision aggregometry, Sysmex CS2500 analyzer

Aims: We report a case of a 59-year-old female who presented with mucinous borderline ovarian cancer, reactive thrombocytosis, chronic anemia and postoperative diffuse bleeding complications.

Methods: Laboratory diagnostics in our patient included: complete blood count, screening and specialized coagulation tests (BCSXP analyzer Siemens Healthineers), platelet function screening test and platelet aggregation testing. Laboratory assays and methods are presented in Table 1, columns 1, 6, respectively.

Results: Results of all relevant laboratory diagnostics at different time points are presented in Table 1.

Preoperative hematological tests revealed anemia and thrombocytosis. Platelet function screening test showed abnormal closure time. Platelet ability to respond to aggregating agonists was found to be slighty decreased (Table 1, column 2). Drug-induced platelet dysfunction was ruled out. Mild reduced plasma VWF-ristocetin-cofactor activity and normal factor-VIII-coagulant activity were found (Table 1, column 2).

Surgical procedure included an exploratory-laparotomy-hysterectomy-ovariectomy-appendectomy. Postoperative capillary diffuse bleeding complications occurs within 24 hours after operation. Significant blood loss anemia was noticed (Table 1,column 3). Reoperative surgery was done. Development of major bleeding in spite of haemostatic treatment during surgery. After administration of replacement-recombinant-activated-clotting-factor VII, acute bleeding was stopped. There was no previous history of bleeding episodes.

Conclusions: Significant bleeding in our patient could be associated with decreased vWF activity and platelet aggregation testing. These laboratory findings are suggestive of AWVS. Further studies will be required to investigate the significance of these laboratory findings and their impact on the phenotype observed in our patient. AWVS should be considered in patients with postoperative bleeding whenever laboratory findings suggest platelet dysfunction.

PB0789 | Coagulation and Inflammation Genes Are Differently Expressed during the Acquired Coagulation Disorder Induced by Snake Envenomation

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Background: Snake venoms are complex mixtures of toxins that activate and/or inhibit coagulation, fibrinolysis and the inflammatory system. Bites by *Bothrops* snakes evoke an inflammatory reaction, marked by an increase in pro-inflammatory cytokines and acute phase proteins (APPs). This envenomation also induces hemostatic disturbances, as thrombocytopenia, consumption coagulopathy (characterized by decreased levels of factors II, VIII, X and fibrinogen) and secondary hyperfibrinolysis. The neutralization of the venom by the specific antivenom therapy allows the restoration of

hemostatic parameters, including fibrinogen levels. However, the hepatic molecular mechanisms underlying these alterations and its interactions are still unclear.

Aims: To investigate the gene expression kinetics of important coagulation and inflammation factors synthetized by the liver in mice injected with *Bothrops jararaca* venom.

Methods: All procedures involving animals were approved by IACUC. Swiss mice were injected s.c. with saline or venom (1.6 mg/kg b.w, s.c.). After 3, 6 or 24 h, liver fragments were collected. Gene expression was assessed by Real-Time Quantitative PCR.

Results: Envenomation induced an important increase in mRNA levels of the positive APPs fibrinogen and haptoglobin, and also the transcription factor STAT3. Interestingly, mRNA levels of tissue factor decreased at 3 h. At 24 h, mRNA levels of FX increased and of antithrombin III and protein C decreased. No alterations were observed regarding the gene expression of the negative APP murinoglobin, the transcription factor NFκB-1 and most hemostasis-related genes, e.g., factors II and VIII, plasminogen and α_2-antiplasmin. Nevertheless, significant correlations were observed among these genes.

Conclusions: Our results indicate that envenomation induces a rapid mRNA synthesis of fibrinogen chains, which could explain the fast recovery of hemostatic parameters in patients after the neutralization of the venom by specific antivenom. Moreover, important genes related to coagulation and inflammation display different patterns of expression and seem to have common regulatory mechanisms.

Funding: FAPESP, CNPq, CAPES, Fundação Butantan.

PB0790 | Experience with Recombinant Porcine FVIII in Patients with Acquired Factor VIII Deficiency Undergoing Major Surgery

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Background: Acquired factor VIII (FVIII) deficiency is a rare and potentially fatal coagulopathy with limited experience in patients undergoing major surgical procedures. Bypassing agents and recombinant porcine FVIII (rpFVIII) are hemostatic drugs used to treat and prevent bleeding in patients with acquired FVIII deficiency.

Aims: Describe 3 clinical cases of patients diagnosed with acquired FVIII deficiency that underwent a major surgical procedure managed with rpFVIII.

Methods: Retrospective record review of 3 acquired FVIII patients at the Gulf States Hemophilia and Thrombophilia Center of Houston, Texas who underwent a major surgical procedure with active inhibitor.

Results: Only one dose of rpFVIII was given preoperatively for each patient. Two patients (#1 and #2) demonstrated an excellent response to rpFVIII with an average estimated blood loss of 950 cc. Poor response experience by Patient #3 was most likely related to antibodies against rpFVIII as the peak FVIII level at 1 hour was only 4.6%. However, this patient experience no excessive bleeding, most likely due to the effect of emicizumab. No thromboembolic complications were reported.

Conclusions: Recombinant porcine FVIII is a safe and effective hemostatic drug for patients with acquired factor VIII deficiency undergoing major surgery. Close monitoring of FVIII levels is important to assess efficacy and to adjust dosing. In our experience lower dosing of rpFVIII than recommended by manufacture are needed for major surgical procedures. Pharmacokinetic study with rpFVIII is recommended.

PB0791 | Prophylactic Potential of Standard and Modified Emicizumab Prophylaxis in 2 Patients with Acquired Hemophilia: A Case Report

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Background: Acquired factor VIII deficiency is a rare coagulopathy caused by an autoantibody (inhibitor) to factor VIII (FVIII) leading to severe, potentially life-threatening hemorrhage. Disease management concentrates on permanently eradicating the autoantibody using immunosuppressants and achieving hemostasis, primarily with bypassing therapy and recombinant porcine FVIII. Emicizumab, a

TABLE 1

	Gender/ Age	Secondary DX	Peak Inhibitor	Inhibitor at time of surgery	Immunosuppressive Treatment	Procedure	Initial dose of rpFVIII	Subsequent rpFVIII doses	Estimated Blood Loss
Patient #1	F/24	Pregnancy	79 B.U.	2.70 B.U.	Prednisone	Cesarean Section	100 mg/kg	-	600 cc
Patient #2	F/53	RA	17.6 B.U.	5.60 B.U.	Prednisone	Cardiac (valve replacement)	100 mg/kg	50 IU/kg q 6 hrs (x5 doses)	1300 cc
Patient #3	M/90	Idiopathic AFib, CAD, CHF	380 B.U.	55 B.U.	Rituximab Mycophenolic acid *Emicizumab	Knee Arthrotomy	100 mg/kg	-	100 cc

monoclonal antibody, designed to promote hemostasis in the congenital factor VIII deficient patient, has shown hemostatic efficacy in both congenital factor VIII deficient patients with and without inhibitors.

Aims: To assess the efficacy of emicizumab prophylaxis in prevention of bleeding symptoms in patients with acquired hemophilia.

Methods: Retrospective record review of a 57 year old female and a 90 year old male with multiple comorbidities diagnosed with acquired factor VIII at the Gulf States Hemophilia and Thrombophilia Center of Houston, Texas on standard and modified off label emicizumab prophylaxis.

Results: Average bleed event prior to emicizumab prophylaxis occurred twice a month. 1st Patient (female) - Standard Protocol: Loading dose ~3 mg/kg/ dose subcutaneous once every 7 days for four doses. Maintenance ~1.5 mg/kg/dose subcutaneous once every 7 days. 2nd Patient (male) - Texas Protocol: Loading dose ~1.5 mg/kg/dose subcutaneous once every 7 days for two doses. Maintenance dose ~1.5 mg/kg/dose subcutaneous once every 21 days. Peak inhibitor titer at start of protocol= female- 6.67 B.U. and male- 150.4 B.U. Following start of emicizumab, both Standard and Texas Protocol, immediate cessation of bleeding episodes were noted.

Conclusions: Emicizumab is a monoclonal antibody that promotes hemostasis without replacing factor VIII, while the use in acquired hemophilia is very limited these cases illustrate its efficacy and safety using standard and modified low dose prophylaxis (Texas Protocol) in acquired hemophilia patients with multiple comorbidities. Further studies in this disease are warranted.

PB0792 | Rutin and Rutin Succinate Improve Murine Survival in an Experimental Model of Acute Hemostatic Disturbances

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Background: *Bothrops jararaca* snake venom (BjV) contains various metalloproteinases and thrombin-like enzymes with procoagulant activity, which induce consumption coagulopathy and bleedings. Understanding the rapid pathophysiological events evoked by snake venoms in hemostasis is a useful tool to study acute hemostatic disturbances in general. We showed previously that the bioflavonoid rutin ameliorates hemostatic parameters in mice injected with BjV.

Aims: Since rutin is not water-soluble, we have currently addressed the mechanisms of action of a water-soluble form of rutin, rutin succinate, to block hemostatic disorders induced by BjV. Thus, we carried out a comparative evaluation of the ability of rutin succinate and rutin to inhibit BjV activities *in vitro* and *in vivo*.

Methods: Procedures involving animals were approved by IACUC. The inhibitory activity of rutin and rutin succinate was tested on the *in vitro* coagulant and fibrinogenolytic activities of BjV, as well as *in vivo*, in C57BL/6 mice injected i.p. with BjV either alone or pre-incubated with rutin or rutin succinate. Survival rates were analyzed, and blood was collected 48 h after envenomation from surviving mice.

Results: Both bioflavonoids showed the characteristic activities described for rutin, i.e, antioxidant and an inhibitor of protein disulfide isomerase. Rutin succinate inhibited the coagulant and fibrinogenolytic activities of BjV *in vitro*. Mice injected with BjV or BjV preincubated with rutin showed decreased RBC and platelet counts, hypofibrinogenemia and hemorrhage at the site of venom injection. Surprisingly, mice injected with BjV preincubated with rutin succinate had normal blood cell counts and fibrinogen levels, and hemorrhage was completely abrogated. Importantly, BjV induced a mortality of 66.7%, and rutin and rutin succinate could completely prevent it.

Conclusions: Rutin and rutin succinate prevented the mortality of mice injected with BjV; moreover, rutin succinate inhibited the coagulant and fibrinogenolytic activities of BjV *in vitro* and venom-induced hemostatic disturbances *in vivo*.

Funding: FAPESP, CNPq, CAPES, Fundação Butantan.

PB0793 | A Novel Therapeutic Approach for Acute Thrombo-hemorrhagic Disorders Evoked by Snake Venom: Potential of Rutin and Rutin Succinate

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Background: Snakebite envenomation - a tropical neglected disease with over 2 million accidents per year, which show a high rate of morbidity - is a type of acquired thrombo-hemorrhagic disease. In Brazil, bites from *Bothrops* snakes have a complex pathophysiology, inducing thrombocytopenia, consumption coagulopathy with hypofibrinogenemia, secondary hyperfibrinolysis, and bleedings in victims. The treatment with antivenom has limitations and the management of the local effects of the snakebite, as local hemorrhage and the inflammatory reaction, still presents a great challenge. On that account, we searched for potential new ancillary therapeutic compounds, as rutin, a safe natural flavonoid, easily available, inexpensive and with broad range of beneficial activities.

Aims: To verify the potential of rutin and its water-soluble form, rutin succinate, as a treatment for the hemostatic disorders and local hemorrhage induced by *Bothrops jararaca* snake venom (BjV).

Methods: All procedures involving animals were in accordance with IACUC. C57BL/6 mice were injected with rutin or rutin succinate (negative controls, i.v.), BjV (1.6 mg/kg b.w., s.c., positive control), or BjV followed by i.v. injection of rutin or rutin succinate (14.4 mg/kg b.w. i.v.) 10 min later. At 6, 24 or 48 h after envenomation, blood was collected and local hemorrhage was evaluated.

Results: Mice showed characteristic hemostatic disturbances of snake envenomation as thrombocytopenia, hypofibrinogenemia, and hemorrhage at the site of BjV injection. Treatment with rutin

or rutin succinate induced a faster recovery of fibrinogen levels at 24 h. Moreover, when administered as a treatment, rutin succinate decreased the local hemorrhage at 48 h.

Conclusions: Our results indicate that rutin and rutin succinate ameliorated local and systemic disturbances induced by the envenomation, showing thereby a great potential as ancillary treatments for snake envenomation and possibly to other thrombo-hemorrhagic diseases. Funding: FAPESP, CNPq, CAPES, Fundação Butantan.

PB0794 | Successful Treatment of a Rare Acquired Thrombin Inhibitor in a Patient with Hemodialysis-dependent End-stage Renal Disease

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Background: Autoantibodies against thrombin are extremely rare and, thus, the diagnosis and management are still challenging.

Aims: We demonstrate a case with major bleeding secondary to acquired thrombin inhibitor which had been treated by multimodal immunomodulation.

Methods: A 50-year-old Thai woman with hemodialysis-dependent end-stage renal disease (ESRD) spontaneously developed subcutaneous hematoma at the right arm, epistaxis and gross hematuria. The initial coagulation tests revealed prolonged activated partial thromboplastin time (aPTT) uncorrectable by mixing study, prolonged thrombin time (TT) and normal prothrombin time (PT). The factor VIII, IX, XI, X, V and prothrombin assays elicited normal or elevated levels (Table 1). Lupus anticoagulants were negative. The thrombin-based antithrombin (AT) activity assays (Berichrom® AT III (A), Siemens Healthineers) were used to evaluate thrombin inhibitor. Prothrombin complex concentrate (PCC), and immunomodulation by prednisolone 1 mg/kg/day, 4 weekly doses of intravenous vincristine 1 mg and plasmapheresis (PEX) using 1.5-plasma-volume fresh frozen plasma and/or human albumin were prescribed to control bleeding and inhibitor suppression, respectively. Subsequent hemodialysis sessions were heparin-free.

TABLE 1 Results of laboratory investigations performed at the diagnosis

Assays	Results	Range in normal controls
Activated partial thromboplastin time (aPTT, second)	39.9	22.93-29.59
Thrombin time (TT, second)	22.8	14.05-18.82
Reptilase time ratio	1.36	0.91-1.22
Clauss fibrinogen level (g/L)	6.72	1.7-4.0
Prothrombin level (IU/dl)	135.1	70-120
Factor VII coagulant activity (IU/dl)	262.7	60-140
Factor VIII coagulant activity (IU/dL)	280.9	50-150
Factor IX coagulant activity (IU/dl)	88.9	70-120
Factor X coagulant activity (IU/dl)	93	60-130

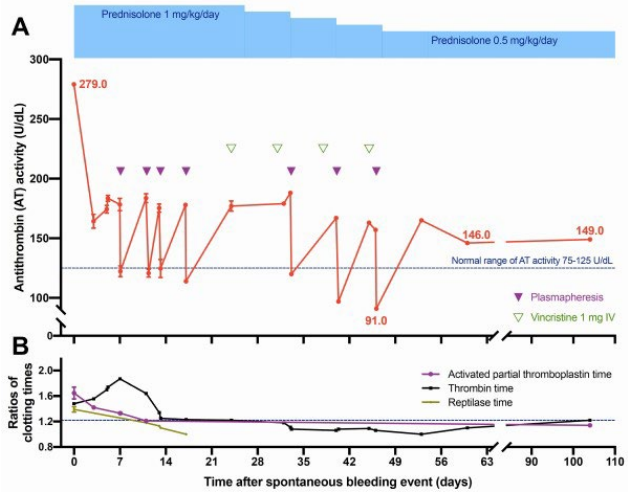

FIGURE 1 Antithrombin (AT) activities for monitoring thrombin inhibitor (A) and clotting time ratios (B). Each treatment modality is illustrated.

Results: The elevated AT activity of 279.0 U/dL at the diagnosis was suppressed to 171-186.4 U/dL during the first week after initiating prednisolone (Figure 1A). Since the abnormal bleeding was hardly controlled by PCC, PEXs could induce transient decreases in AT activities (median of difference -64.1 U/dL, range -70.0 to -50.8, P=0.02). By this integrated therapy, thrombin inhibitor monitored by AT activities was gradually declined within 8 weeks and stably maintained during the follow-up. During prednisolone tapering-off, PCC could be discontinued while clotting times were normalized (Figure 1B). On extensive investigations, there was no identifiable etiology of the inhibitor.

Conclusions: Antithrombin activity assays may be applied to diagnose and follow acquired thrombin inhibitor. In an ESRD setting, corticosteroids, vincristine, and PEX can be safely used for inhibitor suppression.

PB0795 | Acquired Hemophilia A in Young Nulliparous Woman: A Case Report

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Background: Acquired hemophilia A is a rare but potentially life-threatening bleeding disorder caused by auto-antibodies directed mostly against coagulation factor VIII (FVIII). Acquired hemophilia

A usually occurring older adults, and occasionally post-partum. The appearance of FVIII antibodies may be associated with several underlying conditions such as autoimmune diseases, diabetes, malignancies and use of medications; however, its cause is undetermined (idiopathic) in up to 50% of reported cases.

Aims: A new case of acquired hemophilia A in a young nulliparous woman is reported.

Methods: A 18-year-old female was admitted to department of hematology with complaints of one week history of both arm ecchymosis. Family and personal history of bleeding diathesis were negative. Coagulation profile showed prolonged activated partial thromboplastin time (aPTT) of 84.5 seconds (reference range 26 - 42 seconds), which was not corrected via mixing with normal plasma. Results of coagulation test showed that factor IX (91.1%) was normal and FVIII activity was markedly decreased (2.1%), and FVIII inhibitor level was 20.16 Bethesda units (BU). Lupus anticoagulant was negative. Complete blood count was follows: hemoglobin 11.1 g/dL, white cell count 6.28 x 10^9/L, platelet count 249 x 10^9/L.

Results: The patient was treated with prednisolone, and FVIII inhibitor was not detected after 7 days. Three month later, she had a good response and stopped the medication.

Conclusions: Acquired hemophilia A in young age is extremely rare and its outcome seems more favorable than in older adults because the inhibitors usually resolve more quickly and in a higher rate of patients. However, due to acquired hemophilia has a significant mortality, it is important to be prompt recognition and treatment.

PB0796 | Real World Experience on Use of Susoctocog-alfa (rpFVIII) in the Treatment of a Population of Italian Elderly Patients with Acquired Haemophilia A

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Background: Acquired haemophila A (AHA) is a rare coagulation disorder, more frequent in the elderly people, but often its management is difficult due to the concomitant presence of severe co-morbidities. Susoctocog-alfa could be an interesting option of treatment in these patients.

Aims: To assess the efficacy and safety of rpFVIII in the treatment of an elderly population of patients with AHA and concomitant severe co-morbidieties.

Methods: All data of elderly patients presenting AHA, and treated with susoctocog-alfa were retrospectively collected at five Italian Haemophilia Centres.

Results: Nine patients were retrospectively collected, 88.9% were males, mean age 78.7 years. 44.4% were idiopathic AHAs. Seven patients presented muscular bleeding, among these one patient also had haematuria. At diagnosis the remaining two patients presented respectively intracranial haemorrhage and haematuria with concomitant knee haemartrosis. 7/9 patients presented concomitant severe diseases, among these 71.4% were cardiovascular Susoctocog-alfa as first line therapy was used in 1/3 of cases. Median loading dose in both, first or second-line therapy, was 100.0 IU/kg; median subsequent doses were 50 IU/kg with a median frequency of 12 hrs and with a median number of doses of 8. After the loading dose the median FVIII peak was 166%. The rpFVIII-inhibitors were not tested before treatment with susoctocog-alfa, while only two patients developed a low-titre antibodies. Three surgeries were performed under susoctocog-alfa treatment, all without concomitant acute bleeding. rpFVIII was considered effective in all cases and was associated with antifibrinolytics in 22.2% of cases. Low-dose aPCC as short-term prophylaxis to prevent recurrences was started after acute treatment in 55.6% of patients. No adverse events were reported during treatment with this drug or during eight months of follow-up.

Conclusions: Our report showed that susoctocog-alfa may be an effective and safe option for patients with AHA, especially those presenting severe co-morbidities, also if used at a lower dosage than recommended.

DISSEMINATED INTRAVASCULAR COAGULATION

PB0798 | The ISTH DIC Score Predicts Outcome in Non-Septic Patients Admitted to a Cardiovascular Intensive Care Unit

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Background: The International Society of Thrombosis and Haemostasis (ISTH) disseminated intravascular coagulation (DIC) score is widely used to predict survival in critically ill - typically septic - patients.

Aims: To investigate whether the ISTH DIC score can be used to predict the 30-day mortality in non-septic patients in an intensive care unit (ICU).

Methods: In this single-center, prospective, observational study we included all patients ≥18 years of age who were admitted to a medical ICU with a focus on cardiovascular diseases between August 2012 and 2013. The DIC score was calculated at admission (DIC-0 h) and 72 hours thereafter (DIC-72 h) and classified as overt when ≥ 5.

Results: A total of 233 patients were included in this study (61% male; median age 65.9 years). Overt DIC-0 h and overt DIC-72 h were associated with a significantly higher 30-day mortality rate at admission (53.8% vs. 22.2% and 50.0% vs. 18.1%; respectively; p < 0.001). A sensitivity analysis including only non-septic patients demonstrated that overt DIC on admission was an independent predictor of mortality (HR: 2.54; 95%: 1.24-5.19; p=0.010).

Conclusions: This study suggests that the DIC score may be applied to non-septic ICU populations, and indicates that it is a useful tool for survival prediction, regardless of underlying disease.

PB0799 | A New Classification of Sepsis-Associated Disseminated Intravascular Coagulation (DIC) Based on Comprehensive Evaluation of Coagulation and Fibrinolysis

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Background: The functional dynamics of coagulation and fibrinolysis in patients with disseminated intravascular coagulation (DIC) vary extensively owing to various underlying diseases and severities. Conventional measurements of coagulation and fibrinolysis such as TAT, PIC, AT, FDP may not always reflect the precise pathophysiologic condition of DIC.

Aims: We aimed to establish novel classification of DIC with simultaneous assessment of both comprehensive coagulation and fibrinolysis dynamics in patients with sepsis-associated DIC.

Methods: Plasma samples obtained from 57 patients at the time of initial diagnosis with sepsis-associated DIC from September 2016 to July 2019 were evaluated. Comprehensive coagulation and fibrinolysis dynamics were quantitatively evaluated by clot-fibrinolysis waveform analysis (CFWA) and thrombin/plasmin generation assay (T/P-GA) (Nogami.K;Br J Haematol. 2019, Matsumoto T; Thromb. Haemost.2013). The results were expressed as the ratios relative to normal plasma.

Results: CFWA results demonstrated that the maximum coagulation velocity (|min1|) ratio underwent a modest increase to 1.30 ± 0.67 but the maximum fibrinolytic velocity (|FL-min1|) ratio decreased to 0.61 ± 0.33. T/P-GA results showed that the peak thrombin (Th-Peak) ratio moderately decreased to 0.72 ± 0.27, whereas the peak plasmin (Plm-Peak) ratio substantially decreased to 0.46 ± 0.35. |min1| and Th-Peak ratio correlated with one another (R=0.47, P< 0.001). |FL-min1| and Plm-Peak ratio strongly correlated (R=0.72, P< 0.001), suggesting that CFWA reflect thrombin plasmin generation. With |min1|) and |FL-min1| ratio DIC could be classified as follows: coagulation-predominant, coagulation/fibrinolysis-balanced, fibrinolysis-predominant, and consumption-impaired coagulation. Most patients (80.7%) in our cohort displayed coagulation-predominant disease.

Conclusions: A new classification of sepsis-associated DIC based on comprehensive assessment of coagulation and fibrinolysis dynamics would be greatly helpful for the diagnosis, hemostatic monitoring and decision of optimal treatment.

PB0800 | Coagulopathy in Pediatric Patients with Hemophagocytic Lymphohistiocytosis

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Background: Coagulation abnormalities, a well-known complication of hemophagocytic lymphohistiocytosis (HLH), occur in up to 2/3 of adult patients and are associated with increased mortality. Data is lacking in pediatric patients.

Aims: To describe the incidence and characteristics of coagulopathy in pediatric patients presenting with HLH.

Methods: Retrospective chart review was performed on patients 0-21 years of age meeting the required 5/8 HLH diagnostic criteria with evaluation of at least one coagulation parameter from 2006-2019. Coagulopathy was assessed from laboratory results and defined as international normalized ratio (INR)>1.5, ≥10% prolongation of partial thromboplastic time (PTT) from upper limit of normal (ULN), fibrinogen < 1 g, or D-dimer > 3xULN.

Results: 56 patients were identified, with a median age of 8 years [IQR 0-16, STD 7.5]. 95% of patients had thrombocytopenia. 84% of patients had laboratory evidence of coagulopathy. Fibrinogen was obtained in all patients while PT, PTT and D-dimer were less frequently measured (95%, 95% and 54% respectively). Of those evaluated, D-dimers were abnormal 97% of the time, PTT 74% of the time, fibrinogen 63%, and PT 57%. Bleeding and thrombotic complications were less commonly seen than laboratory evidence of coagulopathy (34% and 16% respectively). Mortality was 43%

and was associated with lower platelet count at presentation and nadir (p=0.04 and p=0.003 respectively), peak INR (p=0.01), fibrinogen at nadir (p=0.01), peak ISTH DIC score (p=0.03), major bleeding (p=0.03) and ferritin (p=0.03). Patients with laboratory evidence of coagulopathy and patients with major bleeding were significantly more likely to die than those without (OR 19.8, 95% CI 1.09-359.87, p=0.04, OR 4.2, 95% CI 1.1-15.95, p=0.04 respectively).

Conclusions: Coagulopathy is seen in the majority of pediatric patients with HLH and is associated with increased mortality. Coagulation parameters should be fully assessed in these patients and may help inform prognosis.

PB0802 | A Multicenter Prospective Validation Study on Disseminated Intravascular Coagulation in Trauma-Induced Coagulopathy

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Background: Trauma-induced coagulopathy (TIC) progresses to disseminated intravascular coagulation (DIC), as consequences of dysregulated inflammatory and coagulofibrinolytic responses to the trauma.

Aims: We hypothesized that both DIC and TIC patients elicit same coagulofibrinolytic changes characterized by pathomechanisms of DIC, leading to massive transfusion.

Methods: Severely injured trauma patients with injury severity score≥16 were prospectively included and 276 patients were eligible for the analyses. Platelet counts, global markers of coagulation and fibrinolysis as well as specific markers of thrombin and plasmin generation, anticoagulation, endothelial injury, and inhibition of fibrinolysis were measured immediately at the presentation to the emergency department (0 h) and 3 h later the arrival. The patients were subdivided into those with and without DIC or those with and without TIC using 0 h data. Time courses of these markers and association of various variables with development of DIC and prevalence of massive transfusion were evaluated.

Results: Severity of injury and thrombin generation were associated with the development of DIC. DIC patients showed increased thrombin generation, insufficient anticoagulation controls, endothelial injury, increased fibrinolysis followed by elevation of plasminogen activator inhibitor-1, leading to consumption coagulopathy. These changes were associated with critical bleeding, resulting in more massive transfusion and poorer outcome than non-DIC patients. Same changes in measured specific markers both at 0 h and 3 h were observed in TIC patient.

Conclusions: DIC and TIC evoked same coagulofibrinolytic responses in severely injured trauma patients immediately after trauma and were associated with massive transfusion.

TABLE 1 Laboratory Assessment of Coagulopathy

	Platelets <150,000	Fibrinogen>1 g	INR ≥1.5	PTT prolongation >10%	D-dimer elevation
Measured	56	56	53	53	30
Presenting	45 (80%)	11 (20%)	18 (34%)	28 (53%)	28 (93%)
Developed	8 (14%)	24 (43%)	12 (23%)	11 (21%)	1 (3%)
Total	53 (95%)	35 (63%)	30 (57%)	39 (74%)	29 (97%)

TABLE 2 Coagulation parameters of surviving and deceased patients

	Surviving (n=32)	Decreased (n=24)	P-value
Platelets (mean, presentation)	128,344	72,833	0.04
Platelets (mean, nadir)	70,188	19,479	0.003
INR (mean, peak)	1.74	2.64	0.01
Fibrinogen (mean, nadir)	151.3	91.4	0.01
ISTH DIC score (mean, peak)	6.00	7.31	0.03
Thrombosis (%)	12.5% (4/32)	21% (5/24)	0.40
All Bleeding (%)	25% (8/32)	46% (11/24)	0.10
Major bleeding (%)	12.5% (4/32)	37.5% (9/24)	0.03
Ferritin	8,349	22,924	0.03

PB0803 | Hypofibrinogenemia Is a High Risk for Infectious Type of Disseminated Intravascular Coagulation

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Background: Disseminated intravascular coagulation (DIC) is classified into hematopoietic injury, infectious or basic types in the Japanese Society of Thrombosis Hemostasis.

Aims: We examined the relationship between hypofibrinogenemia and pathophysiology in infectious DIC.

Methods: We analyzed 3663 patients who were treated with thrombomodulin-α (TM-α) as a post-marketing survey for TM-α in DIC. Patients with fibrinogen < 1.5 g/L were considered to have a hypofibrinogenemia.

Results: Hypofibrinogenemia was observed in 10.3% in infectious type of DIC, 27.8% in hematopoietic injury type of DIC and 25.7% in basic type of DIC. The frequency of both bleeding and organ failure symptoms, and the scores of organ failure or DIC diagnostic criteria were significantly higher in infectious type of DIC patients with hypofibrinogemia than in those without hypofibrinogemia, suggesting that a hypofibrinogenemia in infectious type DIC is more progressive and severe DIC.

Although a 28-days survival and resolution rate of DIC were significantly lower in only infectious type DIC with hypofibrinogenemia than those without, there were no significant difference in a 28-days survival and resolution rate of DIC between both groups of hematopoietic and basic type DIC. The mechanism of hypofibrinogenemia may be different in infectious type DIC from hematopoietic injury type DIC.

Conclusions: The hypofibrinogemia in infectious type of DIC is progressive and severe DIC and is frequently associate with bleeding and multiple organ failure, suggesting that the monitoring the fibrinogen level is important for management of infectious type of DIC.

HEMOPHILIA – BASIC

PB0804 | Exploring the Relationship between Condition Severity and Health-Related Quality of Life in Haemophilia A

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Background: Haemophilia A (HA; factor VIII deficiency) is a chronic bleeding disorder characterised by prolonged trauma-related or spontaneous bleeding events into the joint space and muscle tissue. Being present from birth, condition severity is classified according to endogenous factor VIII activity, namely mild (5-40%), moderate (1-5%) and severe (< 1%). There is little comparative research exploring health-related quality of life (HRQoL) across condition severity.

Aims: To examine the relationship between HA severity and HRQoL, using EQ-5D index scores and EQ VAS (visual analogue scale) scores for non-inhibitor adult patients studied in 'Cost of Haemophilia in Europe: a Socioeconomic Survey - II' (CHESS II) study.

Methods: CHESS II is a retrospective burden-of-illness study in adults with mild, moderate, and severe HA or haemophilia B (factor IX deficiency) across France, Germany, Italy, Spain, UK, Romania, Netherlands, and Denmark. Data from the EQ-5D-5L were pooled using the UK-specific EuroQoL value set to allow for comparison across patients from multiple countries. Results are presented as means (± 95% confidence intervals [CIs]).

Results: Of participants enrolled in CHESS II (n=787), 258 (33%) and 249 (31%) adults with HA without active inhibitors provided responses to the EQ-5D-5L questionnaire and EQ VAS, respectively. Data for the overall cohort and by condition severity are presented in Table 1.

Conclusions: This research suggests that individuals with more severe forms of HA report lower HRQoL for both EQ-5D-5L index

TABLE 1 EQ-5D-5L and EQ VAS results by condition severity

	Mild	Moderate	Severe	Total
EQ5D-5L, sample (n)	42	72	144	258
Mean	0.87	0.75	0.69	0.74
CI lower (95%)	0.82	0.71	0.65	0.71
CI upper (95%)	0.92	0.79	0.73	0.77
Visual analogue scale (VAS), sample (n)	42	72	135	249
Mean	80.74	76.38	65.48	71.20
CI lower (95%)	76.64	72.96	62.33	69.04
CI upper (95%)	84.84	79.79	69.01	73.62

score and EQ VAS. Consequently, there appears to be a greater unmet need for people with severe HA compared to less severe forms of HA, which may be addressed if care provision can manage FVIII levels to the extent that clinical manifestations are reduced.

PB0805 | The Role of Genetic and Environmental Factors in Development of Inhibitor in Hemophilia A

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Background: According to previous studies, type of mutation which occurs in Factor VIII gene correlates with the production of inhibitory antibodies.[6] Inhibitors are IgG antibodies which neutralize coagulation factors.[7] Presence of inhibitors has been reported in 8-30% of cases with hemophilia as one of the most complicating health problems in those patients.
Aims: This study is aimed to evaluate the role of environmental and genetic factors in the development of Factor VIII (FVIII) inhibitory antibodies in hemophilia A patients.
Methods: 62 patients with hemophilia A were evaluated for the presence of inhibitor. Then 20 persons were randomly evaluated for BclI/intron18 polymorphism of FVIII gene by RFLP PCR. FVIII deficiency levels were determined.
Results: Digested fragment of BclI gene product (99 bp fragment) was observed in 11 patients. We did not find any significant relation between the development of FVIII inhibitor and onset age, ethnicity, family history of having inhibitors and severity of the disease. BclI/intron 18 polymorphism was associated with both inhibitor level and hemophilia type.
Conclusions: Development of FVIII inhibitor did not show any significant relationship with genetic and environmental factors in studied population. But patients with high level of inhibitor had a cut point in BclI/intron 18.
BclI/intron 18 gene polymorphism can be considered as a genetic marker for developing FVIII inhibitor in the future studies.

PB0807 | Effect of the First Factor VIII Infusions on Immunological Biomarkers in Children with Hemophilia A: Results from the HEMFIL Study

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Background: Inhibitors are the most serious complication in patients with haemophilia A (HA). Studies targeting the initial immune response during the first FVIII exposures are still lacking.
Aims: To investigate the effect of the first FVIII infusions on immunological biomarkers of children with congenital HA.
Methods: Children with severe HA (FVIII < 1%) were enrolled at the time of diagnosis. Plasma samples were collected at enrolment and during treatment until 75 exposure days (ED) or inhibitor development. Samples were divided into three groups, considering the ED to FVIII at blood collection: before any exposure (T0); 1-35 ED; and >35 ED. Specific anti-FVIII antibodies (IgM,IgG1,IgG3,IgG4), chemokines (CCL2,CCL5,CXCL8,CXCL9,CXCL10) and cytokines (IL-2,IL-4,IL-6,IL-10,IFN-gamma,TNF,IL-17) were assessed.
Results: Currently, 70 children with severe HA, median age 10.0 months (IQR,6.0-14.0), have been included. Inhibitor was detected in 27 (38.6%) patients of whom 17 were high-titre. The median time for inhibitor development was 13.0 ED (IQR,8.5-18.0). All included patients were treated with Advate®(Shire,USA). Patients with inhibitors presented increased levels of specific anti-FVIII IgG4 in 1-35 ED stratum in comparison with T0 ($p< 0.01$). At 1-35 ED, plasma concentration of IL-6
($p< 0.01$) and CXCL8 ($p=0.02$) were higher in comparison with patients who did not develop inhibitors. In counterpart, at 1-35 ED, patients who did not develop inhibitors presented a mixed cytokine profile and higher plasma levels of CXCL9 ($p< 0.01$) and CXL10 ($p< 0.01$). Patients with inhibitors presented low number of chemokine/cytokine correlation at T0. However, a rearrangement of connections occurs in the first 35 ED to FVIII, leading to a more intense cross-talking between cytokines and chemokines ($r>0.68$).
Conclusions: Exposure to FVIII seems to trigger a pro-inflammatory response mediated by IL-6 and CXCL8 in patients with HA at inhibitors development. Furthermore, regardless of inhibitor status, the immune system of all patients with HA was modulated after repeated infusions of FVIII.

PB0808 | Small RNAs Influence the Pathophysiology of Hemophilic Synovitis and Arthropathy

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Background: The molecular biomarkers and signaling pathways that contribute to the development of hemophilic arthropathy is not well understood.

Aims: To study the pattern of microRNA(miR) expression from the onset of bleeding to the development of arthropathy, in a murine model of hemophilia A and to further correlate their association with joint disease.

Methods: We characterized a murine model of acute and chronic hemarthrosis(n=3-8 animals per group) by histological analysis and micro-computed tomography(CT). Joint specific RNA from the target joints(n=11 animals per group) were then profiled for small RNA expression by an Illumina HiSeq2500 analysis. A comprehensive bioinformatic analysis was performed, to identify microRNAs associated with acute/chronic hemarthrosis. A limited validation was performed by immunofluorescence detection of Collagen I(COL1A1) expression in the affected joints.

Results: Several microRNAs(~50-105) were dysregulated in the hemarthrosis models. In the acute hemarthrosis model, microRNA that regulate inflammatory mediators such as TLR2/4 and MYD88 dependent activation of macrophages (miR-125a-5p,+12.8-fold) and NF-κB and TNF-α mediated inflammatory cascade (miR-486b-5p, -14.8-fold) were significantly elevated. In the chronic hemarthrosis model, pathologically relevant microRNAs that modulate TGF-β and SMAD signaling (miR-29b-2-5p,+9.3-fold; miR-486a-5p,-0.1-fold), VEGF signaling (miR-34b-5p, +6.3-fold) and Semaphorins such as SEMA3A (miR-223-5p,-0.5-fold) were discovered. A limited validation of one of the cellular targets of miR-218-5p (-1-fold) through immunofluorescence analysis showed a markedly higher expression of collagen-1, in the hypertrophied synovium of the arthritic joint. Such elevated levels of type I collagen may mark the onset of fibrosis in the affected joint and lead to bone remodeling, a phenomenon confirmed by our micro-CT analysis.

Conclusions: This is the first study to comprehensively document the role of microRNAs in hemophilic arthropathy. Our data has also revealed a disease stage-specific microRNA expression, that highlights their potential as therapeutic targets to attenuate the development of joint disease.

PB0809 | Self-Adjustment of Prescribed Dosing Regimens and Physical Activity by Persons with Hemophilia (PwH): Findings from the HemACTIVE Patient Survey

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Background: Prophylaxis with replacement factor is the gold standard for PwH. Adherence to prescribed regimens is low (30%-73%), including missed doses and self-adjustment of dosing regimens. It has been suggested that the desire for independence and freedom from restrictions may drive adherence behavior. As such, children and elderly who depend on others for medical care tend to have better adherence.

Aims: The HemACTIVE patient survey was used to evaluate the relationship between self-adjustment of physical activities vs. self-adjustment of dosing regimens.

Methods: Persons with moderate/severe hemophilia A from the EU, US, and Canada, aged 2-65 years (caregivers for PwH < 18y), were given a 25-minute, IRB-approved, web-based questionnaire.

Results: 275 PwH were enrolled (29% children [< 18y], 29% younger adults [18-30y], 41% older adults [≥31y]; 61% severe hemophilia). Most PwH (77%) reported adjusting activities due to hemophilia, on average 2 days/week. Children adjusted their activities less frequently (63%, 1 day/week) than young adults (75%, 2 days/week) and older adults (90%, 3 days/week). Despite frequent adjustment of activities, complete adherence to prescribed regimens was low, reported by only 46% (of 206 responses). Most PwH reported occasionally or frequently missing doses (41%) or adjustment of dosing schedules (ie, adding infusions [19%] or infusing on different schedule [7%]). Children and older adults were more adherent (~50%) than younger adults (35%). Although most attributed their nonaherence to forgetfulness (47%), many reported managing their treatment around activities (39%), based on need (33%), due to time restrictions (26%), or belief that treatment was not necessary (22%).

Conclusions: PwH frequently adjust their dosing schedules, either infusing on a different schedule or adding more infusions to prescribed regimens. Reasons given for nonadherence suggest that as PwH age into adulthood, they increasingly desire autonomy in their lives and wish for a lifestyle that does not revolve around treatment.

PB0810 | Seeking New Approaches: Aberrant Clots, Fibrin Degradation Products and their Effects on Clot Stability

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Background: In laboratory models fibrin and fibrin degradation products (FDP) demonstrate anti-thrombin effects and enhanced plasminogen activation, suggesting a regulatory role. Haemophilia is characterised by abnormal thrombin generation (TG) producing structurally aberrant clots, of loosely-packed fibres. Fibrinolytic mediators infiltrate porous clots reducing clot stability. To counterbalance, we hypothesised aberrant fibrin and resultant FDP have reduced capacity to bind thrombin and activate plasminogen, thereby promoting clot stability.

Aims: This laboratory study aimed to compare effects of 'normal' FDP versus 'haemophilia' FDP on TG and clot stability.

Methods: Clots were formed in columns containing fibrinogen. 'Normal' fibrin was generated with 2 nM thrombin, 'haemophilia' fibrin with 0.5 nM. Clots were lysed with plasmin (2 nM) and perfusate treated with VFK-chloromethyl-ketone (plasmin inhibitor) and FPR-chloromethyl-ketone (thrombin inhibitor). FDP were concentrated and separated. Six aliquots of each FDP were generated. Normal plasma was spiked with dilutions of FDP and TG initiated with tissue factor (TF,1pM), fluorogenic substrate plus calcium. Fluorescence was converted to thrombin concentration (Thrombinoscope software). Area under the elasticity curve (AUEC) assessed clot stability by thromboelastometry, using TF (1:17000 dilution) plus tissue plasminogen activator (t-Pa, 2nmolar). Normal plasma was spiked with pooled 'normal' versus 'haemophilia' FDP.

Results: Both 'normal' and 'haemophilia' FDP's tended to reduce total thrombin (ETP) and prolong lagtime (figure 1). Reduction in ETP was more pronounced and reached significance with 'haemophilia' FDP ($p < 0.005$ Kruskal Wallis ANOVA, control vs undiluted FDP, Dunn's multiple comparison). Effects of both 'normal' and 'haemophilia' FDP on lagtime was significant ($p < 0.05$ Kruskal Wallis ANOVA). AUEC was reduced in plasma spiked with 'haemophilia' FDP compared to control (table 1).

Conclusions: Contrary to the hypothesis 'haemophilia' FDP had an inhibitory effect on TG and clot stability in this laboratory model. We plan to characterise the FDP profile and mechanisms underlying this phenomenon. Modulating FDP activity may represent a novel therapeutic target.

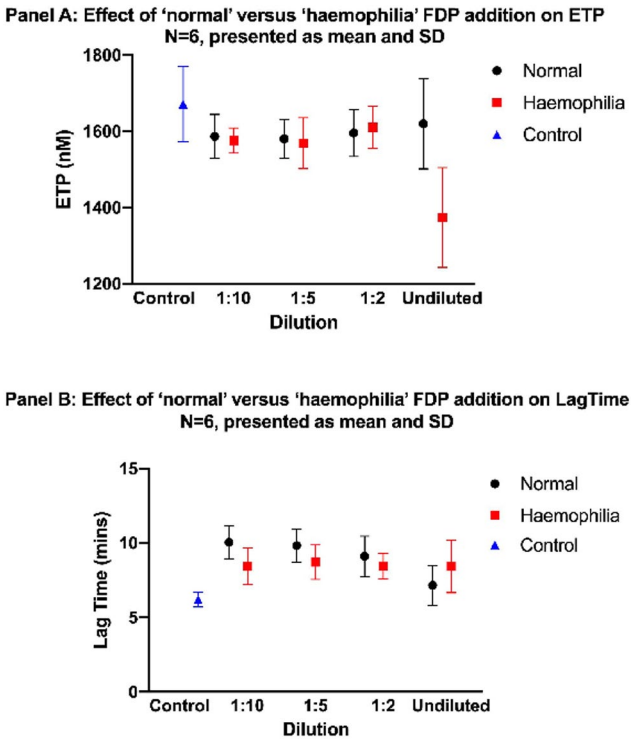

FIGURE 1 Thrombin generation data

PB0811 | X Chromosome Inactivation: A Modifier of Factor VIII and IX Plasma Levels in Haemophilia Carriers

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Background: Haemophilia A and B are X-linked haemorrhagic disorders caused by mutations in *F8* and *F9* genes. Due to recessive inheritance, males are affected, while female carriers are usually asymptomatic and express about half of normal factor VIII and IX activity. The phenotypic expression of the disease in female carriers is extremely variable. This variability might be attributable to several

TABLE 1 TF/t-Pa ROTEM parameters for plasma spiked with FDP versus control. Data presented as mean

N=2	Clotting Time (Seconds)	Alpha angle (Degrees)	MCF (mm)	AUEC *60*mm
Control	209	54.5	24	2376
Normal FDP	485.5	40	26	2597.5
Haemophilia	902	27	19	1929.5

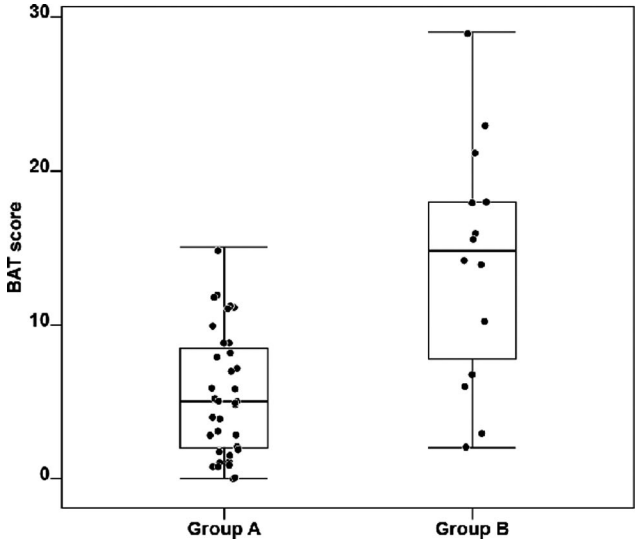

FIGURE 1 Comparison of BAT score between two carriers groups. A: factor level >20 IU/dL and XIC<80:20. B: factor level ≤20 IU/dL and XIC≥80:20

genetic mechanisms, including skewed X chromosome inactivation (XCI).

Aims: To investigate whether the low activity of FVIII or FIX (≤50 IU/dL) could be related to XCI in haemophilia carriers with bleeding symptoms.

Methods: XCI status was determined by indirect DNA-based methylation analysis (HUMARA assay). Bleeding tendency was evaluated using the standardized ISTH bleeding assessment tools (BAT score). BAT score ≥6 has been considered abnormal in adult females.

Results: The HUMARA assay was tested in 73 symptomatic female carriers of haemophilia A and B with a clotting activity of FVIII or FIX ≤50 IU/dL. Approximately 20% of haemophilia carriers showed an extremely skewed XCI (≥90:10), while 33% showed a skewed pattern of ≥80:20.

Data on bleeding manifestations were available for 65 haemophilia carriers.

A statistical association between XCI pattern and FVIII and FIX levels was observed, with a value of Person's r=-0.60. Especially the group of female carriers with very low coagulant activity (≤20 IU/dL) and high XCI pattern (≥80:20) showed a BAT score of 15 (IQR: 8-18), while female carriers with factor level >20 IU/dL and XCI pattern < 80:20 had a BAT score of 5 (IQR: 2-8) (Figure).

Conclusions: Our study demonstrates that XCI could have a great influence on the phenotype and expression of X-linked diseases, but random XCI not fully negate bleeding tendency in haemophilia carriers, underling the complexity of female carriers phenotype.

PB0812 | Impact of APTT Reagents on Factor VIII Assay in Plasma Spiked with a Pegylated Recombinant FVIII: A French Multicentre Study

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Background: Measurement of FVIII may be achieved by one-stage clotting (OSA) or chromogenic assay (CSA), which is the reference method for the EMA. However discrepancies exist when enhanced half-life FVIII products are assayed with different OSA and CSA. In this regard, different results have been published with full-length PEGylated rFVIII (rurioctogog alfa pegol, Adynovi®).

Aims: To compare OSA with CSA using different aPTT activators for the measurement of FVIII activity in plasma samples spiked with Adynovi® or a full-length rFVIIII (octocog alfa, Advate®).

Methods: FVIII-deficient plasma samples spiked with Adynovi® or Advate® at 1, 0.50, 0.25, 0.05 and 0.025 IU.mL^{-1} were analyzed by 11 French laboratories. FVIII activities were measured using CSA (Biophen FVIII:C) and OSA with different aPTT reagents. Calibration curves were performed with SSCLOT4 standard. Measurements were repeated on 3 consecutive days and the following aPTT reagents were tested: CK.Prest® (n= 5); SynthASil® (n =6); SynthAFax® (n = 3), Actin FS® (n = 4).

Results: Final target values were median FVIII levels measured by CSA in the 11 centers. Median recoveries according to aPTT reagents and FVIII products are presented in the table. When FVIII levels were above 0.25 IU.mL^{-1}, the recovery rates obtained were similar whatever the aPTT reagent used, ranging from 0.67 to 0.81 with Adynovi®, and from 0.72 to 0.84 with Advate®. When FVIII levels were close to or below 0.05 IU.mL^{-1}, the recovery rate increased and widely varied from one reagent to another. It was close to 1 with CK Prest, SynthASil and SynthAFax but higher with Actin FS, i.e. between 1.29 and 1.82.

Conclusions: Our data validate the use of CK Prest, SynthASil and SynthAFax for for measurement of ADYNOVI® and ADVATE® by

TABLE 1 Measurements of Adynovi (median values)

ADYNOVI®	*n*	1 IU.ml^{-1}	0.5 IU.ml^{-1}	0.25 IU.ml^{-1}	0.05 IU.ml^{-1}	0.025 IU.ml^{-1}
CSA IU.ml^{-1} (SD)	33	1.05 (9.5)	0.56 (7.3)	0.29 (2.8)	0.056 (0.71)	0.03 (0.59)
Recovery CK Prest (SD)	15	0.79 (0.18)	0.77 (0.03)	0.79 (0.04)	1.07 (0.13)	1.17 (0.23)
Recovery Actin FS (SD)	12	0.71 (0.04)	0.77 (0.09)	0.81 (0.16)	1.29 (1.29)	1.75 (1.16)
Recovery SynthASil (SD)	18	0.75 (0.03)	0.71 (0.08)	0.69 (0.09)	0.93 (0.19)	0.90 (0.27)
Recovery SynthAFax (SD)	9	0.67 (0.06)	0.67 (0.06)	0.67 (0.06)	0.90 (0.11)	0.90 (0.16)

TABLE 2 Measurements of Advate ® (median values)

ADVATE®	*n*	1 IU.ml^{-1}	0.5 IU.ml^{-1}	0.25 IU.ml^{-1}	0.05 IU.ml^{-1}	0.025 IU.ml^{-1}
CSA IU.ml^{-1} (SD)	33	1.13 (9.1)	0.60 (7.3)	0.32 (2.8)	0.055 (0.71)	0.028 (0.59)
Recovery CK Prest (SD)	15	0.083 (0.06)	0.82 (0.05)	0.84 (0.04)	1.09 (0.12)	1.18 (0.30)
Recovery Actin FS (SD)	12	0.72 (0.06)	0.78 (0.07)	0.76 (0.15)	1.39 (0.84)	1.82 (1.32)
Recovery SynthASil (SD)	18	0.81 (0.08)	0.75 (0.97)	0.72 (0.09)	0.96 (0.22)	1.0 (0.29)
Recovery SynthAFax (SD)	9	0.79 (0.04)	0.79 (0.05)	0.82 (0.06)	1.10 (0.12)	1.12 (0.20)

OSA, but not of Actin FS, which overestimates low FVIII levels with both products.

PB0813 | Exploring the Relationship between Common Complications of Haemophilia and Anxiety and Depression in People with Haemophilia A

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Background: Haemophilia A (HA) is a rare congenital bleeding disorder that occurs in people with a limited amount of clotting factor VIII. HA is characterised by prolonged trauma-related or spontaneous bleeding events. Over time, these events may lead to joint deterioration and chronic pain. Currently, there is limited research on the relationship between common complications of haemophilia and incidence of anxiety and depression in people with HA (PWHA).

Aims: To explore the relationship between bleeding events, joint damage, and chronic pain among PWHA with and without a diagnosis of anxiety and/or depression using data from the 'Cost of Haemophilia in Europe: a Socioeconomic Survey - II' (CHESS II) study.

Methods: CHESS II is a retrospective burden-of-illness study in adult patients with mild, moderate, and severe HA and haemophilia B across France, Germany, Italy, Spain, UK, Romania, Netherlands, and Denmark. Data on bleeding events (annualised bleeding rate [ABR]), joint damage (problem joints [PJs]; a new term which reflects long-term morbidity by considering patient-centric factors such as joint-pain and functional-mobility), chronic pain (moderate or severe pain), and prior clinical diagnoses of anxiety and/or depression, were reported by haematologists.

Results: A total of 601 adult PWHA without active inhibitors were profiled, including n=127 (21.1%) with reported anxiety and/or depression, and n=474 (78.9%) without anxiety and/or depression. Mean ABR, mean number of PJs, and number (%) of people reporting moderate or severe chronic pain are presented in Table 1.

TABLE 1 Results

Outcomes	With anxiety and/or depression (n=127)	Without anxiety and/or depression (n=474)
Mean ABR (95% confidence interval [CI])	4.12 (3.56-4.68)	3.09 (2.36-3.83)
Mean number of problem joints (95% CI)	1.04 (0.84-1.24)	0.49 (0.41-0.57)
% with moderate or severe chronic pain, n (%)	54 (42.52%)	112 (23.63%)

Conclusions: This analysis suggests there is an association between common complications of haemophilia and clinically diagnosed anxiety and/or depression. However, further research is needed to explore a broader array of patient characteristics that could influence such diagnoses.

PB0815 | Contributions of the Individual Components of an Activated Prothrombin Complex Concentrate to Thrombin Generation in the Presence of Emicizumab

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Background: While emicizumab has proven remarkably effective as a prophylactic treatment for hemophilia A, some patients still experience bleeding. One treatment for bleeding in patients with inhibitors is an activated prothrombin complex concentrate (APCC; FEIBA) (disfavored in NHF MASAC #255). APCC contains among other things prothrombin, factor X (FX), and factor IX (FIX), as well as activated factors FIXa and FXa. Previous studies have shown that addition of APCCs to hemophilia A plasma containing emicizumab gives a significant increase in thrombin generation [J Thromb Haemost 2018;16:1580-1591].

Aims: The goal of this study was to assess the contribution of individual components of APCC to increased thrombin generation.

Methods: Thrombin generation assays were done in hemophilia A plasma using 1 pM tissue factor, 100 μM lipid, and 420 μM Z-Gly-Gly-Arg-AMC with or without 55 μg/mL emicizumab (clinical steady state levels). To mimic the expected APCC increases in plasma concentration at a dose of 50 U/kg, added: prothrombin 1800 nM; FX 130 nM; FIX 90 nM; FIXa 0.4 nM.

Results: Under these conditions, hemophilia A plasma alone had essentially no thrombin generation. Addition of emicizumab enhanced thrombin generation. Further addition of prothrombin, FIX, and FX gave a small increase in thrombin generation. Addition of FIXa to emicizumab significantly increased thrombin generation (Figure 1). Addition of prothrombin to FIXa and emicizumab increased thrombin generation and increased the peak level of thrombin; none of the other zymogen components significantly altered thrombin generation (Figure 2).

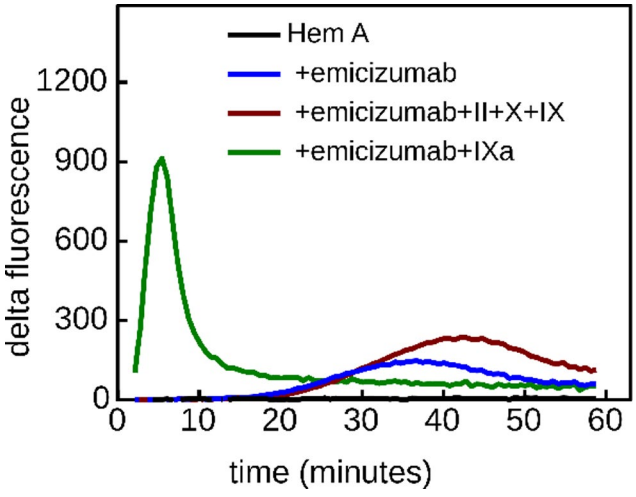

FIGURE 1 Effect of factor IXa on thrombin generation in Hemophilia A plasma with emicizumab

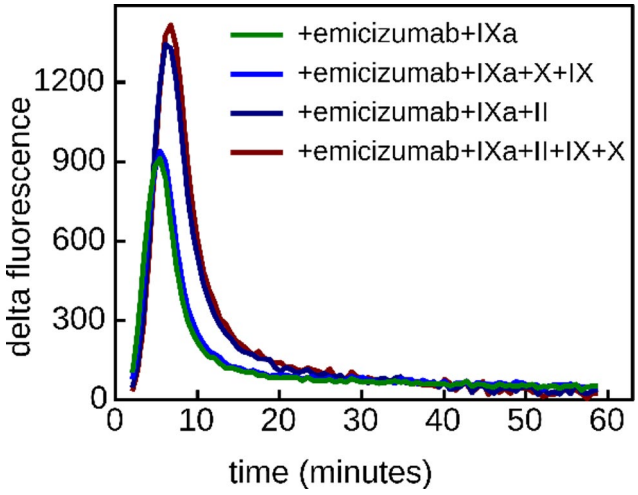

FIGURE 2 Added effect of prothrombin on thrombin generation in Hemophilia A plasma with emicizumab and factor IXa

Conclusions: A previous study of the individual components of APCC in the absence of emicizumab concluded that a combination of FXa and prothrombin had the major effect on thrombin generation [Haemophilia. 2016;22:615-24] and that FIXa did not increase thrombin generation. Surprisingly, given the weak solution affinity of emicizumab for FIXa, with emicizumab, the FIXa provided by APCCs is the most significant contributor to thrombin generation.

PB0816 | Next Generation Sequencing Antibody Repertoire Analysis of Inhibitory Immune Response in Hemophilia A

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Background: Inhibitory antibodies to factor VIII (FVIII) represent a serious complication in hemophilia A (HA) treatment. The development of anti-FVIII antibodies is poorly understood. Serological studies have associated inhibition with antibodies of IgG and IgD classes, and highly inhibiting antibodies with the IgG4 subclass. To our knowledge, B-cell receptor repertoires from patients with HA have not previously been reported.

Aims: To identify antibody repertoire signatures associated with inhibitor development in previously untreated patients with severe HA.

Methods: Whole-blood mRNA was prospectively collected prior to FVIII treatment and every 3-4 exposure days (ED) until inhibitor formation or 20ED. Patients received a single recombinant

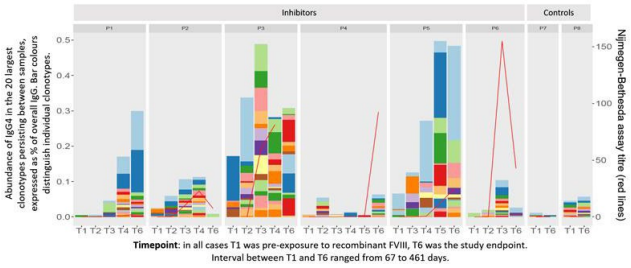

FIGURE 1 Abundance of IgG4 in IgG4-rich clones found in each patient, plotted against inhibition titre

FVIII concentrate simoctocog alfa (ClinicalTrials.gov identifier: NCT01712438). Heavy-chain B-cell repertoires were sequenced from 8 patients (inhibitors=6, controls=2) using Illumina MiSeq, with IgG read coverage including a short length of the CH1 region to determine the isotypic subtype.

Results: Inhibitor formation (n=6) occurred at a median of 7.5ED, with peak titre of 66.1BU (4.3-154.8BU). Repertoire sequencing was performed on up to 6 samples/patient. Antibody responses in all patients were polyclonal and no standout clonal expansions that correlated with inhibition could be identified. We did not identify overall correlations of inhibition with levels of specific IgG subtypes, or with the level of IgD, although patients developing inhibition generally developed higher levels of IgG4 than controls. There were also no strong phylogenetic signatures associated with inhibition. The results suggest that the response to inhibition is diverse rather than stereotyped.

Conclusions: Although we have shown that samples for B-cell repertoire sequencing can be conveniently collected during routine clinical visits, the technique on its own has not identified specific antibody signatures, confirming the complex nature of inhibitor formation. In future work we aim to identify FVIII-specific clonotypes explicitly using additional methods, and track their evolution using the rich information provided by repertoire sequencing.

PB0817 | Examining the Hemophilia Disability Paradox

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Background: In economic evaluations, health-state-utility-valuations (HSUV) for quality-of-life (QoL) estimates inform the cost-effectiveness of new interventions. HSUVs are estimated by eliciting the preferences of people for different health-states. However, people with inherited and long-term health conditions, such as hemophilia, may adapt to their given health-state. This "disability paradox" could mean that QoL estimates for the same health-state derived from people with hemophilia (PWH) may be valued higher compared to the general population (GP).

Aims: This study aimed to explore differences in preferences for the same health-states elicited from PWH and GP and whether there were differences in the preferences elicited across hemophilia severity.

Methods: A discrete choice experiment (DCE_{TTO}) was completed by 283 PWH and 1,900 GP to derive HSUVs between July-to-September 2019 in the USA. Participants indicated their preferences for hypothetical EQ-5D-5L health-states with a duration attribute. A total of 120 DCE_{TTO} choice tasks were administered online, with participants completing 15. Dominated and repeated scenarios were included to test for inconsistencies in responses. Conditional-logit regressions were used with random sampling of GP to match PWH for a balanced comparison. Model estimates were compared and QoL-weights developed to adjust for differences in preferences.

Results: Responses were collected from 1,327 people (1,150 GP, 177 PWH including 104 Severe, 40 Moderate, 33 Mild) after removal of respondents failing consistency-checks. Sampling for age and gender (male-only), mean HSUV difference between PWH and GP was 0.17. By disease severity, mean HSUV differences between severe PWH and moderate PWH with GP were 0.13 and 0.17, respectively. Limited sample size precluded analysis for mild PWH.

Conclusions: These findings demonstrate the presence of disability paradox in hemophilia. QoL estimates for the same health-states derived from PWH may be valued higher compared to GP, indicating under-estimation of the impact of hemophilia.

Disclaimer: HCD Economics were funded by uniQure to undertake this research.

PB0819 | Concizumab Does Not Affect Activated Partial Thromboplastin Time, Prothrombin Time or Factor VIII/IX Activity Measured Using One-Stage Clotting or Chromogenic Substrate Assays

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Background: Concizumab is a humanised, monoclonal antibody in phase 3 clinical development as a once-daily, subcutaneous prophylaxis for patients with haemophilia A and B with or without inhibitors. Concizumab specifically targets the Kunitz-2 domain of tissue factor pathway inhibitor (TFPI), blocking factor Xa binding to TFPI, thereby preventing tissue factor-FVIIa and FXa inhibition. During treatment of patients with haemophilia, physicians may need to measure haemostasic potential using activated partial thromboplastin time (aPTT), prothrombin time (PT) or FVIII or FIX activity assays using an aPTT-based one-stage clotting (OSC) or a chromogenic

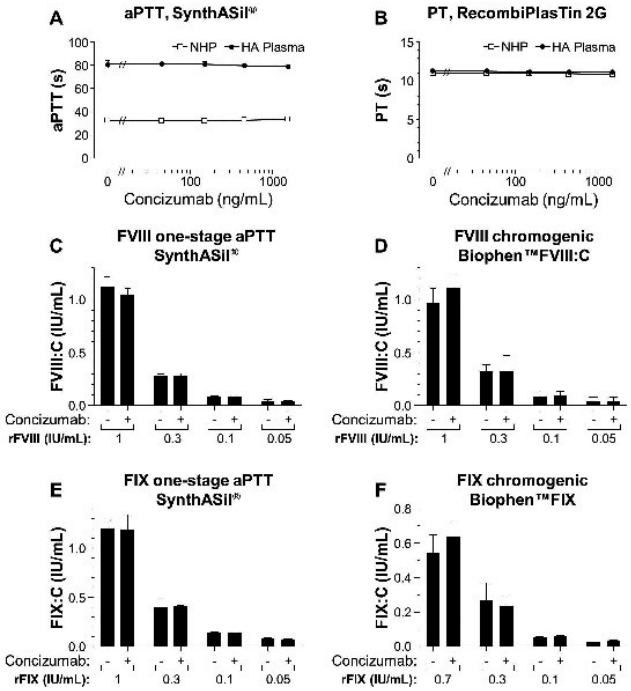

FIGURE 1 A-B: Measurement of aPTT and PT. C-F: Factor measurement in severe haemophilia A or B plasma spiked with or without concizumab (200 ng/mL)

substrate (CS) assay. Thus, knowledge on whether concizumab affects these assays is imperative for optimal patient care.

Aims: To evaluate whether the presence of concizumab influences aPTT, PT, FVIII or FIX activity measurements.

Methods: Normal human plasma (NHP) treated with FVIII-neutralising antibody (to simulate inhibitors) or severe haemophilia A plasma were spiked with various concentrations of concizumab. aPTT and PT were measured on an ACL TOP®550 instrument using three different aPTT (SynthASil®, Actin® FS, STA®-Cephascreen) and PT (RecombiPlasTin 2G, Innovin®, PT HS PLUS) reagents. For FVIII or FIX activity determination, recombinant FVIII or FIX (NovoEight® or Benefix®) were spiked into severe haemophilia A or B plasma with or without 200 ng/mL concizumab and analysed using an OSC (SynthASil®) or a CS (Biophen™FVIII:C or Biophen™FIX) assay.

Results: No relevant interference by concizumab was observed in the aPTT or PT assays (Figure A-B). The presence of 200 ng/mL concizumab did not result in any statistically significant differences in factor activity measurements at any FVIII or FIX concentration using either an OSC or CS assay (Figure C-F).

Conclusions: Concizumab did not affect aPTT or PT measurements. Furthermore, concizumab did not affect FVIII or FIX activity measurements using either an OSC or CS assay.

PB0820 | Unexpected Phenotypic Characteristics Associated with Severe Haemophilia B in Two Paediatric Patients with Deletions Involving the Whole F9 and Contiguous Genes

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Background: About 4-8% of patients with severe haemophilia B (HB) show large deletions of one or more exons of *F9*. Deletions of the entire *F9* often involve vicinal genes and show clinical features exceeding severe-HB, such as intellectual disability, hypopituitarism, seizures, scoliosis, short stature, hypotonia, overweight and bilateral cryptorchidism.

Aims: Understand the molecular basis of complex clinical phenotypes in two paediatric patients with severe-HB and whole *F9*-deletions.

Methods: Both entire *F9*-deletions were primarily detected in hemizygous probands by lack of all 12 PCR-amplification products of *F9*. Dense SNP-array analysis was performed to estimate the gap extents. Guided by each involved SNP-array coordinates, case-specific STS walking strategy allowed amplification and characterisation of the deletion breakpoints by Sanger sequencing.

Results: Two patients with severe-HB, C1 and C2, showed large deletions of 4.39 and 3.9 Mb involving the *F9* and 14 and 15 nearby genes, respectively on Xq26.3-Xq27.2 and Xq27.1-Xq27.2 bands. Both patients present reduced factor IX activity, overweight, global developmental delay and generalized hypotonia. C1 and C2 share a partial or total deletion of *FGF13*, which was associated with overweight in the literature, and they also share a *SOX3* deletion widely related to intellectual disability and global developmental delay. Notably, C2 only differed from C1 in the deletion of *MAGEC2*, which may account for the anal atresia present in C2 and not in C1. *MAGEC2* deletion has never been associated with anal atresia, thus far.

Conclusions: Deletions involving X-linked genes in hemizygous males unequivocally associate with the expression of gene-specific phenotypes, such as *F9* deletions and HB. Our findings provide further support to the association of *FGF13* with overweight and *SOX3* with global developmental delay and generalized hypotonia. In addition, our data suggest for the first time a causative linkage between anal atresia and *MAGEC2* deletion.

PB0821 | *In vitro* Effect of Concizumab on Protein C Activation and Antithrombin Activity

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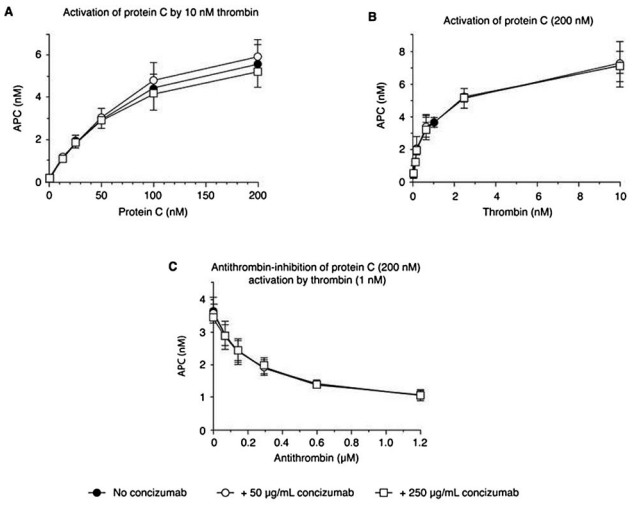
Background: Concizumab is a humanized, anti-tissue factor pathway inhibitor (TFPI) monoclonal antibody in phase 3 clinical development as a once-daily, subcutaneous prophylaxis for hemophilia A or B with or without inhibitors. Activated protein C and antithrombin are two independent negative regulators of coagulation, acting downstream and independent of TFPI.

Aims: To assess whether concizumab binding to endothelial cell-surface-associated TFPI affects protein C activation by thrombin or antithrombin inhibition of thrombin.

Methods: The affinity of concizumab for cell-surface-associated TFPI was assessed in a competition ELISA using human umbilical vein endothelial cells (HUVECs). The effect of concizumab on thrombin-mediated protein C activation on the HUVECs was measured at different protein C and thrombin concentrations in the absence or presence of excess concizumab (50 or 250 μg/mL). The effect of concizumab on antithrombin-mediated thrombin inhibition was measured at different antithrombin concentrations using protein C as thrombin substrate.

Results: Concizumab bound TFPI expressed on HUVECs with a K_D of 0.42 nM (95% CI: 0.33–0.52 nM) and at 50 and 250 μg/mL concizumab (0.3 and 1.7 μM, respectively), all TFPI was saturated. Concizumab (50 or 250 μg/mL) did not affect thrombin-mediated protein C activation (Figure 1A and 1B). An inhibitory anti-thrombomodulin antibody completely blocked the reaction, confirming thrombomodulin-dependency of the assay. No effect of concizumab on antithrombin inhibition of thrombin was observed (Figure 1C).



(A) Thrombin-mediated activation of protein C measured in the absence or presence of 50 or 250 μg/mL concizumab at different concentrations of protein C or (B) thrombin; (C) Antithrombin inhibition of thrombin activity with protein C as substrate in the absence or presence of 50 or 250 μg/mL concizumab.
TFPI, tissue factor pathway inhibitor

FIGURE 1 Downstream effects of concizumab binding to TFPI on protein C activation and antithrombin activity

Conclusions: Concizumab did not affect protein C activation or antithrombin activity in an *in vitro* set-up, consistent with its specificity for TFPI. The data suggest that the function of these downstream major coagulation inhibitors may be maintained in patients with hemophilia receiving concizumab prophylaxis.

PB0822 | Emicizumab Prophylactic Therapy in Patients with Hemophilia A Is Better Measured by Thrombin Generation Assay Than Thromboelastography

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[2]*Virginia Commonwealth University (VCU) School of Medicine, Division of Hematology/Oncology, Richmond, United States*

Background: Emicizumab (Hemlibra®) is a recombinant, humanized bi-specific antibody that functions as a bridge between factor IXa and X in the tenase complex, mimicking the cofactor function of activated factor VIII. The optimal laboratory monitoring of a patient's response to Emicizumab therapy in a clinical setting is still unknown. Emicizumab therapy interferes with many standard coagulation tests, including aPTT based single factor assays. Practitioners are turning to global coagulation tests including thromboelastography (TEG and ROTEM) and thrombin generation assay (TGA).

Aims: To compare TEG, ROTEM and TGA's ability to monitor patients' response to Emicizumab therapy.

Methods: 13 severe hemophilia A (HA) subjects, ages 10-50 yrs were enrolled. Blood was drawn prior to the first Emicizumab infusion and 5 weeks later. Laboratory work-up included plasma Emicizumab levels, TEG and ROTEM in whole blood and TGA in platelet-rich (PRP) and platelet-poor plasma (PPP) prior to and after 5 weeks of treatment.

Results: After treatment, all subjects achieved therapeutic Emicizumab levels (51 ±14 μg/mL) and none had breakthrough bleeds or needed prophy factor VIII. Emicizumab led to normalization of TEG and ROTEM parameters in all patients (Table 1). No changes were observed in TGA lag time. Mean thrombin peak increased from 46 to 90 nM in PRP and from 29 to 102 nM in PPP and mean ETP increased from 852 to 1445 nM/min in PRP and from 431 to 975 nM/min in PPP by week 5 (Table 2).

Conclusions: TGA lag time, ETP and thrombin peak were all impaired at baseline in severe HA. Marked improvements in thrombin peak and ETP after treatment were similar to values in mild HA patients, but lower than controls. Emicizumab treatment normalized ROTEM/TEG parameters in spite of the lower ETP levels noted with TGA, suggesting that ROTEM/TEG may not be as sensitive as TGA to the pro-coagulant effects of Emicizumab.

TABLE 1 TEG and ROTEM results (values represent mean and range); HA- Hemophilia A

	Severe HA (Baseline)	Severe HA (after 5 weeks Emicizumab treatment)	Mild HA (Baseline)	Normal Volunteer without HA (Baseline)
TEG R (min)	25.9 (9.8-81.8)	6.2 (4.1-8.1)	7.6 (6.6-8.6)	5.3 (4.6 -6.1)
TEG K (min)	9.08 (2-33)	1.4 (1-2)	2.5 (2.1-2.9)	1.8 (1.4-2.3)
TEG angle (degrees)	41 (8-63)	70 (62-76)	56 (52-61)	65 (59- 69)
TEG MA (mm)	53 (38-64)	65 (59-72)	57 (55-60)	58 (56 -60)
ROTEM CT (sec)	1699 (710->5400)	443 (321-555)	668 (630-760)	402 (266 -463)
ROTEM CFT (sec)	352 (208-768)	108 (84-176)	191 (154-228)	135 (102-170)
ROTEM angle (degrees)	43 (25-54)	67 (30-74)	56 (50-60)	64 (59-70)
ROTEM MCF (mm)	52 (44-66)	60 (53-66)	54 (51-57)	56 (50-60)

TABLE 2 Thrombin Generation Assay (values represent mean and range); PRP- platelet rich plasma; PPP- platelet poor plasma; HA- Hemophilia A

	Severe HA (Baseline)	Mild HA (after 5 weeks Emicizumab treatment)	Mild HA (Baseline)	Normal Volunteer without HA (Baseline)
CAT Lag PRP (min)	11.7 (7-17)	12.7 (6 -22)	9.4 (8-11)	4.3 (3-5)
CAT ETP PRP (nM/min)	852 (121-1464)	1445 (766-2225)	1584 (1416-1751)	1847 (1600-2172)
CAT Peak PRP (nM)	46 (3-82)	90 (41-152)	87 (79-94)	165 (127-207)
CAT Lag PPP (min)	5.3 (3-10)	5.8 (3 -8)	6.9 (6-7)	3.5 (3-4)
CAT ETP PPP (nM/min)	432 (214-629)	1038 (674-1589)	1285 (1145-1425)	1557 (1539-1576)
CAT Peak PPP (nM)	29 (10-45)	105 (60-172)	164 (152-176)	390 (374-405)

PB0823 | Fibrin Polymerization Ability Is Variable in Patients with Severe Haemophilia and Might Influence their Joint Condition

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Background: Factors affecting joint condition in severe haemophilia (SH) are still on debate. Variability on fibrin polymerization capacity might theoretically influence the bleeding tendency and thus, the joint condition in SH but studies on this regards are lacking.
Aims: To evaluate the correspondence between fibrin polymerization capacity and joint condition in SH patients.
Methods: 17 SH patients (3 inhibitors, median age [p25-p75]= 43.9 [36.0-52.2] years old) were recruited. Joint condition was evaluated using the HEAD-US score. Level of fibrinogen (Fib) (Clauss) and fibTEM (rotational thromboelastometry) was determined. Fibrinogen ability to increase the clot strength was evaluated by the quotient R= Fib/fibTEM maximum clot firmness (MCF_{fibTEM}).
Results: Median value of Fib was 324 [278-351] mg/dl, MCF_{fibTEM} = 15 [11-16] mm and R= 24.6 [20.6-26.6] mg/dl/mm. The patient group was divided into 2 groups taking into account the value of the 25th percentile of each variable. Thus, patients with Fib ≤ 278 mg/dl were considered patients with low fibrinogen level, patients with MCF_{fibTEM} ≤ 11 mm were considered patients with low platelet independent maximum clot strength and patients with a R ≤ 20.6 mg/dl/mm were considered good responders to fibrinogen. Based on this analysis, neither Fib nor MCF_{fibTEM} influenced the joint condition of the patients. However, patients with poor response to fibrinogen (R > 20.6 mg/dl/mm) had statistically significant greater joint damage than the good responders to fibrinogen (R ≤ 20.6 mg/dl/mm) (Table 1). Poor responders to fibrinogen had frequently joint damage in ankles followed by elbows and knees being cartilage the most commonly affected joint compartment. Good responders to fibrinogen only presented milder joint alterations in elbows and ankles.
Conclusions: Fibrin polymerization capacity may be variable between patients with SH and might influence their joint condition. However, larger studies are required to confirm this finding.

TABLE 1 Patients' characteristics. N: number of patients; R: right; E: elbow; C: cartilage; B: bone; L: left; K: knee; A: ankle

Variable		Good responders to fibrinogen	Poor responders to fibrinogen	p-value
N		4	13	-
Age (years)		30.0 ± 18.2	44.9 ± 7.7	0.112
Inhibitors (%)		2 (50)	1 (7)	0.121
HEAD-US	REC	0.8 ± 1.5	3.3 ± 0.8	< 0.010
HEAD-US	REB	0.3 ± 0.5	1.2 ± 0.7	0.020
HEAD-US	LKC	0	2.3 ± 2.0	0.037
HEAD-US	RAC	0	3.0 ± 1.5	0.010
HEAD-US	RAB	0	1.2 ± 0.7	0.011
HEAD-US	LAC	1.0 ± 2.0	3.0 ± 1.7	0.035

PB0824 | Diagnosis and Management of Acute Hemarthrosis and Haemophilic Arthropathy in Spain Using Unified Clinical and Ultrasound Criteria: Preliminary Results

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Background: Currently, the life expectancy of people with haemophilia (PWH) is similar to the general population. The new substitute therapies and in particular extended half-life factors allow to improve the quality of life with a physical activity in accordance with the WHO recommendations. However, the risk of traumatic and subclinical hemarthros remains present. The treatment, the evolutionary control of acute hemarthrosis and the prevention of degenerative processes: arthropathy, remain important in the health control of PWH.

Aims: Perform an analysis of the current status of the most affected joints (elbows, knees and ankles) in PWH using unified clinical and ultrasound criteria, in addition to carry out a 18 months-follow up to assess bleeding incidents, physical function and quality of life.

Methods: Observational, longitudinal, prospective, multicentre study (NO-EPA classification). The subjects will attend a total of two visits to the referral hospital (baseline and 18-months follow up). The following data will be collected: clinical and demographic data, clinical assessment (Haemophilia Joint Health Score, HJHS), ultrasound assessment, self-perceived functional abilities (Haemophilia Activity List (HAL) and Pediatric HAL (PedHAL), Quality of Life (A36 hemophilia-QoL).

Results: At this moment, as preliminary evaluation, we have n=40 PWH, however our objective is to reach n=150 subjects. Through the different clinical evaluation and the ultrasound we can observe how both children and adults, despite treatment, continue to have some subclinical bleeding, affecting their joint health, decreasing their physical activity and worsening their quality of life (Table 1 and 2).

Conclusions: Using unified clinical and base ultrasound criteria to control health joint evolution in PWH will allow us the early detection of subclinical bleeding and the adoption of medical and physiotherapy tailored measures to prevent joint deterioration.

TABLE 1 Clinical and demographic data (ABJR: Annual Bleeding Joint Rate)

	Children (n=4)				Adults (n=36)			
	Min	Max	Mean	Std	Min	Max	Mean	Std
Age (years)	3.0	12.0	8.3	3.9	17.0	68.0	36.4	12.5
BMI (kg/m^2)	15.9	26.0	19.7	4.6	19.3	38.6	25.7	4.7
Type of haemophilia (A/B)	4/0				35/1			
Severity (severe/moderate/mild)	4/0/0				27/3/6			
Replacement treatment (prophylaxis/on demand)	4/0				28/8			
ABJR	0.0	1.0	0.3	0.5	0.0	5.0	0.6	1.1

TABLE 2 Questionnaire and ultrasound data (RE: Right Elbow; LE: Left Elbow; RK: Right Knee; LK: Left Knee; RA: Right Ankle; LA: Left Ankle

	Children (n=4)				Adults (n=36)			
	Min	Max	Mean	Std	Min	Max	Mean	Std
HJHS (total)	0.0	0.0	0.0	0.0	0.0	58.0	23.4	19.5
Effusion Ultrasound (RE/LE/RK/LK/RA/LA)	0/0/0/1/0/0				0/0/8/5/2/2			
Synovitis Ultrasound (RE/LE/RK/LK/RA/LA)	0/0/0/0/0/0				10/13/11/11/17/17			
HAL	91.0	100.0	97.7	4.5	36.6	100.0	78.8	19.4
A36-QoL	106.0	128.0	119.0	9.6	37.0	132.0	99.9	23.5

PB0825 | Association of Adherence and Bleeding Frequency among Severe Hemophilia A Patients on Prophylactic Factor VIII

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Background: Prophylaxis treatment has become the gold standard for treating hemophilia. The initiation of prophylaxis therapy may reduce the bleeding tendency in patients. Recent evidence shows that the prophylaxis regimen reduces bleeds and joint damage. However, reported adherence levels among patients with hemophilia to vary widely (17-93%). The level of adherence and its association with bleeding tendency and co-morbidity have not been well documented.

Aims: This study aims to evaluate severe hemophilia A patients' adherence to prophylaxis regimen and to understand the association of bleeding and factors contributing to non-adherence.

Methods: This is an observational study with retrospective and prospective data collection conducted via patients' interviews and review of patients' medical records. Validated adherence scale (Veritas -Pro), comprises of 24 questions to be answered by each subject was used in this study. Patients with age of above 18 years old who are using prophylaxis regimens will be eligible for inclusion.

Results: A total of 38 patients were recruited. Median bleeding episodes per year was 5 episodes (0 to 19 episodes). 32 (84.2%) of them experienced joint bleeding in the previous month, 22 (57.9%) had confirmed arthropathy, and 25 (65.8%) had other co-morbidities. Median adherence was 36.6% (mean 39%, range 25% to 61%).

Conclusions: The median of bleeding rate of 5 in this study is smaller compared to on-demand group which may reach up to 12 median bleeding rates. This study has provided evidence showing a positive correlation of adherence to bleeding frequency and 65.8% had other co-morbidities.

PB0827 | Testing the Impact of Recombinant von Willebrand Factor and Recombinant Factor VIII on Osteoclastogenesis

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Background: Bone resorption is tightly regulated by receptor activator of nuclear factor κB ligand (RANKL) and osteoprotegerin (OPG), both affecting osteoclast differentiation from macrophages. Previous studies showed that the factor VIII/von Willebrand complex (FVIII/vWF) limits osteoclastogenesis by acting as a scavenger for RANKL and promoting OPG activity. Indeed, osteoporosis due to increased bone resorption was observed in mice with factor VIII deficiency and in a subset of hemophilia A patients. However, impact of recombinant multimeric von Willebrand factor and recombinant FVIII on the regulation of osteoclastogenesis has not been tested yet. Furthermore, modified recombinant FVIII and alternative hemostatic agents may exhibit a differential impact.

Aims: We aimed to establish an in-vitro method to evaluate the impact of recombinant multimeric vWF and various FVIII products on osteoclastogenesis.

Methods: $CD14^+$ monocytes were incubated with 20 ng/ml human macrophage colony-stimulating factor (rhM-CSF). Expanded cells were incubated with RANKL (100 ng/ml), OPG (100 ng/ml), recombinant multimeric vWF (1 U/ml), and recombinant FVIII (1 U/ml). After 14 days, degree of differentiation was evaluated by microscopic imaging and by cells' release of tartrate resistant alkaline phosphatase into the supernatant using a photometric assay (TRAcP).

Results: RANKL initiated significant TRAcP secretion (738±26% of control) inhibitable by OPG (378±59% of control). Addition of recombinant vWF substantially reduced osteoclast formation (125±8% of control). Addition of recombinant FVIII to recombinant VWF reversed its inhibitory effect on osteoclastogenesis (492±62% of control).

Conclusions: We developed an assay to evaluate the impact of recombinant multimeric vWF and recombinant FVIII on osteoclastogenesis. Preliminary results argue for inhibiting effects of recombinant multimeric vWF which may be reversed by recombinant FVIII. Further studies will have to determine whether modified recombinant FVIII products or alternative hemostatic agents exhibit

similar effects. Results from these studies will provide insight into the development of osteoporosis in hemophilia A patients.

PB0828 | Prediction of the Response to FVIII Treatment in Haemophilia A

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Background: Thrombin generation (TG) is frequently measured in haemophilia patients to evaluate their baseline TG level, to monitor their response to therapy and to follow up patients in time. TG is also being used to compare efficacy and potency of potentially new treatment drugs. Many research groups use plasma from healthy controls spiked *in vitro* with an antibody (Ab) in order to mimic haemophilia plasma.
Aims: Our goal was to evaluate whether these *in vitro* mimicked samples have the same TG kinetics as haemophilia A samples and therefor are suitable for research purposes.
Methods: The Calibrated Automated Thrombogram was used to measure TG in plasma samples from healthy controls and haemophilia patients. Samples were spiked *in vitro* with recombinant FVIII (Kogenate, Bayer) and/or FVIII-Ab (PAHFVIII-S, HaemTech). Haemophilia A plasma was spiked with a buffer or with 100% FVIII and 15 μg/ml FVIII-Ab (to mimic haemophilia A again in the presence of the Ab). Plasma from healthy controls was spiked with 6 and 15 μg/ml FVIII-Ab. FVIII was measured using the STA R of Stago (France).
Results: When TG was measured in haemophilia A samples with and without FVIII-Ab, no differences could be observed between both plasma samples. A doses-response of FVIII could be observed for peak height, ETP, time-to-peak and velocity index, but not for lag-time, that seemed to be independent of the FVIII level. The FVIII doses-response in healthy controls with 6 and 15 μg/ml were parallel to each other, indicating that the differences between the two curves was dependent of the remaining FVIII level (94%, 34% and >4% for the samples with 0, 6 and 15 μg/ml FVIII-Ab, respectively).
Conclusions: No substantial differences were observed in TG kinetics, indicating that *in vitro* induced haemophilia samples can be used to determine the response to FVIII treatment in haemophilia A.

PB0829 | Detection of Residual Factor VIII Levels Reveals the Occurrence of Readthrough Over the Majority of F8 Nonsense Mutations

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Background: Nonsense mutations are commonly associated with "null" genetic conditions. However, an event called "ribosome readthrough" may restore the synthesis of full-length proteins through suppression of nonsense mutations and incorporation of an amino acid. This might explain why some *F8* nonsense mutations are associated with moderate haemophilia A (HA).
Aims: To evaluate full-length protein production resulting from occurrence of readthrough over a wide panel of *F8* nonsense mutations.
Methods: Expression of an optimized fusion protein between factor VIII (FVIII) and a high-sensitivity luciferase in HEK293 cells and luciferase assays in media and cell lysates. Evaluation of FVIII in HA patient plasma through Western blotting and ELISA.
Results: *F8* nonsense mutations were subdivided into a high-frequency (12 mutations; patient number, n>10), arising from the highly frequent CGA(arginine)>TGA change, and a low-frequency (44 mutations; patient number n=1-5) group. The latter was rationally classified into two subsets including mutations predicted to be suppressed by reinsertion of the original amino acid, and localized in the B-domain, in which potential amino acid substitutions introduced during readthrough are predicted to be tolerated. Noticeably, the selected mutations (44 out of 216) have been reported in 384/611 (63%) HA patients with nonsense mutations.
Strikingly, expression of all *F8* nonsense variants led to detectable luciferase activity with a different extent (0.3-7%) that appeared to be consistent with the impact of the inserted amino acid on FVIII secretion. The selected panel includes 11 nonsense mutations, found in Italian HA patients, that in preliminary results revealed traces of FVIII protein in plasma.
Conclusions: Data from our expression platform indicate that a relevant number of *F8* nonsense mutations, relatively frequent in HA, undergo readthrough and can be associated with residual protein levels. This might have relevant pathophysiological implications, and might contribute to interpret the variable susceptibility of HA patients to develop inhibitors upon replacement therapy.

PB0830 | Increased Plasmin Generation in People with Severe Haemophilia

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Background: People with severe haemophilia (PWH) exhibit significant variability in bleeding tendency that is independent of individual factor VIII/IX levels. Despite this, the factor(s) underlying this phenotypic heterogeneity remain largely unknown. In this study, we hypothesised that inter-individual differences in fibrinolytic pathway function may contribute to bleeding heterogeneity in PWH.

Aims: To investigate whether endogenous plasmin generation potential in plasma from PWH is different compared to plasma from a healthy control population.

Methods: Platelet-poor plasma was collected from healthy male donors (n=100) and people with severe haemophilia A or B at trough levels participating in the Irish Personalised Approach to the Treatment of Haemophilia (iPATH) study (n=78). The plasmin generation (PG) potential of each sample was measured in modified plasmin generation assays designed to probe the activity of different facets of the fibrinolytic system. Briefly, tissue-plasminogen activator (t-PA)-driven plasmin generation was measured using a plasmin-specific fluorogenic substrate. In addition, exogenous thrombin co-incubated with t-PA enabled individual evaluation of fibrin-dependent PG acceleration independent of each individual's capacity to endogenously generate thrombin.

Results: Unexpectedly, fibrin-dependent PG in plasma from PWH was significantly enhanced when compared to plasma from the healthy control cohort. Notably, enhanced PG activity in the PWH cohort was not affected by haemophilia type (A or B) or by residual factor levels, as evaluation of plasma from the same PWH post-factor replacement did not impact the observed enhanced PG activity.

Conclusions: Assessment of PG potential in a large severe haemophilia A and B cohort using thrombin generation-independent PG assays revealed significantly increased PG in PWH, which was independent of FVIII/FIX levels. Enhanced PG may therefore represent a contributory factor to the heterogeneous bleeding phenotype observed in individuals with severe haemophilia.

PB0831 | Identification of Novel Genetic Risk Factors in the Conserved Haplotype Region Surrounding the LCT Locus on Chromosome 2q21

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Background: Inhibitors development affects about 30% of patients with severe hemophilia A (HA). Different environmental and genetic risk factors are involved in this process.[1,2] Recently, we identified a SNV (rs3754689) in the *LCT* gene potentially linked with this predisposition.[3] Since this variant is benign [4] and is located in a conserved haplotype block, we hypothesized that the association signal captured by this variant is probably located in coinherited, neighboring genes (*R3HDM1*, *UBXN4*, *CXCR4*, *MCM6*, *miRNA-128-1*). *R3HDM1*, *UBXN4* and *miRNA-128-1* have been previously linked with autoimmune disorders, therefore they are potential clinically relevant targets.

Aims: To identify novel genetic risk factors associated with inhibitor development in coding regions of *R3HDM1*, *UBXN4*, *CXCR4*, *MCM6*, *miRNA-128-1* genes in a cohort of Italian patients with severe HA with or without inhibitor.

Methods: A cohort of 246 Italian patients with severe HA with (72) or without (174) inhibitor was subjected to target sequencing by TruSeq Custom Amplicon (Illumina). Data were analyzed according to the guidelines reported by the Broadinstitute (https://software.broadinstitute.org/gatk/best-practices/). Statistical analysis was performed by Plink, PlikSeq. ENCODE project and Enhancer Atlas were consulted to evaluate the role of the variants in the gene expression.

Results: 228 variants passed the quality controls: 56 common (MAF≥1%) and 172 rare (MAF< 1%). Logistic regression of common variants confirmed the protective role of the rs3754689 missense variant previously identified. Moreover, other 4 variants resulted significantly associated (Tab.1). Two of these (rs3213892; rs3816155) are localized in the intron 13 of the *LCT*; a genomic region involved in the regulation of the *UBXN4* expression (Tab.2). Rare variants resulted not associated with the development of inhibitor.

TABLE 2 EnancherAtlas 2.0 results

Peaks	Type	Target Genes	Cell resource
chr2:136552517 -136552517	Enhancer	UBXN4	UBXN4 GM12891,HL-60,Fetal_thymus GM12891,Fetal_thymus
chr2:136552526 -136552526	Enhancer	UBXN4	GM12891,HL-60,Fetal_thymus

TABLE 1 Common variants association

SNP	OR	95% IC	FDR_BH	P<0.05	Position	Gene	Annotation
rs1050115	0.5611	0.3608-0.8724	0.2624	0.01072	exonic	UBXN4	NM_014607:exon4:c.A303G:p.E101E
rs3087343	0.5577	0.354-0.8787	0.2624	0.01356	intronic	MCM6	.
rs3213892	0.5884	0.3829-0.9041	0.2624	0.01498	intronic	LCT	.
rs3816155	0.6096	0.3978-0.9342	0.2687	0.0231	intronic	LCT	.
rs3754689	0.6497	0.4273-0.9879	0.4214	0.04664	exonic	LCT	NM_002299:exon2:c.G655A:p.V219I

Conclusions: This study confirmed the association of the chromosomal region around the *LCT* locus with inhibitor development in patients with severe HA. Further investigations are necessary to evaluate the expression of target genes and miR-128-1.

PB0832 | Uptake of MyABDR in Australia

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Background: Care for people with Haemophilia has improved much over the last two decades for those receiving early regular prophylaxis with clotting factor concentrates. One of the biggest challenges to formally assess the real benefit of prophylaxis is the need to monitor treatment adherence and outcome. *MyABDR* is a secure app for smartphones, linked to the web-based Australian Bleeding Disorder Registry (ABDR), for patients with bleeding disorders to self-record their home treatments and bleeds.

Aims: To review uptake of *MyABDR* among Haemophilia A (HA) patients in Australia.

Methods: *MyABDR* data was derived from the ABDR on patients from whom consent had been obtained. Data was obtained on diagnosis, severity, treatment regimen and interactions (treatments and bleeds) of patients registered to self-record on *MyABDR*. Prescribed treatment regimen was compared against the treatments recorded in *MyABDR* to understand adherence to prophylaxis and adherence to logging in the *MyABDR app*.

Results: There are 2214 HA patients in the ABDR. 31.3% (694/2214) of HA patients in Australia are registered on *MyABDR*, which include 71.6% (499/696) severe and 12.6% (192/1518) non severe HA patients. 455 of the 499 severe HA patients registered on *MyABDR* were prescribed routine prophylaxis. Almost 90% (407/455) of *MyABDR* users commenced routinely recording their prophylaxis treatments with the paediatric group more adherent compared to the adult group. Within 6 months of commencing prophylaxis, time lag of 2 weeks or more in recording prophylaxis treatments in the *MyABDR* app was observed in 17% of users and adherence to logging in the *MyABDR* app dropped by 9%. There were 50.8% (207/407) of *MyABDR* users recording their prophylaxis treatments and reported at least one bleed.

Conclusions: *MyABDR* is a valuable tool to determine relationship between treatment, outcome and patient compliance.

PB0833 | Bone Remodeling Alterations in Haemophilia: A Cell Biology Approach

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Background: Bone disease is a significant complication of haemophilia A (HA). However, the pathophysiology is not exactly known and both processes of bone resorption and bone formation could be hypothesized to be altered. It has been suggested that Factor VIII could directly affect bone cells. Moreover, bone cells express protease-activated receptors (PARs) and FVIII deficiency could play a role also by decreasing thrombin production.

Aims: The aim of this study concerned the identification of the specific role played by FVIII, von Willebrand Factor (VWF) and thrombin on osteoclasts and osteoblasts.

Methods: In vitro assays assessed the osteoclastogenic potential of PBMC (Peripheral Blood Mononuclear Cells) isolated from a haemophilic patient. Osteoclastogenesis of healthy donors-PBMC were analysed in the presence of FVIII (2U/ml), VWF (20 μg/ml), FVIII/VWF (2U/ml) with or without osteoprotegerin (OPG 25 ng/ml), and thrombin (Thb 100 nmol/l). Moreover, the effects of these treatments on osteoblast differentiation and activity were also studied.

Results: PBMC from HA patient showed increased ability to form mature osteoclasts compared to those obtained from healthy controls. Moreover, RNA expression analysis performed on patient's osteoclasts revealed higher levels of RANK, TRAF6, CATHEPSIN K and TCIRG1 genes expression compared to control osteoclasts. VWF appears to play a major role, showing by itself ~45% inhibition of osteoclastogenesis comparable to OPG (the physiologic inhibitor), and even more if is complexed with FVIII (53% inhibition). Thrombin reduces osteoclast differentiation with variable effects (30-50% inhibition).

No alteration of alkaline phosphatase positivity was observed in control osteoblasts treated with Thb and VWF, whereas incubation with FVIII leads to a statistically significant reduction, also revealed in osteoblasts treated with FVIII/VWF.

Conclusions: All these data support that bone loss observed in haemophilic patients could be related to increased osteoclast formation and activity and that coagulation factors directly impact on bone cells.

PB0834 | Reduced Splenic T Cells Count in an Untreated Mouse Model of Severe Hemophilia A

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Background: Hemophilia A (HA) is an X-linked bleeding disorder caused by factor VIII (FVIII) deficiency. Upon replacement of the missing clotting factor by intravenous infusions, around 30% of patients develop neutralizing antibodies (inhibitors). Their formation involves the activation of both the innate and adaptive arm of the immune system and might be related to the absence of immunological tolerance to FVIII.

Aims: Little is known about the development and function of the immune system in HA patients prior to treatment. Discovering any intrinsic alteration would help to understand the pathogenesis of inhibitor formation and to develop new therapies. As such, we sought to analyze the numbers and phenotype of different immune cells in a mouse model of severe HA.

Methods: Single cell suspensions were obtained from several organs collected at different timepoints from HA and age-matched wild-type (wt) mice. Cell numbers were determined by Trypan blue count while percentages and phenotype of immune cells evaluated by flow cytometry.

Results: At week 8 both splenic CD4 and CD8 T cells were significantly lower in HA compare to wt mice. This difference disappeared at later timepoints (52 weeks). Interestingly even CD4 T regulatory cells were decreased in the spleen of HA mice. Thymic count and cell composition were similar between the two groups, suggesting that the difference observed in periphery was not due to a reduced thymic output or an impaired T cell development.

Conclusions: Our preliminary data support the hypothesis that the absence of FVIII could affect the immune cell populations. The decreased T cell count found in the spleen of HA mice is intriguing since they are highly involved in the inhibitor formation. Studies are currently ongoing for understanding the basis and significance of this reduction.

PB0835 | Discrepancies between FVIII Assays in Routine Diagnosis and Treatment Monitoring of Haemophilia A

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Background: Measurement of the clotting activity of Factor VIII (FVIII) can be done either by one-stage clotting (OSA) or by chromogenic substrate assay (CSA). Variability between these 2 methods has been known for several years, particularly for haemophilia A (HA) patients substituted with recombinant products or carrying specific genetic mutations. This variability can have major implications in terms of monitoring and diagnosis of HA and it is currently uncertain whether both assays should be used routinely.

Aims: This study emphasizes the need to use both assays in the management of the haemophilia patient.

Methods: In our institution, all FVIII assays (n=793) between 2015 and 2018 for diagnosis (endogenous FVIII) or monitoring (exogenous FVIII) were performed using both OSA (HemosIL Werfen kit) and CSA (Biophen FVIII:C HYPHEN BioMed). Discrepancy was defined as a twofold difference (ratio OSA/CSA >1.9 or < 0.6) and evaluated for the assay of exogenous FVIII (mainly severe HA patients substituted with standard half-life recombinant FVIII; n=287), mutated endogenous FVIII (male patients and haemophilia carriers of all severities; n=192) and non-mutated endogenous FVIII (healthy and VWD patients; n=268). Clinical discrepancy was defined as discrepant results leading to misclassification of HA patient according to the ISTH classification.

Results: For the assay of exogenous FVIII in treated HA patients we noticed 4.5% and 5.5% of discrepancies with higher CSA and OSA values respectively. For the assay of endogenous FVIII in untreated HA patients, a clearer trend was observed with 10.4% and 16.7% discrepancies with higher CSA and OSA respectively. For non-mutated endogenous FVIII, no significant discrepancy was noted. Considering clinical discrepancy, OSA misclassified 22% while CSA misclassified 17% of HA patients.

Conclusions: Discrepancies between CSA and OSA assays are frequent for both endogenous and exogenous FVIII. For the time being, both assays should ideally be routinely performed for diagnosis and monitoring of HA patients.

PB0836 | Factor VIII Does Not Elicit Danger Signals to Innate Immune Cells in Vivo

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Background: The immune mechanisms underlying formation of alloantibodies towards infused Factor VIII (FVIII) in severe hemophilia A (HA) patients are not completely understood. The contribution of $CD4^+$ T cells to inhibitor development implicates a key role for antigen presentation. Following antigenic challenge, innate immune cells upregulate activation markers to promote robust adaptive immune responses, however in vitro studies indicate this does not happen with FVIII alone. Whether upregulation of activation markers occurs in response to FVIII in vivo is unclear.

Aims: To evaluate evidence of early FVIII immunogenicity involving innate immune cell activation in HA mice.

Methods: C57BL/6 HA mice (≥8 weeks age) received either a single intravenous infusion of 0.2 μg (80 IU/kg) FVIII or an intensive dosing protocol of 0.6 μg (240 IU/kg) FVIII for three consecutive days. Saline-treated mice were used as controls. Spleen cells were harvested 6- or 18-hours following the single FVIII infusion and 24-hours after the intensive dosing protocol. Spleens were harvested and expression of activation markers CD40, CD86, and MHC class II on splenic B lymphocytes ($B220^+$), dendritic cells (DCs, $CD11c^+$), and macrophages ($CD11b^+$) were analyzed by flow cytometry.

Results: In response to a single FVIII challenge, no significant changes were observed in total B cells or macrophages expressing any activation markers. Transient increases in CD40 expression

were seen on DCs with a slight increase in the total population of CD40-expressing DCs 6-hours after FVIII challenge (p=0.0358). These effects did not persist at 18-hours after FVIII administration. Following the more intensive treatment protocol, innate immune cells remained unchanged at 24-hours post-treatment with no significant changes in activation markers.

Conclusions: This is the first study to demonstrate that FVIII does not upregulate activation markers shortly after a single infusion or repeated intensive exposure to FVIII in HA mice, suggesting a more complex and temporal mechanism involved in inhibitor formation.

PB0837 | Two-year Update on the WFH World Bleeding Disorders Registry

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Background: The World Federation of Hemophilia (WFH) World Bleeding Disorders Registry (WBDR) is the only global registry collecting standardized clinical data on a large population of people with hemophilia (PWH).

Aims: The goals of the WBDR are to enroll at least 10,000 PWH from more than 50 countries.

Methods: The WBDR is a prospective, longitudinal, observational registry of patients diagnosed with hemophilia A and B. All hemophilia treatment centres (HTC) contribute data on basic demographics, diagnostics, and clinical variables included in the Minimal Data Set and may choose to contribute to the Extended Data Set. An international data integration component was established, aiming to facilitate data transfer from existing patient registries to the WBDR.

Results: Since launching the WBDR in January 2018, 80 HTCs from 38 countries have received ethics approval. Over 4400 PWH have been enrolled, including 771 patients imported from the Czech National Hemophilia Program Registry to the WBDR. Patients registered with the WBDR represent all regions of the world and all World Bank gross national income categories. Most patients enrolled are male (99%), with hemophilia A (86%) and severe disease (52%). The most frequent severity category among hemophilia A and B patients was severe (51% and 43%, respectively). The median age of patients in the registry is 20 years, with a ratio of children to adult participants of 45%:55%.

Conclusions: Following a successful launch, the WBDR is establishing itself as the leading global hemophilia registry. The real-world data collected through the WBDR can now be used to generate evidence to improve the quality of care worldwide.

The WFH thanks the many dedicated health care providers and patients who are part of this important initiative.

The WBDR is supported by our Visionary Partners: SOBI and Takeda; and our Collaborating Partners: Bayer, CSL Behring, Grifols, Pfizer, and Roche.

PB0838 | *In vitro* and *in sillico* Analysis of *F9* Gene Missense Mutations at Exons 2 and 3 Causative of Severe Hemophilia B

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Background: Factor IX (FIX) glycoprotein undergoes numerous post-translational modifications including gamma-carboxylation of 12 glutamic-acid residues in Gla-domain, which is directed by the FIX propeptide and the gamma-glutamyl-carboxylase enzyme. Mutations in propeptide and Gla-domain generate severe deficiency of FIX activity in hemophilia B (HB) patients.

Aims: Analyze the effect of *F9* gene missense mutations at exon 2 (p.Glu35Gly) and 3 (p.Lys51Glu, p.Phe55Cys, p.Glu79Gln) on the structure/function of the FIX protein.

Methods: Missense mutations causing severe HB referred in the http://www.factorix.org/database and which did not have a prior functional study, were selected through the correlation of the reported clinical severe phenotype and the output prediction of six *in silico* tools. *F9* cDNA-intron 1 was cloned into the plasmid pcDNA3.1 (+/-) to induce the mutations by site-directed mutagenesis. The plasmids carrying the mutants and wild-type FIX proteins were transfected into HEK-293 cells. After 48 hours of expression, the effect of inhibitors of intracellular transit was evaluated by the production of the secreted proteins to the culture medium and the intracellular proteins obtained from cellular lysates, which were quantified by ELISA assay.

Results: The four mutants decreased their extracellular concentration respect to the wild-type FIX. In order to know the effect of these mutations on intracellular transit, the main degradation pathways were inhibited. In presence of NH_4Cl, an inhibitor of lysosomal degradation, mutant proteins p.Glu35Gly and p.Lys51Glu increased their intracellular concentration. In presence of Brefeldin A, an inhibitor of protein transport from the endoplasmic reticulum to Golgi apparatus, mutant proteins p.Phe55Cys and p.Glu79Gln increased their intracellular concentration.

Conclusions: p.Glu35Gly and p.Lys51Glu mutant proteins are degraded mainly by the lysosomal pathway; p.Phe55Cys and p.Glu79Gln mutant proteins are degraded mainly in pre-Golgi compartment. The structural predictions gave an overview of the mutants behavior which was extended by *in vitro* analysis.

PB0839 | Animal Model Construction for Hemophilic Arthritis: Evaluation of Hemophilia Joint by Ultrasound Detection

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Background: To find an ideal method for modeling hemophilic arthritis which is easy to operate, fast to construct, and the lesions of model can be sensitively detected by ultrasound.

Aims: To establish the animal model of hemophilic arthritis via injecting blood or iron dextran into knees of rabbits and evaluate the effect of different modeling method by ultrasound detection.

Methods: 25 rabbits were divided into three groups:Rabbits in Group A was injected with 1 ml blood, 3 times a week for 36 times. Group B was injected with 1 ml blood, twice a week for 24 times. Rabbits in Group C was injected with 1 ml iron dextran and divided into 1/2/3 month group(C1/C2/C3group), each group have 5 rabbits. Ultrasound was used to monitor the changes in synovium and cartilage. At the end of modeling,specimens were harvested and pathological of knees were observed under microscope.

Results: At the end of the modeling, the thickness of synovium in group A (4.5 ± 0.5 mm) measured by ultrasound was thicker than that in group B(2.4 ± 0.4 mm) and C(4.1 ± 0.4 mm). Synovium in group A and B increased in thickness and grade of CDFI during modeling. In group C, the thickness and blood flow signal of synovium increased in the first month. With the prolongation of the modeling time, the synovium thickness slightly thinned and the blood flow signal weakened. At the end of modeling, the damage of cartilage in groups A and C can be detected by ultrasound. The pathological changes can be observed in each group under microscope.which were similar to hemophilic arthritis.

Conclusions: The animal model of hemophilia arthritis can be established by injecting blood or iron dextran. Intraarticular injection of iron dextran is an effective method to establish hemophilic arthritis models for relatively easy operation and sensitive ultrasound detection in changes of synovitis and cartilage.

PB0840 | Feasibility of Using Hospital Episode Statistics in England to Assess the Resource Use and Outcomes in Haemophilia

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Background: Haemophilia is a bleeding disorder characterised by a deficiency of clotting factor FVIII/FIX. Limited evidence has been generated on the hospital activity for haemophilia in the secondary healthcare setting in England. Information about these patients could provide a greater understanding of the burden of haemophilia in secondary healthcare settings.

Aims: This study aimed to assess the feasibility of utilising NHS England's hospital episode statistics (HES) to assess resource use and clinical outcomes in the secondary healthcare setting for haemophilia.

Methods: The HES dataset contains details of diagnoses and procedures for hospital admissions, outpatient appointments and A&E attendances at NHS hospitals in England. The dataset was queried from 2009-2018 (HES Years) for haemophilia patients that ever-used secondary healthcare in England over the 10 years which encompasses all ages (adults/paediatrics) and all haemophilia severities. Specific ICD-10 codes were queried for the patient counts: D66 (Hereditary Factor VIII deficiency) & D67 (Hereditary Factor IX deficiency). In addition, OPCS-4.7 procedure codes (X81-X98) were assessed to understand which high cost drugs are being frequently coded for haemophilia admissions in secondary care.

Results: The number of Haemophilia A and Haemophilia B patients captured over the 10 years is 16,940 and 2,473 respectively. Hospital activity in 2018 indicated that people with haemophilia A and B were coded 3,466 and 504 times respectively within the year. The most frequently coded high cost drug in the haemophilia cohort was X83 (High cost for other cardiovascular drugs) which encompasses blood products.

Conclusions: The feasibility of using the HES database is promising with large numbers of distinct patients across a 10-year period for haemophilia A and B. Coding of high-cost blood products as a procedure will assist in identifying hospital activity of different subsets of haemophilia patients. Further analysis of the dataset is planned to further understand resource use and outcomes in haemophilia.

PB0841 | Evaluation of Intron 22 and Intron 1 Inversions of Factor VIII Gene in Haemophilia A Patients

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Background: Haemophilia A is X-linked recessive bleeding disorder caused by either qualitative or quantitative defect of coagulation Factor VIII, a component of intrinsic pathway of clotting.

Aims: To detect mutation in intron 22 and 1 in haemophilia A patients by Inverse Shift Polymerase Chain Reaction (IS-PCR) technique and to correlate intron 22 and 1 inversion mutation with development of Factor VIII inhibitors.

Methods: This Cross sectional analytical study was conducted at Diagnostic and Research Laboratory Liaqat University Hospital (LUH) Hyderabad, Medical Research Centre Liaqat University of Medical and Health Sciences (LUMHS) Jamshoro and National Institute of Blood Diseases (NIBD) Karachi from January 2019 to June 2019. The sample size was 73, calculated by Open Epi software.

The sampling technique was non-probability purposive. Diagnosed cases of haemophilia A, irrespective of their age and gender, either not transfused Factor VIII or at least one week after the transfusion of Factor VIII were included. In this study, IS-PCR method was used for inversion mutation and Bethesda Assay for inhibitor detection.

Results: The majority of participants were severe haemophiliacs, followed by moderate and mild haemophiliacs. The presence of Intron 22 inversion mutation was prevalent in severe category i.e. 45%. However, Intron 1 inversion mutation was not identified. Factor VIII inhibitors were present in 4 (66%) severe and 2 (33%) moderate haemophiliacs and in 5 (83%) of them intron 22 inversion was present. Joint deformity was present in majority of Haemophilia A patients 62 (85%), with knee joint being predominantly involved followed by elbow, ankle, shoulder, wrist and hip joint.

Conclusions: There is a significant correlation of severity of Haemophilia A with intron 22 inversion mutation and development of Factor VIII inhibitors. Furthermore, intron 22 inversion mutation exhibit significant relation with development of Factor VIII inhibitors.

PB0842 | Methodological Validation of the Measurement of Ultra-low Levels of Factor VIII Activity by Chronometric APTT-based Assay

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Background: The nomenclature defines the severity of hemophilia A according to the factor VIII coagulant activity (FVIII:C) level. For FVIII:C < 1 IU/dl, hemophilia is classified as severe and for values between 1 and 5 IU/dl, it is classified as moderate hemophilia. It is often difficult to conclude for values close to 1 IU/dl while the prophylactic treatment may vary depending on the biological severity of hemophilia.

Aims: The aim of this study is to validate FVIII:C method that would allow reliable results for ultra-low values.

Methods: The intra-laboratory reproducibility was evaluated by performing FVIII:C with a chronometric method on 10 consecutive days. The bias was assessed by inter-laboratory comparison of FVIII:C results of 4 spiked plasmas performed in 8 French hemostasis laboratories.

Results: Concerning the reproducibility, for plasmas (0.0; 0.3; 0.7; 1.0; 1.5 and 3.0 IU/dl), the results (mean [range], coefficient of variation) were respectively: 0.00 IU/dl [0.00-0.00], incalculable; 0.18 IU/dl [0.10-0.30], 43.82%; 0.60 IU/dl [0.40-0.80], 19.25%; 0.95 IU/dl [0.80-1.10], 10.23%, 1.33 IU/dl [1.10-1.70], 16.64%, 2.64 [2.00-3.70], 17.69%. Concerning bias determination, the results (mean, bias) for each plasma (0.0, 0.5, 1.0 and 1.5 IU/dl) were respectively: 0.19 IU/dl, 5.3%; 1.01 IU/dl, 5.9%; 1.90 IU/dl, 8.9%; 2.60 IU/dl, 6.5%. According the FVIII:C level, the measurement uncertainty was: 0.6 ± 0.25 UI/dL; 1.33 ± 0.48 UI/dL; 2.64 ± 0.94 UI/dL.

Conclusions: In this study, the very small overlap between values allowed us to specifically measure FVIII:C < 1 IU/dl. This could possibly distinguish "extremely" or "modestly" severe hemophilia A. To definitely validate an acceptable uncertainty limit, we have now planned to implement a national external quality control in 25 French haemostasis laboratories.

PB0843 | Platelet Activation in Patients with Severe Haemophilia A

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Background: Haemophilia A is an X-linked hereditary bleeding disorder that occurs predominantly in males and is a result of a deficiency in coagulation factor VIII. The disease is classified as mild, moderate or severe, depending on residual factor VIII activity, with each severity having a different phenotype (pattern of bleeding). Within the category of severe haemophilia A, however, the phenotype also differs. There have been several postulations regarding the differing phenotypes, one of which proposes that increased platelet activation status accounts for a milder bleeding phenotype in some patients, despite similar levels of residual factor VIII. However, previous studies have shown conflicting results in this regard.

Aims: To compare platelet activation in patients with haemophilia A and normal, healthy controls. In addition, the status of platelet activation in patients with severe haemophilia A was investigated to determine whether there was a relationship between increased platelet activation and severity of the bleeding phenotype.

Methods: The principle methodology involved measuring markers of platelet activation: P-selectin(CD62P), activated glycoprotein IIbIIIa (PAC-1), monocyte-platelet aggregates, using immunophenotyping by flow cytometry. Other variables were also measured or obtained from the history e.g.: ABO blood grouping, platelet count, age of patient, age of first bleed, presence or absence of arthropathy and number of joints involved.

Results: Increased expression of only P-selectin was demonstrated in the patient group (n = 19) compared to the control group (n = 14). There was no relationship between the expression of any of the platelet activation markers and severity of the phenotype i.e. presence of arthropathy, number of joints involved.

Conclusions: The study was unable to confirm an association between an increase in platelet activation status and a milder bleeding phenotype in patients with severe haemophilia A. A limiting factor was the small patient sample available. Larger studies are warranted to more accurately investigate platelet activation in these patients.

PB0844 | Inflammation in Hemophilia Is Marked by Up-regulation of LPS-binding Protein

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Background: Impaired clotting in hemophilia can cause activation of the innate immune system resulting in abnormal macrophage differentiation, delayed wound healing and protracted joint inflammation.
Aims: To assess inflammation in hemophilia.
Methods: We collected citrated plasma from 60 adult patients with hemophilia A/B, an average age of 38 years and a BMI of 27.3 kg/m2. Male blood donors served as controls (n = 54; age, 38 years; BMI, 27.1).
Results: ELISA testing of plasma samples revealed upregulation of C-reactive protein (CRP) and the pro-inflammatory adipokine leptin in hemophilia patients compared to healthy blood donors. Both parameters were high in a specific subset of patients with severe hemophilia and comparatively low in patients with mild hemophilia. Upregulation of CRP and leptin was particularly pronounced in overweight hemophiliacs compared to weight-matched controls suggesting that adipose tissue inflammation is aggravated by the underlying clotting defect. Implicating endotoxin translocation as a trigger for inflammation in hemophilia, we found markedly elevated levels of lipopolysaccharide-binding protein in conjunction with upregulated hepcidin in normal-weight hemophiliacs compared to controls, but no additive effects in overweight patients. Finally, we analyzed plasma from hemophilia B patients before and after transition from a conventional standard-half-life factor IX concentrate to a prophylactic therapy with an elongated half-life (EHL) FIX. Following up on the EHL prophylaxis after > 6 months, we observed in hemophiliacs a return of hepcidin plasma levels back to baseline values of healthy controls indicating that an effective prophylaxis can control bleeding as well as inflammation.
Conclusions: We demonstrate a specific link between hemophilia and low-grade inflammation that involves increased lipopolysaccharide levels in the blood circulation and subsequent adipose tissue inflammation. In addition, we present evidence that low-grade inflammation is caused by the underlying clotting deficit and that treatment with EHL factors ameliorates inflammation.

PB0845 | Differences in the Prediction of the Time above Target in Hemophilia A Patients Using a Chromogenic Assay and a One-stage Assay in Model-informed Precision Dosing

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Background: Monitoring of Haemophilia A therapy includes the accurate determination of factor VIII activity (FVIII:C) with specific assays. Importantly, the results obtained in different assays can alter depending on the methodology, drug-type, assay conditions, phenotype and severity of disease. Therefore, individualized therapy through model-informed prediction of pharmacokinetic parameters (i.e. time above target (TaT)) can be challenging, if assay discrepancies are insufficiently accounted for.
Aims: Comparison of the prediction of the time above target in Hemophilia A patients with a published population pharmacokinetic model using two FVIII assays.
Methods: Pre-dose FVIII:C and five post-dose samples (0.25-48 h) of 35 patients receiving a B-domain deleted (BDD) or a full-length product were measured on an ACL TOP in a chromogenic assay

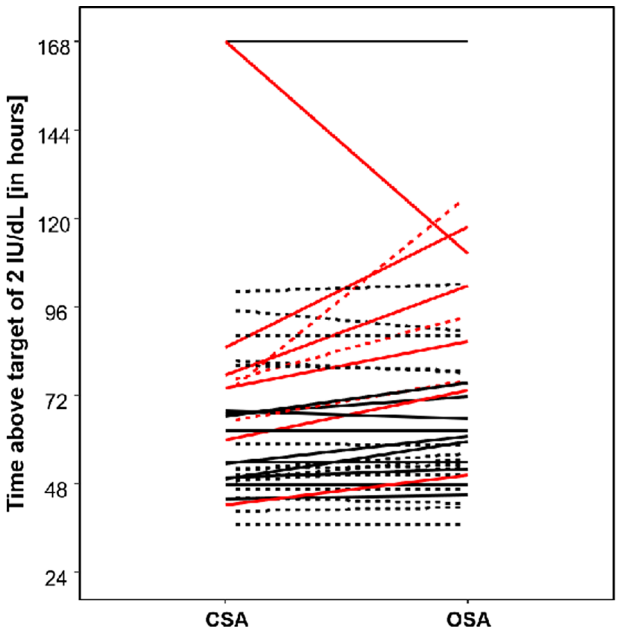

Figure 1 Predicted time above the target (TaT) of 2 IU/dL per patient using a chromogenic assay (CSA) and a one-stage assay (OSA) in model-informed precision dosing; patients received either a B-domain deleted product (dotted lines) or a full-length product (solid lines); red lines indicate a difference of more than 12 h TaT between the two assays

FIGURE 1 Predicted time above target

(CSA) using BIOPHEN FVIII kit and a one-stage APTT assay (OSA), with HemoSil SynthaSil APTT reagent, respectively.

Secondly, the most accurate population pharmacokinetic model identified in a previous study (PAGE 28 (2019) Abstract 8970) was used to forecast TaT (FVIII:C > 2 IU/dL) by supplying a previous trough concentration measured with either the CSA or OSA.

Results: For BDD the OSA was lower by 4% (mean, 36%SD) when compared to CSA, and for full-length products, the OSA was 6% higher (mean, 34%SD). A concentration dependency in both drug-types was observed, where CSA was higher than OSA at higher concentrations and *vice versa* at smaller concentrations.

The predicted TaT varied substantially among the two assays with differences larger than 12 h in 26% of the patients (Figure 1, red lines). While predictions in patients receiving the BDD product were in good alignment (Figure 1, dotted lines), predictions in full-length patients were mostly larger using OSA measurements.

Conclusions: Both assays can be used to predict the TaT in patients receiving the BDD product, while further improvements are needed in patients receiving a full-length product.

PB0846 | Decreased Platelet Surface Phosphatidylserine Predicts Increased Bleeding in Patients with Severe Factor VIII Deficiency

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Background: Bleeding phenotypes among individuals with severe factor VIII (FVIII) deficiency are variable, even with routine prophylactic FVIII administration. Activated platelets, in addition to their role in primary hemostasis, play a major role in coagulation by providing a phospholipid surface for assembly of the prothrombinase complex, thereby greatly enhancing the rate of thrombin generation.

Aims: To determine whether platelet function is associated with past and/or future bleeding in patients with severe FVIII deficiency receiving FVIII prophylaxis.

Methods: Following IRB-approved, written informed consent, platelet function in the presence or absence of *in vitro* activation by various agonists was assessed in 34 patients (median age 12.6 years) by multiple end points: platelet surface expression of phosphatidylserine, platelet surface glycoprotein (GP) VI, platelet surface activated GPIIb-IIIa, platelet surface P-selectin, the percentage of coated platelets, and the percentage of platelet-derived microparticles. A clinically significant bleeding episode was defined as one that required clinical intervention or pharmacotherapy such as additional FVIII replacement or antifibrinolytic agents. Laboratory investigators were blinded to clinical findings and clinical investigators were blinded to laboratory findings until completion of data collection.

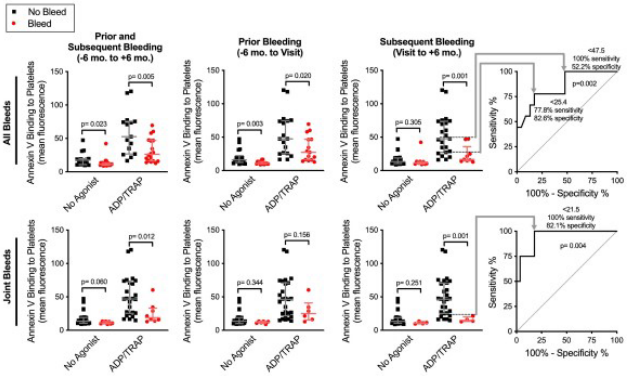

FIGURE 1 Association of platelet surface phosphatidylserine expression with overall, prior, and subsequent bleeding in patients with severe FVIII deficiency

Results: Decreased platelet surface phosphatidylserine expression (identified by geometric mean fluorescence of annexin V binding), both in the presence and absence of adenosine diphosphate (ADP) and thrombin receptor activating peptide (TRAP), demonstrated a significant association with both prior and subsequent bleeding in any location and also specifically with joint bleeds (Figure). No significant difference between patients with and without bleeding was observed for any of the other endpoints.

Conclusions: Lower platelet surface phosphatidylserine expression (as measured by annexin V binding to platelets) is associated with prior bleeding and, more importantly, is predictive of future bleeding in patients with severe FVIII deficiency receiving FVIII prophylaxis. A larger, multicenter study is needed to confirm the clinical utility of platelet surface phosphatidylserine as a biomarker for future bleeding.

PB0847 | ELISA-based Quantification of intracellular FVIII Protein in IPS Derived Vascular Endothelial Cells from HA-patients with Nonsense Mutations

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Background: Development of inhibitory antibodies against FVIII are a great burden in the treatment of Hemophilia A (HA). *F8* null mutations, like nonsense mutations, have a higher risk for inhibitor formation than other mutation types. The location of a specific nonsense mutation influences the risk for inhibitor development. *F8* nonsense mutations located in a region coding for the FVIII heavy chain (A1-A2) have a lower risk to develop inhibitors, while *F8* mutations located in the FVIII light chain (A3-C1-C2) have higher risk. By knowing this we generated IPS cells from ten severe HA-patients with different null mutations and differentiated the cells into vascular endothelial cells (vEC).

Aims: Our aim is to quantify F8 mRNA and intracellular FVIII protein in our patient-specific IPS derived vECs in order to understand the mutation-specific effect on inhibitor development.

Methods: Overlapping RT-PCR and Illumina human ht12-v4 expression array were used to analyze F8 mRNA expression in IPS differentiated vECs. Intracellular FVIII protein was quantified in wild type (Cm) and four patient vEC samples (L705X [StopA2], R1941X [StopA3], P-I22I [intron 22 inversion], P-LDA2 [Deletion Ex7-9]) using ELISA with different FVIII mAbs (GMA012/A2, GMA8011/LC, GMA8018/C1). Reference curves were prepared in the same sample lysis buffer.

Results: Full-length F8 mRNA was amplifiable for patients with nonsense mutation, showing highest mRNA expression for Cm and R1941X. Intracellular FVIII protein is detectable for Cm1 and P-I22I (20 mU/ml) when using mAbs GMA8018 and GMA8011. R1941X is only detectable with GMA8018 (10 mU/ml). P-LDA2 and L705X indicate an absence of protein.

Conclusions: We are able to quantify mU of intracellular FVIII in wild type and two HA-patient derived vEC samples. Absence of C1-specific ELISA signal in high-risk inhibitor mutation R1941X might indicate the existence of truncated protein.

PB0848 | The Relationship between Societal Costs Associated with Haemophilia and Disease Severity: A Regression Analysis Using CHESS II Data

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Background: Haemophilia (deficiency or absence of clotting factor) is a genetic bleeding disorder characterised by prolonged trauma-related or spontaneous bleeding events and associated complications. Haemophilia has been found to pose an economic burden on patients, caregivers, and the wider health care system. There is limited research, on the wider (non-drug related) costs of haemophilia and levels of severity, specifically across different levels within the mild range.

Aims: To assess the relationship between societal costs and severity levels, using interim real-world data from the 'Cost of Haemophilia in Europe: a Socioeconomic Survey - II' study (CHESS II).

Methods: Data were extracted from the CHESS II interim dataset, a societal perspective, prevalence-based study in mild, moderate, and severe adult haemophilia A and B patients across France, Germany, Italy, Spain, UK, Romania, Netherlands, and Denmark (n = 787). Disease severity was categorised according to baseline endogenous factor VIII/IX activity (IU/dl or %). 304 patients provided societal cost data. Mild haemophilia was subcategorized by endogenous factor VIII/IX expression (>5-20%, n = 14; >20-40%, n = 25). Societal costs consisted of healthcare system costs (excluding costs of treatment) and patient-centric costs, such as caregiver hours and time missed from work. A generalized linear model (GLM) was developed to investigate variation in societal costs across severity groups, adjusting for covariates age, BMI and country.

Results: The GLM model provided adequate fit, the average marginal effect at the mean was calculated from regression outputs. Analysis found that when compared to moderate and severe patients (0-5%, n = 265), subgroups of mild haemophilia '>5-20%' and '>20-40%' were €7,170 (p = 0.058) and €13,111 (p < 0.05) less costly, respectively. This analysis controlled for age, BMI and country effects.

Conclusions: This analysis suggests that a higher endogenous factor VIII/IX expression (>20-40%) may have a significant impact on reducing total per-patient cost from the societal perspective, accounting for age, BMI and country effects.

PB0849 | Modelling the Cost of Equivalent trough Level across Gene Therapy and Factor IX Replacement Therapy in Haemophilia B

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Background: Haemophilia is a genetic disorder caused by the deficiency of the body to produce FVIII (haemophilia A) or FIX (haemophilia B) clotting factors. Current standard of care in haemophilia is regular infusions with factor replacement therapy, with novel treatments seeking to minimise the administration burden of treatment while providing greater protection against bleeding episodes and joint damage.

Aims: The aim of this analysis is to quantify the consumption and cost of factor IX replacement therapy required to achieve a trough level equivalent to the hypothetical steady-state expression of a gene therapy over various alternative scenarios.

Methods: Time to FIX trough level (IU/dL) equivalent to hypothetical gene therapy expression was calculated based on published clinical trial outputs, assuming 33.8 hours for standard half-life (SHL) treatment and 82.1 hours for extended half-life (EHL) products. Gene therapy expression was modelled in 30, 50 and 100 IU/dL scenarios. The dose frequency required to maintain each trough level was modelled in order derive annualised factor consumption (Figure 1). Cost of achieving an equivalent trough level was calculated using cost per IU in three markets (Germany, Canada and the USA) and annualised. The unit costs for each market were €1.27, CAN$0.90 and USD$1.64 for SHL and €1.41, CAN$1.85 and US$3.14 for EHL.

Results: The annual cost of factor product to maintain a FIX trough level equivalent to the hypothesised gene therapy expression scenarios varied from €907,370 to €15,648,929 in Germany, CA$1,029,837 to CA$11,089,792 in Canada and from $1,747,605 to $20,187,119 in the USA (Table 1). This was across assumed expression of 30, 50 and 100 IU/dL.

TABLE 1 Annualised cost across three markets, Canada, Germany and the USA

	SHL Yearly Cost			EHL Yearly Cost		
Country	30 (IU/dL)	50 (IU/dL)	100 (IU/dL)	30 (IU/dL)	50 (IU/dL)	100 (IU/dL)
Germany (€)	1,974,807	3,430,172	15,648,929	907,370	1,576,070	7,190,254
Canada (CA$)	1,399,470	2,430,831	11,089,792	1,029,837	1,788,791	8,160,717
US (US$)	2,547,501	4,424,922	20,187,119	1,747,605	3,035,530	13,848,517

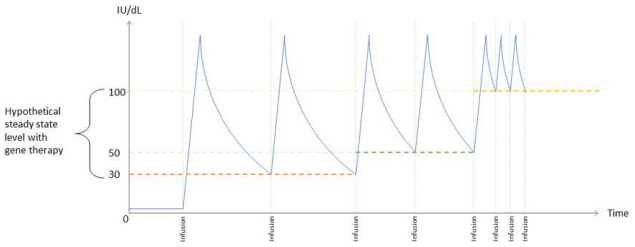

FIGURE 1 Infusion frequency diagram

Conclusions: This analysis demonstrates the magnitude of cost required to achieve equivalent trough level across factor replacement therapy and a hypothetical steady-state gene therapy in haemophilia B patients in Germany, Canada and the USA.

PB0850 | Intracellular Visualization of FVIII Protein in Selected Knockouts in the Conventional and Alternative Secretion Pathways

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Background: FVIII is an essential protein that plays a major role in the coagulation cascade. FVIII is produced by a special subpopulation of endothelial cells. Interestingly the mode and route(s) of the secretion of FVIII are still not well characterized. Early pioneer work describes the role of LMAN1, MCFD2, Bip, Calnexin, Calreticulin and COP vesicles in the intracellular trafficking of FVIII. Additionally, we previously reported the involvement of ATG8 gene family in the F8 secretion. CRISPR/Cas9 knockouts of several proteins within the F8-secreting HEK cells modulate the secreted levels of F8 for LMAN1 KO by -75%, MCFD2 KO by -35%, Calnexin KO by + 80%, Calreticulin KO by -40%, GABARAP KO by -40%, GABARAPL1 KO by + 70%, GABARAPL2 KO by + 100%, ATG7 KO by + 35-100%. We also suggested the presence of two secretory pathways:
1) traditional one that involves COPII vesicles and
2) an alternative pathway that involves ATG8/autophagy related proteins.
Aims: To visualize the intracellular FVIII routes in different knockouts.
Methods: We visualized the path of FVIII protein by co-staining it in combination with different markers for COPII (Sec31a antibody) that acts in the ERGIC compartment, the lysosomes (LAMP1 antibody), the autophagosomes (LC3B antibody) and the trans-Golgi (TGN46 antibody).
Results: Our staining results show absence of co-localization in the Golgi for the LMAN1 and MCFD2 as well as a visual decrease for GABARAP and GABARAPL1 knockouts, while co-localization with autophagosomes after treatment with chloroquine in all knockouts and to a less extent in wild type was also obvious.
Conclusions: These results strongly support the involvement of autophagy machinery with in F8 intracellular trafficking.

PB0851 | Proteomic Profile of Blood Outgrowth Endothelial Cells (BOEC) from a Severe HA Patient with the Reintroduction of FVIII

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Background: Hemophilia A (HA) is a monogenic disease linked to chromosome X which causes FVIII deficiency. As current therapies have important side effects, gene therapy has been addressed. Previously, we engineered and characterized integrating lentiviral vectors to re-introduce FVIII (LVF8) into Blood Outgrowth Endothelial Cells (BOECs) of a severe HA patient with a splice mutation (c.6273G>A; p.K2091K), to restore FVIII activity by gene addition. As this is not a direct gene correction, it is unknown the global influence this will have.
Aims: To apply a proteomic approach to understand the intrinsic protein profile of BOECs from a severe HA patient (c.6273G>A) following the reintroduction of FVIII (LVF8).
Methods: To confirm protein expression and secretion of FVIII in the presence of LVF8 (HA-LVF8), we performed western blots (WB) for FVIII on the lysates and secretome. Intrinsic protein changes were investigated by proteomics using mass spectrometry (MS), and 2D-electrophoresis (2DE), including a matched healthy control. Protein spot data was analyzed by PDQuest software.
Results: The WB for FVIII confirmed the production and secretion of FVIII from HA-LVF8. Proteomic analyses by 2DE comparing HA to HA-LVF8 and a healthy control showed that 29.5% of proteins were altered overall, of which of these only 15.7% returned to the levels observed in the healthy control. Similarly, of the 1127 proteins identified by MS, the greatest differences were seen for HA-LVF8, with

27.1% significantly ($p < 0.05$) altered with respect to HA and 31.7% ($p < 0.05$) with respect to control, while only 7.5% ($p < 0.05$) protein changes were seen between HA and control.

Conclusions: The collective study of intrinsic protein changes highlights that that greatest protein alterations are seen with the reintroduction of FVIII. *In vitro* studies are currently ongoing in immortalized BOECs from HA (c.6273G>A) and controls to address the FVIII direct effects.

PB0852 | The Synergistic Effect of Emicizumab and Factor VIIa on Thrombin Generation Is Dependent on Factor VIIa Activation of Factor IX

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Background: Patients with hemophilia A can be treated with emicizumab, a bivalent antibody which enhances factor IXa (FIXa) activation of factor X (FX). Breakthrough bleeding in patients with inhibitors has been treated safely with recombinant FVIIa (rFVIIa). At therapeutic levels rFVIIa can activate FX and FIX by both TF-dependent and lipid dependent mechanisms.

Aims: The goal of this study was to assess the role of rFVIIa activation of FIX on thrombin generation in the presence of emicizumab.

Methods: Thrombin generation assays were done in hemophilia AB plasma (HemAB), prepared from hemophilia B plasma depleted of FVIII by adding a neutralizing antibody. Emicizumab was added at 55 µg/mL (clinical steady state levels). rFVIIa was added in at varying doses with 25 nM corresponding to a clinical dose of 90 µg/mL.

Results: In the HemAB plasma with 1 pM tissue factor, there was essentially no thrombin generation (Figure 1). Addition of emicizumab

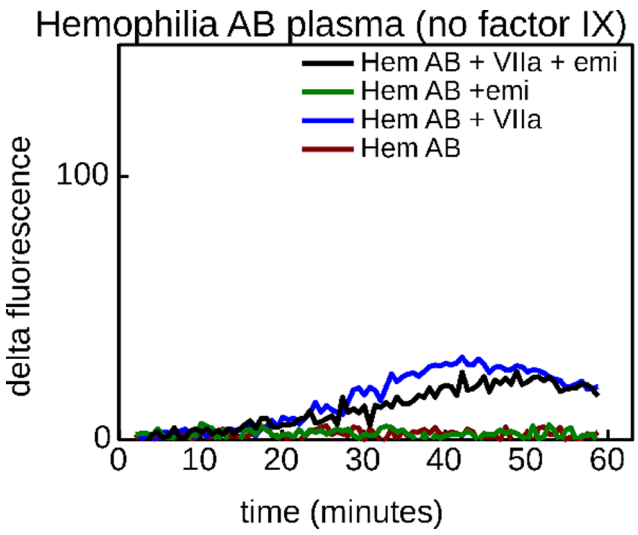

FIGURE 1 Effect of 25 nM factor VIIa on thrombin generation in Hemophilia AB plasma with and without emicizumab

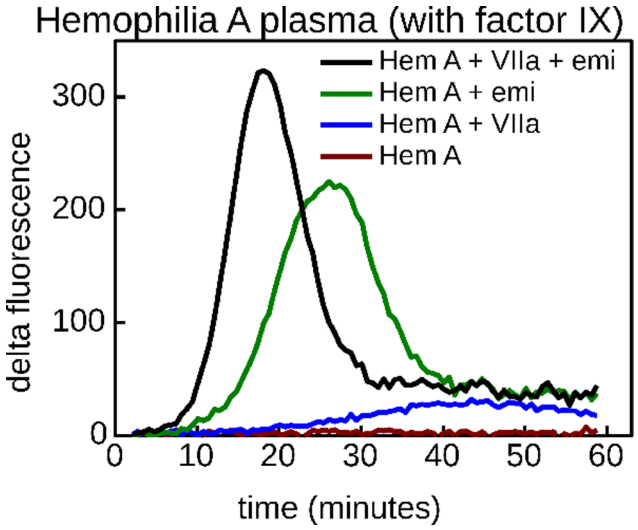

FIGURE 2 Effect of 25 nM factor VIIa on thrombin generation in Hemophilia A plasma with and without emicizumab

did not increase thrombin generation. Addition of rFVIIa gave an increase in thrombin generation, which did not change by addition of plasma levels of FIX. In contrast emicizumab with FIX gave measurable thrombin generation through FVIIa/TF activation of FIX (Figure 2). An additional increase in thrombin generation was observed by the combination of rFVIIa and emicizumab in the presence of FIX.

Conclusions: In hemophilia A or B plasma, rFVIIa activation of FX is the only source of the FXa needed for thrombin generation; FIX activation does not contribute to thrombin generation. In the presence of emicizumab, while rFVIIa can still activate FX, the emicizumab complex with FIXa is an additional source of FX activation. Thus rFVIIa activation of FIX explains the synergistic effect in thrombin generation observed when combining rFVIIa with emicizumab. The generation of FIXa at a site of injury is consistent with the safety profile observed in clinical use.

PB0853 | APC: A New Suspect in the Hunt for a Mechanism of Bone Disease in Hemophilia

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Background: Increased rate of fragility fracture and decreased bone mineral density are now well-recognized comorbidities of hemophilia. In osteoblasts from FVIII-deficient mice, this pathology is reversible via *in vivo* or *ex vivo* factor replacement. Decreased thrombin generation was first suspected to cause this pathology; however, our work suggests thrombin impairs differentiated function in osteoblasts *in vitro*.

Aims: We seek to demonstrate decreased active protein C (APC) generation, not thrombin, is a driver of bone disease in hemophilia.

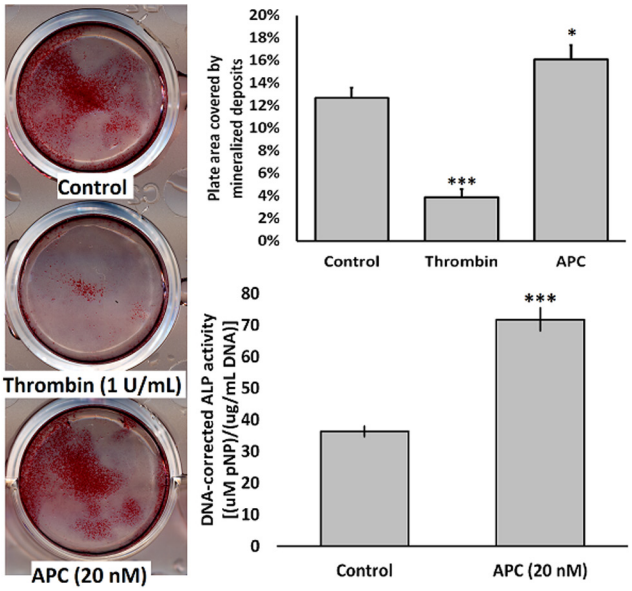

Figure 1: (Left) Mineralized HOS TE85 cultures stained with alizarin red. (Top right) Percent HOS TE85 culture plate area covered by mineralized deposits. (Bottom right) Alkaline phosphatase activity in HOS TE85 cultures. (* $p < 0.05$, *** $p < 10^{-4}$). Error bars are SEM.

FIGURE 1

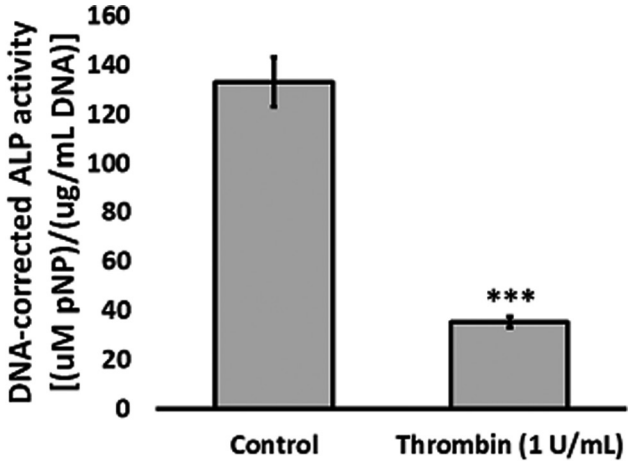

Figure 2: Alkaline phosphatase activity in murine osteoblast cultures. (*** $p < 10^{-5}$)

FIGURE 2

Methods: Bone marrow stromal cells are isolated from FVIII deficient mice and wild-type controls and enriched for an osteoblast phenotype. Human osteosarcoma-TE85 (HOS-TE85) cells are acquired from continuous cultures. Cells are cultured with α-MEM containing 10% FBS, penicillin, streptomycin, ß-glycerophosphate, and ascorbic acid. Calcified deposits are stained with alizarin red. DNA, protein, and alkaline phosphatase (ALP) in cell lysate are measured via spectrophotometric assays. Statistical comparisons are made using Student's T-test.

Results: In murine In HOS TE85, thrombin causes a ~70% decrease in mineralization compared to controls ($p < 10^{-4}$, Figure 1) while APC addition results in a ~30% increase ($p < 0.05$). APC also results in a ~100% increase in ALP activity ($p < 10^{-4}$, Figure 1). Thrombin addition to murine osteoblast-like cells *in vitro* results in a ~70% decrease in ALP activity ($p < 10^{-5}$, Figure 2).

Conclusions: Thrombin causes HOS TE85 and murine osteoblasts to fall behind controls in proliferation and ALP expression, ultimately failing to mineralize. APC, however, appears to upregulate osteoblast differentiated function, namely ALP expression and mineralization in HOS TE85. This suggests decreased APC generation is a cause of bone disease in hemophilia and a potential therapeutic target. These experiments are ongoing and new findings will be presented. Understanding this mechanism is vital to preventing bone loss in hemophilia without raising the risk of inhibitor formation or other complications.

PB0854 | The Annual Global Survey Continues to Close the Gap in Data Collection of Bleeding Disorders

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Background: In 1998, the World Federation of Hemophilia (WFH) started the Annual Global Survey (AGS) as a surveillance project on people with bleeding disorders. This has since grown to be a valuable monitoring tool, providing impactful information to the bleeding disorders community.

Aims: The 20th anniversary of the Report on the AGS included the patterns and trends over time, as well as the progress in patient identification, treatment and care globally.

Methods: The Report on the AGS is an annual cross-sectional survey. Each year WFH National Member Organizations (NMOs) are asked to complete the survey including questions on number of identified patients, level of hemophilia care and factor use for patients with hemophilia A and B, von Willebrand disease and other bleeding disorders, in their country. The survey in 2019 was sent to 140 NMOs.

Results: In 2019, a record high 125 countries participated and a total of 337,641 patients with bleeding disorders were identified. Treatment of bleeding disorders continues to advance and as a result, in 2019, the AGS began to differentiate the reporting of standard half-life and extended half-life products, allowing the data to be more reflective of the changing landscape in treatment. Factor use in high-income countries has grown at a much faster rate than in lower-income countries and differences in levels of care still exist. Between 2002 and 2019, the median factor VIII IU per capita increased from 3.88 to 6.19 among high income countries, and from 0.01 to 0.04 among low income countries.

Conclusions: For over 20 years, the AGS has been an important source of data on bleeding disorders and the data continues to be effectively used for advocacy and priority setting leading to better care of people with bleeding disorders around the world.

PB0855 | Non-Human Glycans Regulate FVIII Inhibitor Formation in Mice

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Background: Infusion of recombinant fVIII products can represent a life-saving intervention in patients with hemophilia A. However, patients can develop neutralizing antibodies to fVIII, otherwise known as inhibitors, which prevent fVIII function and directly increase morbidity and mortality. The development of inhibitors can vary depending on the type of recombinant product employed, with previous studies suggesting that 2nd generation baby hamster kidney (BHK)-derived fVIII products display higher immunogenicity than 3rd generation Chinese hamster ovary (CHO)-derived fVIII. However, the underlying mechanisms responsible for these differences remain incompletely understood.

Aims: To define the underlying mechanism responsible for differences in immunogenicity of 2nd generation versus 3rdgeneration recombinant fVIII products.

Methods: CHO and BHK cells, in addition to the 2nd generation and 3rd generation recombinant fVIII products they produce, were analyzed for glycan content by lectin-based analyses and mass spectrometry for glycan content. fVIII microarrays were also developed and employed to examine lectin and serological engagement 2nd generation and 3rd generation recombinant fVIII products. Finally, 2nd generation and 3rd generation fVIII products were infused into WT or a1-3 galactosyl transferase knockout recipients, followed by assessment of anti-fVIII antibody formation by ELISA and Bethesda approaches.

Results: Our results demonstrate that BHK cells express higher levels of the non-human carbohydrate a1-3 galactose (aGal) than CHO cells, suggesting that aGal incorporation onto fVIII may result in anti-aGal antibody recognition that could positively influence inhibitor development. Consistent with this, BHK-derived fVIII exhibited increased levels of aGal, which corresponded to increased reactivity with anti-aGal antibodies. Infusion of BHK-derived, but not CHO-derived, fVIII into aGal knock out mice, which spontaneously generate anti-aGal antibodies, results in significantly higher inhibitor formation.

Conclusions: These results demonstrate that the increased levels aGal on BHK-derived fVIII can influence inhibitor formation and suggest that post-translational modifications of recombinant fVIII products with non-human carbohydrates may influence fVIII inhibitor development.

PB0856 | A Comparative Analysis of the Immune Response to Homologous Coagulation Factors V and VIII

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Background: One-third of severe hemophilia A patients develop (f)actor VIII alloinhibitory antibodies conferring the most significant treatment-related complication. From a protein therapeutic standpoint, FVIII is considered highly immunogenic. Further, compared to other coagulation factors, FVIII autoantibody formation is the highest. Collectively, this raises the possibility that the biochemical nature of FVIII is distinct from an immune perspective. The homology of FV and FVIII would suggest that these proteins may illicit a similar immune response; yet this is not supported by the very low prevalence of FV autoantibodies.

Aims: To evaluate the immune response to homologous B-domain deleted forms of human FVIII and FV proteins in mouse models.

Methods: B-domainless FVIII or FV (58% sequence homology) protein were administered via tail vein injection at a dose of 5 μg/mouse weekly for 5 weeks, then every-other-week in 3 murine models on the C57BL/6 background. The models were: wild type (WT) mice; WT-mice treated with an antisense oligonucleotide to knock down FV to < 5% of normal (FV-ASO); and transgenic mice with no plasma FV and low platelet FV (plt-FV). Anti-factor IgG concentration and Bethesda assays of functional inhibition were measured in longitudinal plasma samples.

Results: In WT and FV-ASO mice, the FV immune response was undetectable/low (< 10 BU), while the FVIII immune response was robust (>200 BU). The FV immune response remained low in plt-FV mice; surprisingly, the FVIII immune response was ~10-fold higher compared to WT and FV-ASO mice infused with FVIII (>2000 BU; Fig. 1).

Conclusions: To our knowledge, these data are the first to compare the immune response to FVIII relative to its homologous protein, FV, and demonstrate a markedly increased murine immune response to FVIII relative to FV. This preliminary data also support plasma FV may be relevant to FVIII immunogenicity, suggesting shared FV/VIII epitopes may be particularly important for FVIII tolerance.

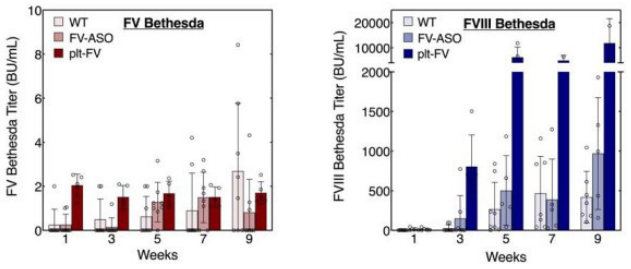

FIGURE 1 FV vs. FVIII Immune Response in Murine Models

PB0857 | Differential Systemic Transcriptional Responses to Induced Joint Bleeding in Wild-type and Factor VIII-deficient Mice

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Background: Little is known about systemic responses to joint bleeding and Factor VIII (FVIII) replacement in hemophilia.
Aims: To quantify systemic gene expression changes in wild-type and FVIII-deficient ($FVIII^{-/-}$) mice at baseline and after induced hemarthrosis, +/- FVIII replacement.
Methods: Hemarthrosis was induced by sub-patellar puncture in wild-type BALB/c (WT) mice or $FVIII^{-/-}$ mice on a BALB/c background +/- recombinant human FVIII (rhFVIII) or rhFVIII-Fc prophylaxis. Spleens were harvested at baseline ($FVIII^{-/-}$ mice) and day 3 or 2 weeks post-injury. Gene expression analyses were performed by RNA sequencing followed by functional enrichment analyses.
Results: Knee injury caused gross hemarthrosis in untreated $FVIII^{-/-}$ mice only (day 2 hematocrit: 27%), resulting in 5381 differentially expressed genes (DEG) on day 3 (log_2 fold-change (log2FC) > 1.0 or < -1.0; $p < 0.05$) with enrichment of cell cycle and immune pathways. Injury in WT mice elicited 1307 DEG and the response was absent in $FVIII^{-/-}$ mice administered rhFVIII(-Fc), suggesting transcriptional responses are associated with massive hemarthrosis and altered with FVIII replacement. Numerous genes (log2FC>1.5; $p < 0.05$) and pathways relating to hemostasis, platelet activation, and neutrophil degranulation were upregulated in WT but not $FVIII^{-/-}$ mice on day 3. Moderate gene upregulation (log2FC>0.5; $p < 0.05$) also caused enrichment of platelet activation pathways in uninjured $FVIII^{-/-}$ mice on day 3 after rhFVIII treatment. Most transcriptional responses returned to baseline by 2 weeks.
Conclusions: Hemarthrosis in $FVIII^{-/-}$ mice causes a large, transient systemic transcriptional response involving immune pathways, which is altered with FVIII treatment. Differences between $FVIII^{-/-}$ and WT mice suggest responses are mostly driven by bleeding rather than injury alone, and that FVIII deficiency may compromise platelet activation after joint bleeding. These findings warrant further investigations into hemostatic and immune modulatory functions of FVIII.

PB0858 | Molecular Analysis of Factor VIII Genes in Patients with Hemophilia A; Identification of Specific Mutation in Colombian Patients with Inhibitors

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Background: Hemophilia A in an X-linked bleeding disorder caused by mutations in the FVIII factor gene. It has been shown that genetic factors constitute a risk factor for the development of inhibitors.
Aims: The objective of this study was to identify the specific variations of the FVIII genes of patients of with Hemophilia A and to determine if this mutation helps to predict by literature whether it has a risk of generating inhibitors.
Methods: Descriptive cross-sectional study where whole blood samples were collected from 12 Colombian patients of the IPS "Integral solutions SD" sent to the Molecular Hemostasis laboratory in Bonn, Germany, where the genetic material (DNA) was analyzed.
Results: Twelve patients with a clinical diagnosis of severe hemophilia A who presented or present inhibitors with an age range between 6 and 48 years with a median age of 13.5 were analyzed. The inversion of intron 22 in 6 patients (50.0%) was found in the molecular analysis, followed by a small duplication in 2 patients (16.7%) and 1 patient for the inversion of intron 1 (8.3%) as well as for a large deletion (8.3%), a nonsense mutation (8.3%) and a splice-site (8.3%) figures similar to other studies. 58.3% of the patients evaluated have mutations with a high risk of inhibitor development.
Conclusions: 83.3% of the patients evaluated presented null mutations, however the presence of high inhibitor titers was 66.7%, being more frequent in patients with inversion of intron 22. By Knowing the type of mutation and its risk factor of generating inhibitors will allows the designing an individualized treatment regimen in factor replacement therapy.

PB0859 | A Generic Recombinant Factor VIIa Is Comparable to the Branded Novoseven in In-vitro and Pharmacokinetic Studies in Primates

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Background: Recombinant factor VIIa (NovoSeven®, NovoNordisk, Bagsvaerd, Denmark) is used in the management of bleeding in patients with hemophilia. A generic version of NovoSeven® is also developed for the same indication (AryoSeven™, AryoGen, Tehran, Iran).
Aims: The purpose of this study was to compare several batches of generic of rFVIIa, namely AryoSeven™, with multiple commercially available batches of branded NovoSeven® to determine biosimilarity.
Methods: Four commercially available lots of Novoseven® were obtained through US and European sources and compared to 6 clinical batches of AryoSeven™. Samples were profiled in vitro using surface enhanced laser desorption ionization (SELDI) mass spectrometry, gel electrophoresis (GE), immunoblotting studies, and procoagulant activity as measured by a clot based method. Thrombin generation studies were carried out using NHP supplemented with 0-1.0 ug/ml rFVIIa. The pharmacokinetic profiles representative batch each of the NovoSeven® and AryoSeven™ were studied in primates after a 50 ug/kg bolus IV.
Results: The molecular profiles, GE and immunoblotting studies of the two groups of agents showed a comparable profile with identical

sharp peaks at 50 kDa and 29 kDa. In the clot based functional assays performed using citrated whole blood and retrieved plasma, the two FVIIa products produced comparable procoagulant effects in the PT and aPTT assays In the primate pharmacokinetic studies at a dose of 50 ug/kg, both groups exhibited similar pharmacokinetic profiles in terms of biologic half-life, C_{max}, volume of distribution and other parameters.
Conclusions: The pharmacokinetic profile of the NovoSeven® and AryoSeven™ as studied in primates by measuring the factor VIIa level for up to 4 hours post-administration were found to be comparable. These results support the hypothesis that NovoSeven® and AryoSeven™ are biosimilar. Further clinical validation studies are warranted.

HEMOPHILIA - CLINICAL

PB0860 | Long Term Joint Outcomes through Ultrasound for Hemophilia: First Interim Results from Prospective J-DaUPHIN Study

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[2]*IMSUT Hospital of the Institute of Medical Science, Department of Joint Surgery, Tokyo, Japan*

Background: Early-stage joint damage can be evaluated using musculoskeletal ultrasonography (MSKUS). Yet, the relationship between the MSKUS and long-term outcome has not been investigated.
Aims: Provide preplanned interim results (registration; visit1 and 6 months; visit2) of J-DaUPHIN (Joint Damage Ultrasonography Patients Hemophilia IN Japan) study.
Methods: Prospective, observational, non-interventional, ethics-approved study conducted at Ogikubo hospital. Inclusion criteria was for subjects who are congenital hemophilia without inhibitors, over 6 years of age, received prophylaxis with the infusion records at least 6 months before registration. The MSKUS (joint subject to elbows, knees and ankles of Arnold stage I to III), adherence to prophylaxis, AjBR spontaneously (s-AjBR) and HJHS are planned to perform every half-year for 3 years. Adherence = actual infusions/pre-planned infusions per 6 months. S-AjBR= doubled spontaneous bleedings by home infusion record per 6 months.

TABLE 1 HEAS-US score at registration (Visit 1) and 6 months (Visit 2)

	R-Elbow		L-Elbow	
	Visit 1	Visit 2	Visit 1	Visit 2
Joints	121	59	123	57
Median (min-max)	0.0 (0-4)	0.0 (0-8)	0.0 (0-4)	1.0 (0-4)
	R-knee		L-knee	
Joints	146	66	152	71
Median (min-max)	0.0 (0-4)	0.0 (0-2)	0.0 (0-6)	0.0 (0-4)
	R-ankle		L-ankle	
Joints	81	33	83	36
Median (min-max)	0.0 (0-6)	0.0 (0-5)	0.0 (0-3)	0.0 (0-4)

Results: Results are as of Mar 31, 2019 cutoff. In total 183 patients were registred. Median age was 33 years old. Number of joints were elbows (R 121, L 123), knees (R 146, L 152) and ankles (R 81, L 83). The number of registered ankles were small, because many patients had end-stage arthrosis. The median HEAD-US scores were 0 (Table1).
Wilcoxon signed-rank test was performed for adherence rate to prophylaxis, s-AjBR and HJHS in 98 patients who reached visit 2 (Table 2). There was no significant change in adherence and HJHS. However, s-AjBR in Hemophilia A was not change, s-AjBR in Hemophiia B was significantly worsened. The reason seems 3/17 patients had frequent bleedings after start of this study.Additionally, despite total 209 bleedings detected by MSKUS, only 44 bleedings (21%) were written in patients' record; indicated possibility of detection of asymptomatic bleedings.
Conclusions: Early results from this prospective/ongoing study about long-term outcomes of ultrasound did not offer the improvement of outcomes. We are still working on.

TABLE 2 Comparison table between visit 1 and visit 2

Adherence rate of prophylaxis	Hemophilia A			Hemophilia B		
	Visit 1	Visit 2	p value	Visit 1	Visit 2	p value
N	81	81		17	17	
Median (min-max)	98.72 (42.3-173.1)	98(28.8-190.0)	0.9332	95.83 (53.8-176.9)	100 (42.3-181.5)	0.4169
Annual spontaneous joint bleeding rate						
N	81	81		17	17	
Median (min-max)	0 (0.0-180.6)	0 (0.0-130.3)	0.2067	0 (0.0-6.0)	0 (0.0-52.2)	0.0371
Hemophilia Joint health score						
N	69	69		16	16	
Median (min-max)	15.0 (0-46)	15.0 (0-40)	0.1556	14.5 (0-47)	14.0 (0-44)	0.7168

PB0861 | Healthcare Resource Utilization and Cost Burden of Hemophilia B in the US

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Background: Prior studies have investigated the economic burden of hemophilia B, but focused on outcomes within the overall study sample without stratifying by disease severity or clinical profile.
Aims: To quantify the healthcare resource utilization and costs associated with hemophilia B, both overall and by clinical profile, in the US.
Methods: Adult male patients were selected from the MarketScan Commercial and Medicare Supplemental database (06/2011-02/2019) if they had ≥ 2 diagnoses of hemophilia B and continuous enrollment during the 1-year baseline before and 1-year follow-up period after a randomly selected hemophilia B diagnosis date (index date). Patients were matched 1:1 to a control group without bleeding disorders based on birth year, region, insurance plan type, and index year. Clinical profile was categorized as severe, moderate-severe, moderate, or mild using a claims-based algorithm that incorporated frequencies of factor IX therapy claims and hemorrhage events during baseline. Resource use and cost outcomes during follow-up were compared between patients with hemophilia B versus matched controls using McNemar's tests for categorical variables and Wilcoxon signed-rank tests for continuous variables.
Results: Patients with hemophilia B (N = 454) and matched controls (N = 454) were 46 years old on average. Resource use was significantly higher among patients with hemophilia B than controls in terms of inpatient, outpatient, emergency department, and specialist visits and prescribed opioid use (all $p < 0.05$). Average annual total costs were $205,783 among patients with hemophilia B (vs. $8,052 for controls; $p < 0.001$) and increased by clinical profile, ranging from $83,291 (mild) to $643,979 (severe). Across all clinical profiles, patients with hemophilia B had significantly higher medical, pharmacy, and total costs than controls (all $p < 0.001$).
Conclusions: Hemophilia B is associated with substantial healthcare resource use and costs in the US. Such significant burden highlights that unmet needs remain in hemophilia B.
Financial support: uniQure provided funding for this research.

PB0862 | Hemophilia A with Spontaneous Left Frontotemporal Epidural Hematoma (EDH) and Left Frontotemporoparietal Subdural Hematoma (SDH)

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Background: Spontaneous intracranial hemorrhage is a rare emergent hemorrhagic event and challenging condition to treat in hemophilia patients.
Aims: This case report is discussed to look at the management of hemophilia in hospital with limited examination of coagulation factors.
Methods: Case report data obtained from patient medical records.
Results: A 17-year-old male diagnosed with hemophilia A came to hospital with loss of consciousness at his home. Three days before he had headache on the left side, vomiting without nausea and gradually drowsiness. No prior history of trauma. The patient's family history is unknown. On examination in emergency unit the blood pressure, heart rate and respiratory rate was normal; and neurological examination revealed he was somnolent with sign of left facial nerve damage. A brain CT-scan revealed a left frontotemporal epidural hematoma, left frontotemporoparietal subdural hematoma (both volume ± 25 cc) and midline shift > 5 mm. Initial laboratory test revealed hemoglobin of 11.4 g/dL (normal range, 13.5-17.5 g/dL), white blood cell (WBC) count of 11,800/mm^3 (normal range 4,400-11,300/mm^3), platelet count of 461,000/mm^3 (normal range 150,000-450,000/mm^3) and activated partial thromboplastin time (aPTT) of 92.5 s, (control 22.9 s). Factor VIII activity cannot be tested immediately when patient arrived because of limitation in laboratory (factor VIII activity testing only running once a month). He planned to get surgery if aPTT ≤ 2 times normal limit, meanwhile he got close monitored and head up 30°. He got factor VIII or recombinant factor VIIa alternately and monitored with serial aPTT. On day nineteen factor VIII activity was 3.30% (normal range 50-160%) and aPTT was 58.1 s (control 25.3 s). His sense back to alert since day four. Patient got better although without surgery.
Conclusions: In limited resource and capability to do factor assay; the serial aPTT, closely monitoring, and following clinical symptoms were important in management hemophilia patient with spontaneous intracranial hemorrhage.

PB0864 | Use of Thromboelastography and Thrombin Generation Assay in a Patient Treated with Emicizumab

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Background: Emicizumab, a bispecific monoclonal antibody replacing the function of missing activated factor VIII, has been approved as prophylaxis for patients with haemophilia A and FVIII inhibitors. However, this molecule has a strong interference effect on assays based on the activated partial thromboplastin time, such as one-stage assays for FVIII, complicating the treatment monitoring.
Aims: The authors report the case of a 47 years-old patient with severe hemophilia A and FVIII inhibitor, who benefited from a switch toward emicizumab. Clinical tolerability was immediately very good. Biological tests were assessed after treatment initiation, especially thromboelastography (TEG®, Haemonetics), and thrombin generation assay (TGA).

Methods: The TEG® assay, made with the CRT reagent (activating simultaneously the intrinsic and extrinsic coagulation pathways) showed a normal coagulation profile. Regarding TGA, initiated with tissue factor, the peak was decreased compared to a healthy population (118 nM/min; normal values 200 - 416) and was more than 50% lower compared to the level measured before the switch and during immune tolerance induction (253 nM/min).

Results: Two months after emicizumab initiation, a replacement of his knee arthroplasty was indicated. A perioperative substitution with eptacog alpha and tranexamic acid was applied. Immediate follow-up showed a hematoma, requiring an arthroscopic knee washout with intensification in eptacog alpha injections. Biological monitoring, with TEG® and TGA, was also performed throughout the perioperative period. While TEG® parameters remained very similar to the values observed before the surgery, the lag time of TGA was shortened according to the dose of eptacog alpha.

Conclusions: TGA seems to be a relevant assay to evaluate the effect of eptacog alpha druing a surgery under emicizumab. Although TGA is not a test appropriate to urgent situations, its integration in the monitoring tests for a surgery under emicizumab seems to be interesting, but needs to be evaluated on a larger population of patients.

PB0865 | Finding the Efficacy of Low Dose Prophylactic Regimen in Children Diagnosed with Severe Hemophilia

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Background: Hemophilia A and B are rare inherited bleeding disorders caused by deficiency of coagulation factor VIII (FVIII) and factor IX (FIX), respectively. These disorders are followed by recurrent episodes of bleeding, primarily into the joints and muscles.

Aims: Our study aims to find the efficacy of prophylactic treatment regimen for patients with severe hemophilia

Methods: Children between 4-8 years with severe hemophilia A (FVIII < 1%) without inhibitors and a HJHS score of < 5 were offered prophylaxis with a plasma-derived clotting factor concentrates pdCFCs. at a lower dose of 10-15 IU/kg 2-3 x/week, depending on weight and response. Their bleeding rate over 12 months prior to initiating prophylaxis was documented. All bleeding was documented while on prophylaxis, with special emphasis on joint or muscle bleeds. Inhibitors were monitored every 5-10 exposures.

Results: A total of 28 children were treated with this prophylactic regimen. The median age was 6 years old.

The median annualized bleeding rate (AdBR) prior to prophylaxis was 4 (0-15). No patient had any muscle or CNS bleeding during this period. Two patients developed transient inhibitors (low titer 1.1 BU, 2.5 BU & 2.0 BU respectively) after 7, 17 and 100 exposures. These resolved within 4-12 weeks with continuing prophylaxis and have remained negative since. None of the other patients developed inhibitors after a median of 85 exposures (9 to 124). Compliance was complete except for one patient who missed the prophylactic treatment twice.

Conclusions: It is evident that decrease in bleeding rate, preventing destruction of joint and its function and improvement in quality of life can be achieved through low dose prophylaxis regimen. Therefore low dose prophylaxis may be considered as an initial step for pro-phylaxis regimen, particularly in resource limited settings.

PB0866 | Endothelial Specific Isoform of Type XVIII Collagen (COL-18N): A Marker of Vascular Integrity in Hemophilic Arthropathy

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Background: Hemophilic (HF) arthropathy is a major complication in hemophilia. Collagen IV, XV and XVIII are responsible for maintaining the integrity of the vessel wall in the joint. Following joint remodelling and damage, the short isoform of collagen type XVIII is degraded, releasing measurable fragments.

Aims: Our goal was to quantify the measurable fragments of the endothelial specific isoform of type XVIII collagen (COL-18N) in severe hemophilia A patients and to assess its relation to the clinical and radiological data as well as hemophilia joint health score (HJHS), functional independence score for hemophilia (FISH), and hemophilia quality of life.

Methods: This cross-sectional study included 50 severe hemophilia A patients recruited from the Pediatric Hematology / Oncology unit, Children's Hospital, Ain Shams University, Cairo, Egypt. Quantification of serum collagen type XVIII was done by ELISA. Assessment of joint status clinically using FISH and HJHS scores and radiologically by X-rays and ultrasound was performed.

Results: Hemophilia A patients had significantly higher median collagen type XVIII levels compared to the control group. Inhibitor positive hemophilia A patients and inhibitor negative as well as hemophilia A patients on NSAID and those not had comparable collagen type XVIII levels. Patients with ≥ 2 target joints had significantly higher collagen type XVIII level compared to those with one or those without target joints(p=< 0.001). There were significant positive correlations between collagen type XVIII level and the total HJHS, hem QOL, the Ultrasound total score and annual bleeding rate (p = 0.000, p = 0.006, p = 0.014, p = 0.000).

Conclusions: Our results demonstrate the high level of serum collagen type XVIII in haemophilia A patients and argued its benefit as a potential marker for monitoring the development of hemophilic arthropathies and tailoring the optimal treatment to prevent further joint damage.

PB0867 | Incidence, Presentation and Management of Pseudotumors in Hemophilics: A Systemic Review

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Background: Pseudotumors are a rare complication (1-2%) of Hemophilia. They present as chronic, slowly expanding, encapsulated cystic masses, and most are located in the long bones, pelvis and maxillofacial region. Pseudotumors pose significant morbidity and management challenges.

Aims: Systematic review of literature to identify incidence, presentation, management and outcome of Hemophilia patients with pseudotumors.

Methods: Literature search (1961-2019) on pseudotumor formation in Hemophilia A and B patients was done using PubMed, Embase and Cochrane. The search strategies consisted of MeSH headings, keywords and text words related to hemophilia pseudotumor management. The results are expressed as P value with 95% Confidence Interval.

Results: 92 articles met our inclusion criteria (15 case series, 68 case reports and 9 review articles). 125 Hemophilia patients with pseudotumor formation were identified. Mean age was 28.5 years. Presenting features included large tumors of long bones (57%), abdominal or pelvic mass(19%), mass in the maxillofacial region(24%). Diagnostic modalities included X-ray, ultrasonography, Computed Tomography (CT) scan and Magnetic Resonance Imaging (MRI).16% of pseudotumors responded to conservative treatment with FVIII concentrates (P = 0.02), 64% required surgical intervention i.e arterial embolization and surgical excision (P = 0.016), and 12% were treated with radiotherapy (P = 0.03). In 15% of patients with masses in long bones, amputation was done due to intractable bleeding. Recurrence over 1 year period was 14%.

Conclusions: Although rare, pseudotumors pose significant morbidity in Hemophilia. It is always best to prevent these complications rather than trying to salvage later. Most of the developing countries lack the economic resources to meet the factor VIII needs of their Hemophilia patients. The need of the hour is to manage these patients early in specialized inter-disciplinary clinics designed specifically to meet the needs of hemophilic population.

PB0869 | Limited Port-A-Cath Need for Hemophilia Patients in the Past: Experience in a Single Center

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Background: Hemophilia treatment as prophylaxis requires frequent venous work. Port-a-cath (port) may be required to facilitate reliable venous access especially in young children. As a center, we have preferred less frequent injections for prophylaxis like Canadian approach. We did not use ITI programs due to reimburment reasons as well.

Aims: In this report, we evaluated our clinical experience with Ports in children with severe hemophilia A and vWD Type3.

Methods: Fifteen ports were placed in 9 boys with severe hemophilia A and 1 girl with vWD Type3 for last 30 years. The ages at the time of initial catheter placement ranged from 9 months to 10 years; Five were under age 3 (mean: 3.3 years). Two patients had high titer inhibitor. Indications for catheter placement were primary or secondary prophylaxis in patients who needed multiple infusions. We did not use heparin solutions and used only saline flash for preventing thrombosis.

Results: Subjects were admitted to hospital for a mean of 3.5 ± 1.3 days for port-a-cath insertion. While no complications were experienced during insertion, only one patient developed post-operative hematoma at the port catheter site. Totaly 15 ports remained in place for 925 months (mean 65; range 2-156 months). In one inhibitor case (10%), port-a-cath was removed 2 months after due to catheter infection and sepsis. In another one case catheter was replaced 5 years after insertion due to thrombosis. In 3 cases, catheters were replaced due to deformation without any complication.

Conclusions: Generally, one third of patients had problems with thrombosis and infection. Other patients were happy with port during long-term prophylaxis. The lack of catheter-related infections may be due to less frequent device access, strict adherence to aseptic technique. Nowadays, we hope to proceed with SC agents with prophylaxis.

PB0870 | BAY 81-8973 prophylaxis in Patients with Hemophilia A: Interim Usage and Safety Results from the TAURUS Real-World Study

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Background: BAY 81-8973 (Kovaltry®) is an unmodified, full length, recombinant factor VIII (FVIII) indicated for prophylaxis and

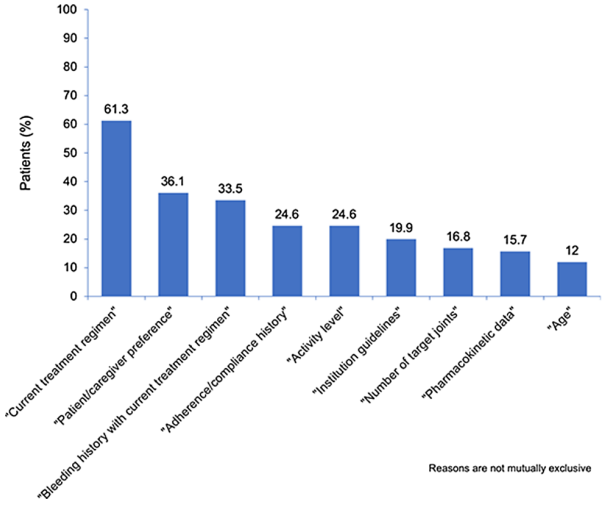

FIGURE 1 Reasons for initial dose/dosing frequency

treatment of bleeds in patients with hemophilia A. Safety and efficacy were established in the LEOPOLD clinical trials.

Aims: To highlight interim findings on the real-world usage and safety of BAY 81-8973 from the TAURUS study.

Methods: TAURUS (NCT02830477) is an international, open-label, prospective, non-interventional study enrolling patients of all ages with moderate/severe hemophilia A (FVIII ≤ 5% or ≤ 1%) and ≥ 50 exposure days to any FVIII, receiving BAY 81-8973 prophylaxis in standard clinical practice. This interim analysis reports switching behavior and safety.

Results: At data cutoff (June 2019), 264 patients had received ≥ 1 prophylactic dose of BAY 81-8973. The median age at baseline was 25.0 years (97.3% were previously treated with FVIII). 191 patients fulfilled inclusion criteria and had ≥ 1 follow-up assessment after receiving BAY 81-8973. At baseline, 60.7% received prophylaxis > 2.5 × /week and 36.6% received prophylaxis ≤ 2.5 × /week (data missing for 5 patients). Three patients switched from ≤ 2.5 × /week at baseline to > 2.5 × /week at cutoff; eight patients switched from > 2.5 × /week to ≤ 2.5 × /week. The most frequent reason for the initial switch to BAY 81-8973 was "physician decision" (61.8%); reasons for initial dose/dosing frequency are shown in
Figure 1. The median prescribed weekly dose was 56.9 IU/kg.

The safety population included 263 patients. 22.1% experienced an adverse event (AE): all were treatment emergent and none were related to inhibitor development or positive inhibitor measurement. One AE (pruritis in one patient) was drug-related and led to discontinuation of BAY 81-8973. No other AEs were drug-related. Serious AEs were reported in 20 patients (7.6%), but none were drug-related.

Conclusions: Data analyzed in this interim report confirm a reassuring safety profile and indicate that prophylaxis regimens before and after initiation of BAY 81-8973 were similar and remained stable during the treatment period.

PB0871 | Prophylaxis Outcome in Patients with Severe Hemophilia in Southern Iran: Near Five-Year Experience from a Single-Center Study

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Background: Bleeding complications in Hemophilia A and B vary from the most common form, hemarthrosis, which is disabling in long term, to a life-threatening intracranial hemorrhage. Considering bleeding complications and according to World Federation of Hemophilia guideline, prophylactic factor replacement therapy should be the goal of therapy.

Aims: We aimed to evaluate the impact of prophylactic regimen on the occurrence of bleeding symptoms in patients with severe hemophilia.

Methods: In this prospective study, 53 patients with severe hemophilia (45 hemophilia A and 8 hemophilia B) who were involved in standard prophylactic regimen from April 2015 to 2019 were investigated. All patients were registered at the Hemophilia Clinic Center of Shiraz University of Medical Sciences, shiraz, southern Iran. Prophylaxis was started from April 2015.

TABLE 1 Demographic and clinical characteristics of 53 hemophilia patients

Variables	Value
Age (year), mean ± SD	9.7 ± 4.48
Age of diagnosis (year), mean ± SD	1.60 ± 1.41
Age of starting infusion?? (year), mean ± SD	2.71 ± 2.21
Weight (Kg), mean ± SD	23.84 ± 12.23
Type of hemophilia N(%)	
Hemophilia A	45 (84.9%)
Hemophilia B	8 (15.1%)
Type of prophylaxis N(%)	
Primary	17 (32.1%)
Secondary	33 (62.3%)
Tertiary	3 (5.7%)
Duration of prophylaxis (month), mean ± SD	47.09 ± 11.12
Hemathrosis N(%)	
Simple	41 (77.4%)
Target	5 (9.4%)
Hospital admission (day), mean ± SD	0.51 ± 1.5
Bleeding episodes, mean ± SD	
Before prophylaxis	18.5 ± 10.3
After prophylaxis	5.7 ± 3.7

Results: The mean age of the patients was 9.71 ± 4.48 (1-19.) years. The mean age of diagnosis and mean age of starting infusion were 1.60 ± 1.41(1-7) and 2.71 ± 2.21(1-7) respectively. Number of patients received primary, secondary and tertiary prophylaxis were 17 (32.1%), 33 (62.3%), and 3 (5.7%) respectively. The duration of prophylaxis was 47.09 ± 11.12 months. Nine patients discontinued prophylaxis due to age > 15 years (N = 5) or not being cooperative (N = 4). All patients were inhibitor negative. The mean of bleeding episodes in the remained patients (n = 44) significantly decreased after prophylaxis from 18.5 ± 10.3 to 5.7 ± 3.7 episode (P < 0.001). Mean hospital admission days was 0.51 ± 1.5 (0-7) days.

Conclusions: This study indicates that the use of prophylaxis in patients with severe hemophilia significantly reduced the numbers of bleeding episodes. Accordingly, it decreases costs of hospitalization, emergency and outpatient visits, costs from work absenteeism, and hemophilic arthropathy, which is known as a long-term complication of hemarthrosis. Consequently, prophylaxis treatment improves the health quality of life of patients with hemophilia.

PB0872 | Clinical Review of Patients with Haemophilia (PwH) in a Malaysian Tertiary Center

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Background: Haemophilia is a group of inherited bleeding disorders, affecting the coagulation factors responsible for haemostasis. With

TABLE 1 Bleeding events among PwH

Annual bleeding event (mean±SD)	4.91 (±6.07)
Types of bleeding	**N (%)**
Musculoskeletal (At least one episode)	226 (86.9)
Presence of target joints	209 (80.4)
Psoas bleeding	41 (15.8)
Intracranial bleeding	38 (14.6)
Surgical bleeding	25 (9.6)
Gastrointestinal bleeding	20 (7.7)

TABLE 2 Treatment characteristics for PwH

Characteristics	Mild; *n* (%)	Moderate; *n* (%)	Severe; *n* (%)
Haemophilia A			
On-demand CFC	27 (96.4)	18 (64.3)	29 (19.6)
Prophylaxis CFC	1 (3.6)	10 (35.7)	123 (80.4)
Haemophilia B			
On-demand CFC	7 (100.0)	6 (60.0)	7 (21.9)
Prophylaxis CFC	0 (0)	4 (40.0)	25 (78.1)
Mean CFC	**Dose (IU/kg/week)**		
PwH A	41.3 (± 19.1)		
PwH B	48.6 (± 21.5)		

the prevalence of 5.1 per 100,000 population, clinical data among PwH in Malaysia are scarce.

Aims: To evaluate the socio-demographic, clinical, and treatment characteristics among PwH in our haemophilia treatment centre (HTC).

Methods: A retrospective review was conducted in Ampang Hospital, Malaysia to include patients with the diagnosis of haemophilia A (HA) and haemophilia B (HB) from 2008 to 2018. Demographic data, clinical characteristics, prophylaxis state, and treatment outcomes were evaluated.

Results: 260 patients were recruited with median age of 31 (IQR;2-84) years. The median age at diagnosis was 2 (IQR;1-73) years and all patients were male. 52.7% were Malays followed by Chinese (34.6%), and Indian (10%). 211 patients were diagnosed with HA with 72.5% of them had severe disease, while 49 patients had HB (severe disease, 65.3%). About two-third had significant family history. Bleeding events among PwH are illustrated in table 1. Chronic arthropathy and synovitis collectively accounted for more than half of the musculoskeletal complications with only 39% of them underwent intervention such as radioisotope synovectomy and arthroplasty. 30.1% of the patients had contracted hepatitis C followed by hepatitis B (1.8%), and HIV (0.8%). 12.7% of the patients developed inhibitor with 12 patients successfully underwent immune tolerance induction. Treatment with clotting factor concentrate (CFC) is illustrated in table 2. 15 patients with inhibitor were treated with on-demand/prophylaxis bypassing agents. 5 patients were treated with emicizumab and 2 patients were recruited in futisiran clinical trial.

Conclusions: Despite the availability of treatment in our centre, significant proportion of patients with severe haemophilia still refused prophylaxis factor replacement. It is worthwhile to further evaluate their quality of life especially among patients with recurrent bleeding events.

PB0873 | Real-World Cost of Emicizumab

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Background: Emicizumab is a bispecific antibody indicated for use in patients with hemophilia A (HA). The pharmacokinetic and delivery qualities make it attractive for use, especially in children. However, data about the financial impact is limited. Partners for Kids (PFK) is a pediatric accountable care organization that assumes financial and medical responsibility for low-income children in central and southeastern Ohio, and its claims data provide an opportunity to evaluate the financial impact of changes in medical care.

Aims: To present a case series of PFK patients on emicizumab to describe the total cost of medical care.

Methods: We interrogated the PFK claims database for healthcare costs of all patients who transitioned to emicizumab from January 1, 2016 to December 31, 2019. PFK data were available for five categories: inpatient, outpatient, emergency department, office, and pharmacy. Cumulative costs for all categories were compiled into total healthcare cost. Total cost, for each PFK patient on emicizumab were compared for 12 months before emicizumab (pre-switch) and during/after emicizumab initiation (post-switch). Emicizumab loading phase costs were included in the post-switch category.

Results: There were 10 patients with claims data pre- and post-switch. Median age at emicizumab initiation was 10.9 years, 7 had severe (< 1%) HA and 3 had moderate HA. One patient had an active inhibitor. Pre-switch, 8 patients were on routine prophylaxis and 1 was on intermittent prophylaxis with FVIII and 1 was on immune tolerance therapy. Total healthcare cost pre-switch averaged USD $50,681.00 per patient per month. Post-switch, total cost averaged USD $48,996.00 per patient per month. The absolute cost difference observed in our cohort pre- and post-switch was driven largely by lower inpatient and pharmacy costs in the post-switch period.

Conclusions: Our real-world analysis of the financial impact of emicizumab prophylaxis in a cohort of pediatric patients with HA demonstrates a reduction in healthcare costs.

PB0874 | The Role of Ultrasonography versus MRI in the Diagnosis of Joint Problems in Hemophilic Children

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Background: Hematomas and recurrent hemoarthrosis are a common problem in hemophilic patients from an early age. Early diagnosis is critical in preventing hemophilic arthritis. Therefore, early detection and proper follow up is critical. Compared to MRI, ultrasonography (us) has advantage of the lack of need for sedation in young children, absence of susceptibility artefacts for haemosiderin deposition, high cost and risk of hemosiderin deposition of hemophilic joints, lower cost of examination and easier.

Aims: To diagnose joint lesions in children with haemophilia, assess the role of US in the diagnosis and follow up of these lesions.and To assess specificity and accuracy of US compared to MRI in diagnosing of Joint problems in haemophilic children.

Methods: A cross sectional study was done for 95 hemophilic patients attending hematology outpatient clinic during period from 2016 to 2018 at Pediatrucs and Radiology Departments of Zagazig University-Egypt. The following data were collected from studied patients and their family after their consent demographic., clinical data, joint examination as well as standard muscloskeletal ultrasonography and MRI scan following Denver Scoring system.

Results: There was a high statistical significant agreement between MRI and Ultrasonographyy in Denver classifications of affected joints. Ultrasonography was excellent to detect hemoarthrosis and synovial hyperplasia in ankle, knee and elbow joints with sensitivity 85.7, 97,6% and 100 respectively.

Conclusions: The ultrasonography is useful for diagnosis joint bleeding as well as follow up of health of joints.

PB0875 | A Real-World Qualitative Analysis of the Burden of Hemophilia B and Patient Experiences with Prophylactic Nonacog Beta Pegol

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Background: In Canada, factor IX (FIX) replacement concentrates for patients with hemophilia B (PwHB) are subject to the national competitive procurement process. In April 2018, the introduction of nonacog beta pegol (N9-GP), in all provinces except Quebec, for once-weekly prophylactic therapy in adult PwHB resulted in a switch of products for many patients on prophylaxis.

TABLE 1 Patient characteristics

Patient characteristic	Median (range) (*N* = 10)
Age at study inclusion/age at diagnosis, years	45 (22-67) / 1.3 (0-16)
Length of time on prophylaxis treatment with N9-GP, months	12 (6-13)
Number of on-demand infusions of factor treatment before N9-GP, infusions per year	10 (0-44)
Number of on-demand infusions of N9-GP, infusions per year	1.5 (0-28)
Hemophilia severity	**n patients**
Mild (> 5%-40% FIX levels)	1
Moderate (1-5% FIX levels)	1
Severe (< 1% FIX levels)	8
Prior treatment before switching to N9-GP	Extended half-life FIX: 6. Standard half-life FIX: 4

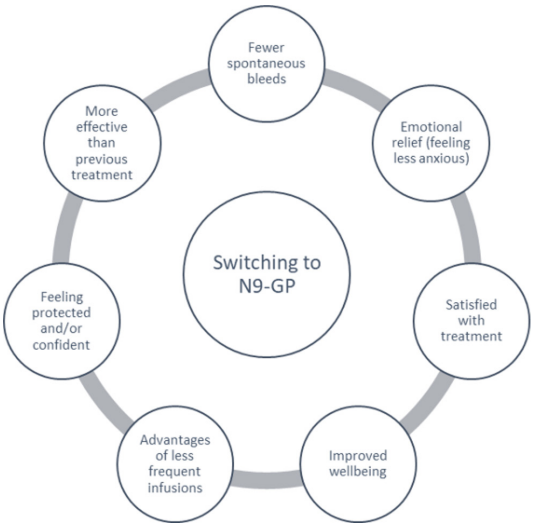

FIGURE 1 Spontaneously-reported themes captured relating to switching to N9-GP, based on responses to open-ended questions

Aims: To qualitatively evaluate the disease burden among PwHB and the experiences among patients who have switched to prophylactic N9-GP therapy in Canada.

Methods: A semi-structured telephone interview was conducted between January and September 2019 in adult male PwHB, who contacted the study organizers to participate and were receiving prophylactic N9-GP for ≥ 3 months. Interview transcripts were analyzed thematically to identify key concepts.

Results: Ten PwHB participated in the interviews, representing approximately 10-15% of PwHB receiving N9-GP in Canada. The median age was 45 years, and 8/10 patients had severe hemophilia. The median annual number of on-demand infusions decreased from 10 to 1.5 infusions following switch to N9-GP (Table). All patients described previous hemarthroses and impaired quality of life (QOL) during their lifetime, including impacts on physical function, daily function, self-care, social life and emotional wellbeing. Based on responses to open-ended questions, when considering the

experiences after switching to N9-GP, common themes (described by > 50% of patients) included advantages from the reduced infusion frequency and fewer spontaneous bleeds. Most patients spontaneously reported a positive experience with the transition to N9-GP and described increased confidence compared with previous therapy (Figure). However, patients suggested that changes could be made to improve the convenience of N9-GP medication packaging.

Conclusions: Understanding patient experiences with new therapies is pertinent as hemophilia treatment options expand. Overall, patients reported favorable experiences after switching to prophylactic N9-GP and decreased need for on-demand treatment. It is likely that the extended half-life of N9-GP contributes to QOL improvements.

PB0876 | Efficacy and Safety of Antihemophilic Factor (Recombinant) in Previously Treated Chinese Patients with Severe or Moderately Severe Hemophilia A: Results from a Phase 4 Study

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Background: Antihemophilic factor (recombinant; rAHF) is indicated in patients with hemophilia A of all ages to treat and prevent bleeding.

Aims: The efficacy and safety of standard prophylaxis versus on-demand rAHF treatment were assessed in previously treated Chinese patients with severe or moderately severe hemophilia A.

Methods: This open-label, sequential, interventional, post-approval study (NCT02170402) conducted in China included patients with hemophilia A of any age with factor VIII (FVIII) levels ≤ 2%. Ethics committees' approval and patient informed consent were obtained. Patients received 6 months' on-demand rAHF treatment followed by 6 months' rAHF standard prophylaxis (20-40 IU/kg every 48 ± 6 hours). The primary outcome measure was the percentage reduction in annualized bleeding rate (ABR) during prophylaxis versus on-demand treatment in the per-protocol analysis set (PPAS; patients who completed 6 months ± 2 weeks each of on-demand treatment and prophylaxis with ≥ 80% compliance to dose and frequency of prophylaxis). Secondary outcome measures included ABR by cause and location, and rAHF hemostatic efficacy, immunogenicity, and safety.

Results: Of 72 patients who received ≥ 1 rAHF dose, 61 were included in the PPAS. Mean total ABR for on-demand rAHF treatment was 58.3 (95% CI, 52.5-64.7; median [range], 53.9 [16.7-121.7]) versus 2.5 (95% CI, 1.5-3.7; median [range], 0.0 [0.0-22.1]) during prophylaxis, representing a mean ABR reduction of 95.9%. Similar findings in favor of prophylaxis were observed for ABR according to bleed cause and location (Table). rAHF hemostatic efficacy was rated by patients as excellent/good in 96.1% of treated bleeds. Transient low-titer FVIII inhibitors (0.6-1.7 BU) in 4 patients resolved before study end. No unexpected safety signals were observed.

Conclusions: The reduction in ABR during standard prophylaxis versus on-demand rAHF treatment in previously treated Chinese patients with severe or moderately severe hemophilia A was comparable to that described in other ethnicities.

TABLE 1 Intra-patient comparison of ABRs in the rAHF on-demand and prophylactic treatment periods (PPAS)

		Cause of bleed		Location of bleed		
	Total ABR	Spontaneous	Traumatic	Joint	Non-joint	Target joint
ABR for on-demand rAHF treatment period (n = 61)						
Mean (SD)	58.3 (24.5)	35.5 (28.8)	13.4 (14.9)	41.4 (24.5)	19.2 (16.5)	18.4 (20.9)
Median (range)	53.9 (16.7-121.7)	33.6 (0.0-119.7)	8.2 (0.0-63.0)	34.2 (4.3-117.7)	15.0 (0.0-72.6)	8.5 (0.0-91.3)
ABR for prophylactic rAHF treatment period (n = 61)						
Mean (SD)	2.5 (4.5)	0.5 (1.5)	1.3 (2.8)	1.9 (3.9)	0.9 (2.4)	0.9 (2.2)
Median (range)	0.0 (0.0-22.1)	0.0 (0.0-6.7)	0.0 (0.0-17.7)	0.0 (0.0-22.1)	0.0 (0.0-15.7)	0.0 (0.0-13.3)
Percentage reduction in mean ABR	95.9	97.4	90.3	95.4	95.3	95.1

ABR, annualized bleeding rate; PPAS, per-protocol analysis set; rAHF, antihemophilic factor (recombinant).

PB0877 | Real Life Use of Emicizumab in Pediatric Patients without Inhibitors

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Background: Emicizumab has been available in Germany for pediatric patients without inhibitors for a year now.We describe five patients who were switched and why.We give details about the Emicizumab levels and our monitoring protocol.

Aims: We want to show which patients were switched, their monitoring and outcome with regard to bleeding and patient satisfaction.

Methods: Five of our patients were switched from FVIII prophylaxis to Emicizumab,one a PUP, age range 8 months to 15 yrs. Reasons for switching: no venous access (port implantation mandatory in 3 patients), unwillingness to tolerate infusion via an indwelling port in one case, unwillingness to continue infusion vie a vein or the port in another case.

A further patient cannot infuse himself because he squints. We measure the Emicizumab level in the blood after the loading period before we start the medication every two weeks, I.e. after 6 weeks of treatment and then every 3 m.

Emicizumab levels were all in the desired range, though recommended dose was not implemented due to vial size in some cases. Even a lower dose every two weeks resulted in levels of 60 ng/ ml.

Twice parents gave factor,in one case due to a forehead hematoma. One dose was given after the child had cut himself with scissors.All patients have emergency cards and an emergency supply of FVIII factor at home, one to two doses.

Patient satisfaction and care giver satisfaction is high, though, the smaller children cry briefly when injected.

Results: We report satisfying results with minor bleeding. The PUP who has been treated now for 10 months never had a bleeding event and has not yet needed factor VIII.

Conclusions: We have switched five of our patients and report smooth and easy to implement switching processes with our management protocol.

PB0878 | Longitudinal Analysis of rFVIIIFc Use and Efficacy in the UK: A Report from the National Haemophilia Database

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TABLE 1 Within-patient analysis before and after switching to rFVIIIFc

	Pre-switch (n = 158)	Post-switch (n = 158)	Within-patient change
Age* med. (IQR)	24 (11;43)	-	-
Infusion frequency (week) med. (IQR)	3.2 (2.6;3.6)	2.3 (2.1;2.7)	-26% (-34;-5%) p < 0.001
Clotting factor consumption (IU/kg/week) med. (IQR) n = 152**	81 (55;100)	68 (52;89)	-12% (-30;5%) p < 0.001
ABR med. (IQR)	2.6 (0;7.2)	2 (0;4)	-1 (-3.2;0.4) p < 0.001
AJBR med. (IQR)	1 (0;5)	1 (0;3)	0 (-1.9;0) p < 0.001
Bleed-free rate	28%	32%	(X^2= 1.4; p = 0.23)

* as of the first treatment with rFVIIIFc **patients without weights were excluded

Background: Prophylaxis with rFVIIIFc in severe haemophilia A (HA) may reduce infusion frequency (IF) and clotting factor consumption (CFC) with improved bleeding outcomes in comparison with standard rFVIII. It is unclear if there is a cumulative effect on bleeding outcomes with time.

Aims: To describe bleeding outcomes associated with switching from standard rFVIII to rFVIIIFc prophylaxis over a three-year follow-up period in the UK.

Methods: All patients with ≥ 6 months rFVIIIFc follow-up in the UK were identified. Trial patients and patients with an inhibitor history or < 6 months Haemtrack (HT)-compliant data were excluded. Age, IF, CFC, ABR and AJBR were calculated and a subgroup analysis of those with 3 years follow-up was performed. Within-patient change in ABR over time and pre and post-switch comparisons were analysed using Wilcoxon signed rank testing, bleed-free proportion was analysed using the X^2 McNemar test.

Results: The criteria were met by 198 patients; 183 with 2 years and 70 with 3 years follow-up. Within-patient analysis of 158 with ≥ 6 months pre- and post-switch HT data demonstrated significant reductions in IF and CFC and ABR and AJBR following switching (table 1). This was most marked in patients with a pre-switch ABR > 5. rFVIIIFc utilisation was stable over three-years follow-up. The proportion of bleed-free patients increased significantly from 36% to 53% over three years follow-up (X^2=6.1 $p < 0.01$) (table 2). ABR reduced non-significantly over time. The results of the subset with three years follow-up were representative of the whole group.

Conclusions: The data suggest that rFVIIIFc may allow a reduction in infusion frequency and improved bleeding outcomes in comparison to rFVIII. There was a progressive increase in the proportion of bleed-free patients over time.

TABLE 2 Subgroup analysis of patients with 3 years follow-up data and within-patient analysis of change in ABR over time (lower table)

	n = 54 with ≥ 6 months pre-switch HT data	Year 1 n = 70	Year 2 n = 70	Year 3 n = 70
Infusion frequency (week) med. (IQR)	3.4 (2.6; 3.7)	2.2 (2.0; 2.7)	2.2 (1.9, 2.6)	2.3 (2.0, 2.7)
Clotting factor consumption (IU/kg/week)	81 (55;105)	75 (60, 95)	75 (57, 96)	81 (62, 101)
ABR med. (IQR)	1.1 (0; 6)	1 (0, 3.8)	1 (0, 3)	0 (0, 2.9)
Bleed-free rate	29%	36%	41%	53%
AJBR med. (IQR)	1 (0; 3.8)	1 (0, 2.8)	0 (0, 2)	0 (0, 1.5)
Within-patient change in ABR (Y3 vs Y1)	-	0 (-1,1), p = 0.37		
Within-patient change in ABR (Y3 vs Y2)	-	0 (-1, 0.5), p = 0.78		
Within-patient change in ABR (Y2 vs Y1)	-	0 (-1, 0), p = 0.94		
Within-patient change in ABR (Y3 vs rFVIII pre-switch)	-1 (-2.5, 0), p < 0.01			

PB0879 | Real-World Effectiveness Evaluation of the Recombinant Factor VIII Fc Fusion Protein in Adolescents and Children with Hemophilia A in Japan: "Fc Adolescent and Children Treatment" Study (FACTs) Part 1 Interim Analysis

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Background: The Phase 3 A-LONG/Kids A-LONG and ASPIRE extension studies evaluated the efficacy and safety of the extended half-life recombinant factor VIII Fc fusion protein (rFVIIIFc); however, no patients in Japan were enrolled in Kids A-LONG or the study assessing rFVIIIFc in previously untreated patients (PUP). The prospective, multicenter, observational FACTs study is designed to evaluate the effectiveness of rFVIIIFc in adolescents and children with hemophilia A (HA) in Japan (Part 1) and summarize the experience of immune tolerance induction (ITI; Part 2).

Aims: To present the first real-world interim data from Part 1 of the FACTs study.

Methods: Study participants provided written informed consent, and local Research Ethics Committees provided study approval for all study sites. Data from patients < 18 years of age with HA treated with rFVIIIFc were collected at baseline and every 6 months up to 2 years, including pre-study treatment data. The regimen was at the investigator's discretion.

Results: As of August 2019, 64 PUPs and minimally treated patients (MTP) were enrolled in the study (Table 1).

Prior to the study, 3 out of 6 patients with a history of high-titer inhibitors underwent ITI with factor VIII (FVIII) and 39 (61%) patients received prophylactic treatment with FVIII (21 [54%] received FVIII treatment ≥ 3 times/week). During the study, 28 (54%)

TABLE 1 Patient treatment and disease history

	All patients (N = 64)
Median (min–max) age at rFVIIIFc start, years	7 (0–17)
Hemophilia severity, n (%)	
Severe	43 (67.2)
Moderate	17 (26.6)
Mild	2 (3.1)
FVIII exposure prior to rFVIIIFc treatment, n (%)	
0–3 days	9 (14.1)
4–20 days	6 (9.4)
21–100 days	3 (4.7)
101–150 days	5 (7.8)
≥151	29 (45.3)
Not available	12 (18.8)

FVIII, factor VIII; max, maximum; min, minimum; rFVIIIFc, recombinant factor VIII Fc fusion protein.

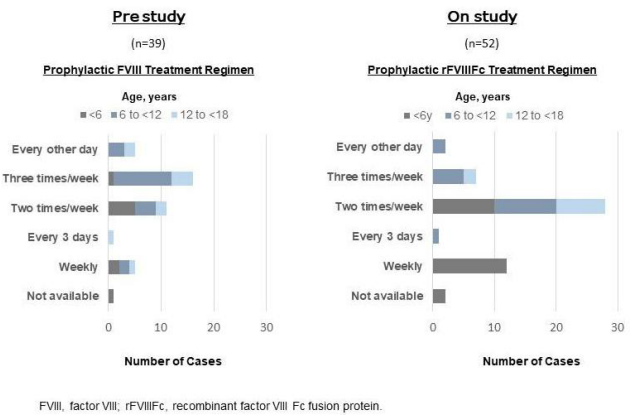

FIGURE 1 Injection frequency in patients receiving prophylactic treatment prior to and during study

patients received rFVIIIFc prophylaxis two times/week; 12 (50%) patients < 6 years of age received weekly rFVIIIFc (Figure 1). Forty-five (70%) patients had no bleed events during the first 6 months of treatment. The median annual spontaneous bleed rate was zero during the study. Three patients developed inhibitors to rFVIIIFc during the study

(PUPs, n = 2; MTP, n = 1) and shifted to ITI with rFVIIIFc.

Conclusions: These interim data in Japanese pediatric patients are consistent with previously reported clinical efficacy and safety data from patients with HA treated with rFVIIIFc.

PB0880 | Incidence of Neutralizing Antibody (Inhibitor) in Previously Untreated Patients (PUPs) with Severe Hemophilia A

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Background: Nearly 20% of Hemophilia A patients develop Inhibitor to Factor VIII. Risk of inhibitor development is maximum upto 50 EDs. Enough literature has been published discussing various aspects of Inhibitor development in hemophilia A patients. However not enough data is available about incidence of Neutralizing Antibody in patients with Hemophilia A PUPs (No exposure to blood/plasma products or CFC).

Aims:

1) To Assess Incidence of Inhibitor in PUPs with severe Hemophilia A.

2) To assess F 8 mutation in PUPs who have Inhibitor.

3) To assess anamnestic response after 1st exposure and before second exposure in PUPS who had Inhibitor to FVIII

Methods: Data was collected between April 2016 -September 2019. All were diagnostic samples. Very detailed history was elicited to confirm PUP status

Number: 116:

Age: 4 months - 60 months.

Family H/O hemophilia A: 52/116 9 (44%).

History of Inhibitor in family: 16/116 (13.7%).

Results: 4/ 116 (3.4%) patients had Inhibitor at PUP state. One patient had High Titer (7.2 NBU) and three had Low titer (2.1,2.8, 1.9 NBU) Inhibitor. All 4 patients had Intron 22 Inversion mutation of F 8 gene. All four patients had family history of Inhibitor. All 4 patients had anamnestic High Responder state to one exposure of CFC with Inhibitor Titers of 32.6,76.5,116.2 & 98.4 NBU.

Conclusions: Exposure to maternal blood & thus Factor VIII antigen,at time of delivery could be the explanation for Inhibitor development in PUP. Scientific community needs to collect systematic data to address this issue.This can have great influence on factor replacement policy for these patients. These patients could be the candidates for Non-factor therapies for Hemophilia A or ITI at PUP status. Larger data needed.

PB0881 | Successful Multiple Surgeries in Adult Patients with Haemophilia A (HA) Treated With Turoctocog Alfa Pegol (N8-GP) in the Pathfinder 3 trial

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Background: Managing patients with HA undergoing major surgery requires maintaining factor VIII (FVIII) activity at target levels. pathfinder 3 assessed N8-GP, an extended half-life glycoPEGylated recombinant FVIII, during major surgery in patients (≥12 years) with severe HA. Informed consent was obtained; the study was approved by relevant ethics committees. Thirty-five patients undergoing 49 surgeries were included: haemostasis was successful (defined as an 'excellent' or 'good' response) in 47/49 (95.9%) surgeries (two had a 'moderate' response) with reduced dosing frequency (Hampton *et al*, WFH-MSK, 2019; ISTH symposium, *Thromb. Haemost.*, 2019; Novo Nordisk data on file).

Aims: To report intraoperative haemostatic response of three patients undergoing multiple (n = 11) major surgeries in pathfinder 3.

Methods: Patients received peri-operative N8-GP for surgery and were assessed over the surgical period: d0, once daily (d1-6); and once during d7-14. Surgical procedure, haemostatic response, N8-GP consumption and adverse events (AEs) were reported. Haemostatic response was defined as follows: excellent, better than expected; good, as expected; moderate, less than optimal but response maintained; poor, bleeding due to inadequate response (regimen change required to restore haemostasis).

Results: A 50-year-old patient (patient 1) underwent three consecutive, elective procedures; a 57-year-old patient (patient 2) underwent

TABLE 1 Details of major surgeries*

Table 1. Details of major surgeries*

Patient	Type of surgery**	Time performed after first surgery	Intraoperative haemostatic response
Patient 1	• Laparotomy/pseudotumour excision (surgery 1)	–	Good
	• Right TKR (surgery 2)	7 months	Good
	• Left TKR (surgery 3)	28 months	Excellent
Patient 2	• Removal of infected knee arthroplasty (surgery 1)***	-	Excellent
	• Bilateral knee prostheses (surgery 2)†	6 months	Excellent
	• Removal of infected knee prosthesis (surgery 3)	19 months	Excellent
	• Knee arthrodesis (surgery 4)***	24 months	Good
Patient 3	• Arthroscopic elbow synovectomy (surgery 1)	–	Good
	• Bilateral ankle arthroscopy/debridement (surgery 2)	4 months	Excellent
	• Right ankle arthroscopy/debridement (surgery 3)	48 months	Good
	• Laparoscopic cholecystectomy (surgery 4)	58 months	Excellent

*Major surgeries were defined as any invasive operative procedure requiring several days of substitution therapy and/or a procedure in which at least one of the following occurred: a body cavity was entered, a mesenchymal barrier (e.g., pleura, peritoneum or dura) was crossed, a fascial plane was opened, an organ was removed or normal anatomy was operatively altered (Hampton *et al*, 2017).

**There were 10 orthopaedic surgeries and one non-orthopaedic surgery.

***Emergency surgeries.

†One spontaneous, mild/moderate knee bleed after the bilateral knee prosthesis insertion was successfully treated with one N8-GP dose.

TKR, total knee replacement.

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four consecutive procedures (two elective, two emergency); and a 30-year-old patient

(patient 3) underwent four consecutive elective procedures (Table 1). Haemostasis was successful for all surgeries. N8-GP consumption was per-protocol during the operative period; no procedures required an intra-operative dose of N8-GP. N8-GP was well tolerated; all AEs reported for patients 1 (n = 7) and 2 (n = 5) were mild and unrelated to treatment; no AEs were reported for patient 3.

Conclusions: N8-GP is effective and convenient with a good safety profile when used during surgery in patients with severe HA undergoing multiple major procedures.

PB0882 | Resolution of Target Joints (TJs) in Children with Severe Haemophilia A (HA) Treated with Turoctocog Alfa Pegol (N8-GP) Prophylaxis: Results from the Pathfinder 5 Trial

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Background: TJs in haemophilia increase disease burden, impacting resource utilisation and costs. pathfinder 5 evaluated the safety/efficacy of prophylactic N8-GP, an extended half-life glycoPEGylated recombinant factor VIII, in previously treated children (< 12 years) with severe HA. Patients received N8-GP ~60 IU/kg (range: 50-75 IU/kg) twice weekly for prophylaxis. Written informed consent was obtained; the study was approved by relevant ethics committees. TJ (≥3 bleeds/consecutive 6-month period) resolution was assessed according to strict criteria (Meunier *et al*, 2017).

Aims: To report the proportion of resolved baseline TJs, using protocol- and International Society of Thrombosis and Haemostasis (ISTH)-defined criteria, throughout the trial.

Methods: TJs from patients participating in both main and extension phases of pathfinder 5 (n = 63) were assessed at baseline, during and at trial end; TJ resolution was defined in the protocol as no bleeds/consecutive 12-month period. The ISTH definition: ≤2 bleeds/consecutive 12-month period (Blanchette *et al*, 2014). Annualised bleeding rate (ABR) in TJs for the whole population (n = 68) was also analysed.

TABLE 1 Characteristics of patients participating in both the main and extension phases of pathfinder 5 with no TJ bleeds over the trial period

Table 1. Characteristics of patients participating in both the main and extension phases of pathfinder 5 with no TJ bleeds over the trial period

Patient number	Patient age (years)	TJ location	Prior treatment	If on prior PPX, age at initiation (years)	EDs prior to trial
Patient 1	3	Right knee	On-demand rFVIII	–	51
Patient 2	4	Left ankle and left knee	On-demand rFVIII	–	54
Patient 3	5	Right knee and left knee	rFVIII PPX	5	66
Patient 4	10	Left knee	rFVIII PPX	1	1399
Patient 5	7	Right ankle	rFVIII PPX	1	192

EDs, exposure days; PPX, prophylaxis; rFVIII, recombinant factor VIII; TJ, target joint.

TABLE 2 Target joint ABRs in all patients (n=68) receiving N8-GP prophylaxis in pathfinder 5

Table 2. Target joint ABRs in all patients (n=68) receiving N8-GP prophylaxis in pathfinder 5

	Younger children (0–5 years) [n=34]	Older children (6–11 years) [n=34]	Total
Mean (SD) ABR	0.03 (0.14)	0.15 (0.38)	0.09 (0.29)
Median (IQR)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)
Range	0.00, 0.78	0.00, 1.50	0.00, 1.50
Poisson estimate of ABR (95% CI)	0.04 (0.01–0.20)	0.14 (0.07–0.31)	0.09 (0.04–0.20)

ABR, annualised bleeding rate; CI, confidence interval; IQR, interquartile range; N8-GP, turoctocog alfa pegol; SD, standard deviation.

Results: Twelve patients had 16 TJs at baseline (age: 3-11 years; four [33.3%] were aged ≤ 5 years), all of which resolved during the trial according to both protocol- and ISTH-defined criteria. One of these patients developed two new TJs (one of which developed after 6 months in trial, resolved at 20 months, was redefined as a TJ at 28 months and resolved again at 44 months), whilst his reporting of bleeds varied from the other patients. Five patients (41.7%) with seven TJs at baseline had no TJ bleeds during the trial (mean 4.5 years) (Table 1). Median TJ ABR was 0.00 (Table 2).

Conclusions: N8-GP prophylaxis resolved all baseline TJs in previously treated children in pathfinder 5. These findings support early prophylaxis with N8-GP and its benefits in children with severe HA, even those with pre-existing TJs.

PB0883 | FIXa-Triggered Thrombin Generation Assay for Monitoring Very Low Levels of FVIII Activity in Hemophilia A

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Background: It has been well documented that assay discrepancies between the severity of bleeding and factor VIII (FVIII) level in severe hemophilia A, lead to inaccuracy over the actual level after factor infusion. One of the possible explanations may be that the current assays are not sensitive enough to quantify FVIII level when it is less than 1%. The potentially close to physiological approach, thrombin generation assay (TGA), may be used to monitor coagulation function in these patients, but the relationship between main parameters and the FVIII level (< 0.01 IU/mL) is not linear.

Aims: To find the optimal protocol of TGA to estimating the coagulation function in severe hemophilia A patients.

Methods: Hemophiliac Platelet-Poor-Plasma (commercialized FVIII -deficient plasma) was mixed with normal plasma. Different concentrations of TF or FIX were used as triggers to generate thrombin and

the TGA parameters were recorded. The correlations between FVIII level and TGA parameters were estimated using linear regression.

Results: An optimal level of FIXa to trigger the thrombin generation instead of TF is 0.6 nM with phospholipid (4 μM) when the FVIII level is lower than 0.01 IU/mL. There was a linear relationship between FVIII levels (<0.01 IU/mL) and TGA parameters. The Peak thrombin value rose sharply with a growing level of FVIII until it reached to 0.01 IU/mL; the ETP augmented dramatically before the FVIII level rose to 0.1 IU/mL. After the cutoff point, the Peak thrombin value and ETP entered a plateau period.

Conclusions: low level of FIXa-triggered TGA is more sensitive with high or low concentrations of FVIII than currently used protocols. It could be helpful for tailoring in hemophilia prophylaxis, adapting replacement in surgical situations, monitoring bypassing agents in patients with inhibitors, and measuring efficacy of gene therapy. But these in vitro results need ex-vivo studies to confirm.

PB0884 | Total Elbow Replacement: Financial Burden Impact

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Background:

Aims: To describe two clinical cases of patients with hemophilia who underwent total elbow replacement and its financial impact.

Methods: Patient A: A 35-years-old patient with severe hemophilia A (PwHA) on prophylaxis treatment. He has had recurrent minor spontaneous bleeding episodes in the right elbow (RE). Physical exam showed a RE with a range of motions (ROM) of 95° flexion; -90° extension. Xray showed a major defect in the humerus-ulnar joint. A multidisciplinary team (MTD) stated patient needed a total elbow replacement (TER). Patient B: A 25-years-old PwHA, from Ecuador, used to have a ROM in his RE of 90° flexion; -45° extension. He suffered a complex comminuted radial head fracture+supracondylar humerus fracture+posterior subluxation in his RE after traffic accident. He was wearing a cast at the moment of physical exam.

Results: Both patients underwent surgical correction. No complication occurred, except for uncontrol pain in patient A. Both patients underwent early physical rehabilitation and after 6 weeks both patients presented a slightly improvement in ROM of the elbow. Two years after surgery and final follow-up, patient A had a RE with ROM of 140° flexion; -50° extension and patient B had 110° flexion; -20° extension in his RE.

Conclusions: There are three major aspects we want to emphasize:

1) Early referral to a major hemophilia treatment center could avoid bleeding complication and improve quality of life in hemophilia patients;

2) Risky orthopedic surgery for hemophilia patients are feasible in South-America and

3) Complex surgery procedures such as TER could have a positive financial impact on health care systems especially in Middle/Low-income countries.

PB0885 | Major Surgery in Emicizumab Haven 1 Trial Patients: The New Zealand Experience

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Background: Emicizumab has revolutionised the management of severe haemophilia A with FVIII inhibitors. The sentinel Haven 1 trial showed an 87% bleed reduction on prophylaxis. Experience in surgery is limited mainly to minor procedures.

Aims: To report the surgical experience in two NZ Haven1 trial patients undergoing major surgical procedures with high dose FVIII and activated FVII (Novoseven) respectively.

Methods: The patients (trial arms A&D) had an elective excision of an expanding anterior thigh pseudotumour (37 × 7 × 8 cm) causing erosion of the femoral cortex, and acute open laparotomy for an NSAID-induced duodenal ulcer, at trial weeks 114 and 176 repectively.

Results: Neither patient had any other bleeding nor treatment side effects on trial. The pseudotumour predated trial entry by 3 years, required regular aspiration for symptom relief and persisted despite dose escalation to 3 mg/kg. The baseline inhibitor 0.6 BU allowed surgery with FVIII 67000 units by c.i. over 14 days without requirement for bypass therapy. Perioperative blood loss was 700mls, drains were removed at 48 hours and peak bovine chromogenic FVIII 319%. The inhibitor titre was 8.9BU on day 15. There were no perioperative complications. Patient 2 (inhibitor 42BU) became hypotensive secondary to haematemesis (Hb58 g/L) and required multiple red cell and platelet transfusions. Bleeding was uncontrollable endoscopically, despite high-dose PPI and TxAc, necessitating early open laparotomy to oversew an *H.Pylori* negative chronic duodenal ulcer. Novoseven 422 mg was administered over 16 days. Regular monitoring for MAHA was negative, there was no recurrence of bleeding (Hb 94 at discharge) and normal midline laparotomy wound healing. Both patients remain well on Emicizumab prophylaxis.

Conclusions: Major surgery can be safely performed in patients on Emicizumab receiving high dose FVIII replacement where the inhibitor can be swamped or using high doses of Novoseven. Neither patient developed microangiopathic haemolysis nor other thrombotic complication despite TxAc and intensive transfusion support.

PB0886 | Prophylaxis with Turoctocog Alfa Pegol (N8-GP) Maintains Improvements in Health-Related Quality of Life (HRQoL) and Treatment Satisfaction (TS) over ≥ 4.5 Years in Patients with Haemophilia A (HA)

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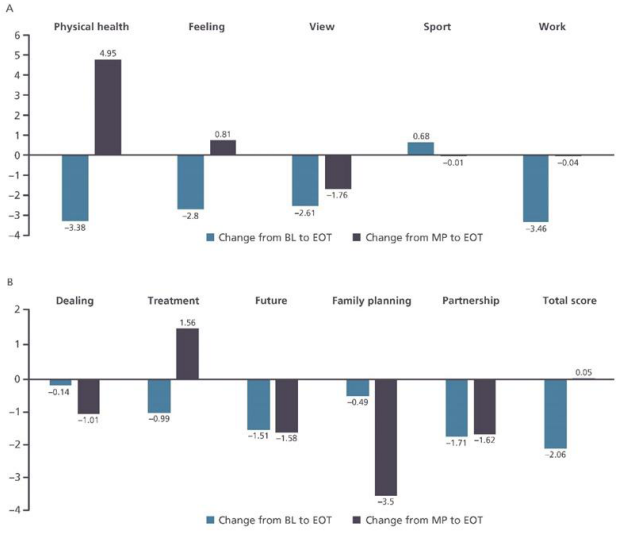
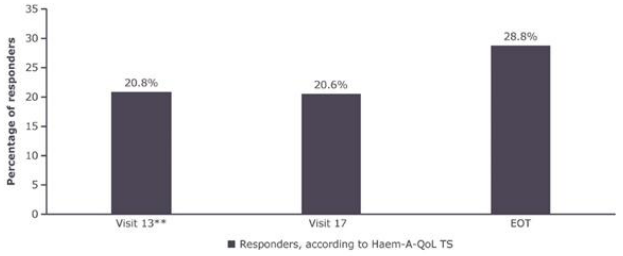
Background: N8-GP is a glycoPEGylated extended half-life recombinant factor VIII developed for prophylaxis/treatment of HA. In the main phase (MP) of pathfinder 2, a pivotal trial that evaluated N8-GP in adults/adolescents with severe HA, HRQoL was improved/maintained from baseline (BL) and a high level of TS reported.
Aims: To examine HRQoL and TS in adults (≥17 years) receiving N8-GP prophylaxis in pathfinder 2 from BL to end of trial (EOT).
Methods: pathfinder 2 was a phase 3, multinational, open-label, partially randomised trial. Prophylaxis cohorts: N8-GP (50 IU/kg) every 4 days (Q4D) or biweekly (MP); N8-GP (50 IU/kg) Q4D or N8-GP (75 IU/kg) every 7 days (randomised extension phase). Informed consent was obtained; the study was approved by relevant ethics committees.
Patient-reported outcome questionnaires administered throughout the trial included: Haemophilia Quality of Life Questionnaire for Adults (Haem-A-QoL, scored 0-100; lower=better HRQoL), EuroQol 5 Dimensions-3 Levels (EQ-5D-3L, scored 0-1; visual analogue scale [VAS] scored 0-100; higher=better HRQoL); Hemophilia Satisfaction questionnaire (Hemo-SAT, scored 0-100; lower=higher TS).



BL, baseline; EOT, end of trial; Haem-A-QoL, Haemophilia Quality of Life Questionnaire for Adults; HRQoL, health-related quality of life; MP, main phase.

FIGURE 1 Change in HRQoL from BL to EOT and from MP until EOT, according to Haem-A-QoL scores



* Response threshold established by Wyrwich et al. *Haemophilia* 2015;21(5):578–584;
** Visit 13 = end of MP.
EOT, end of trial; Haem-A-QoL, Haemophilia Quality of Life Questionnaire for Adults; MP, main phase.

FIGURE 2 Percentage of responders for Haem-A-QoL total score improved over time*

Results: Adults (n = 154; median age: 30 years; range: 17-66) had a median treatment duration of 54.7 months (range: 2.6-77.7). Mean (SD) Haem-A-QoL total score was improved/maintained from BL-EOT (-2.06 [11.53]) and from MP-EOT (+0.05 [9.43]) (Figure 1). Overall HRQoL was improved/maintained (EQ-5D-3L mean [SD] change from BL-EOT: index + 0.01 [0.16], VAS + 3.36 [12.04]; from MP-EOT: index -0.01 [0.19], VAS + 0.18 [12.08]). Improvement in TS was maintained to EOT (Hemo-SAT general satisfaction mean [SD] change: -2.59 [13.28] from BL-EOT; +0.81 [12.00] from MP-EOT). Haem-A-QoL total score response rate (threshold: -7 points) increased from 20.8% (MP) to 28.8% (EOT) (Figure 2).
Conclusions: Prophylactic N8-GP for HA had a positive impact on maintaining and improving HRQoL for 4.5 years or longer in the pathfinder 2 trial.

PB0888 | Treatment of Hemophilic Arthropathy with Hyaluron

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Background: Hyaluronate is an important component of articular hyaline cartilage; participating in its nutrition and recovery, it helps to ease joint mobility and eliminate pain. The binding of hyaluronic acid to a specific component of cartilage tissue - chondroitin sulfate - helps to retain water in the cartilage, which increases its elasticity and improves its shock-absorbing properties.
Aims: The purpose of the study was to study the effects of hyaluronic drugs in hemophilic osteoarthritis.
Methods: In 35 patients with hemophilia, to improve joint function and to compensate for the deficiency of intraarticular fluid, intra-articular administration of preparations containing hyaluronic acid was used. Of 35 patients, 10 patients had X-ray osteortrosis of the III-IV stage, 20 patients of the III stage and 5 patients of the II-III stage. The age of patients ranged from 17 to 55 years. Severe dysfunction of the joints was observed in patients with stage III-IV osteoarthrosis.

Results: Hyaluronic acid preparations have elasticity and viscosity, the therapeutic effect is associated with the replenishment of viscosity. Preparations containing hyaluronic acid were administered intraarticularly 2.5 ml 1 time per week No. 3. It is noted that the introduction of the drug does not cause adverse effects, inflammation and relapse of hemarthrosis. Joint functions have improved in 80% of patients.

Conclusions: Thus, these drugs improve the physiological and rheological status of the tissues of the affected joint and protect the articular cartilage from mechanical damage. The introduction of hyaluronic acid into the joint cavity eliminates (reduces) these negative consequences, replenishing the amount of synovial fluid, increasing its viscosity. Friction of the articular surfaces decreases, the shock-absorbing function improves, movements in the affected joint become less painful, their amplitude increases.

PB0889 | A French Multicentre Prospective, Non-Interventional Study (B-SURE) Evaluating Real-World Usage and Effectiveness of Recombinant Factor IX Fc Fusion Protein (rFIXFc) in People with Haemophilia B: Baseline Data

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Background: Safety and efficacy of the extended half-life rFIXFc were established in phase 3 trials in patients with severe haemophilia B (HB). These trials showed low annualised bleeding rates (ABR) with 7 to ≥ 14 days prophylaxis dosing interval as well as efficacy in treatment of acute bleeds and perioperative management. rFIXFc was launched in France March 2018. The B-SURE study will evaluate real-world effectiveness and usage of rFIXFc in HB patients in France.

Aims: To describe baseline data from the ongoing B-SURE study of rFIXFc.

Methods: B-SURE (NCT03655340), a 24-month prospective, non-interventional, multicentre study, enrolled HB patients in France between September 2018 and September 2019. Eligible patients had received previous FIX treatment and were prescribed on-demand or prophylactic rFIXFc treatment prior to or at enrolment. Patients are routinely monitored according to local practice. At enrolment, baseline characteristics and 6 months retrospective data prior to switch to rFIXFc were collected, including ABR, joint ABR, factor usage and reason for initiating rFIXFc.

TABLE 1 Baseline demographic data and characteristics

At enrolment	N*=90
Age [years], mean (min-max)	34 (4-84)
<18 years, n (%)	31 (34)
≥18 years, n (%)	59 (66)
Severity of haemophilia	
Severe	80 (88.9)
Moderate	5 (5.6)
Mild	5 (5.6)
At least one target joint at enrolment, n (%)	26 (28.9)
Prior to rFIXFc initiation	**n (%)**
History of inhibitors (tolerized prior to rFIXFc initiation)	2 (2.2)
Prophylaxis FIX regimen	64 (71.1)
On demand FIX regimen	24 (26.7)

*Ninety-one patients enrolled, but data for one patient not available for this analysis

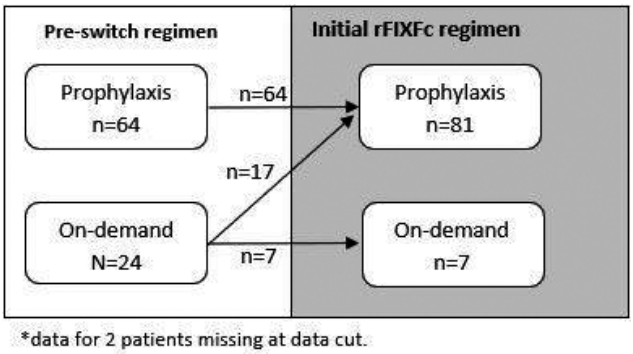

FIGURE 1 Treatment regimen pre- and post- switch to rFIXFc*

Results: Ninety-one male patients were enrolled at 21 Haemophilia treatment centres in France. Baseline data and characteristics are shown in Table 1 and treatment regimens before and after initiation of rFIXFc are shown in Figure 1.

The main reasons for initiating prophylactic treatment with rFIXFc was to 'Reduce injection frequency while maintaining protection from bleeds' (47%) and 'Protection from bleeds' (39%). Prior to rFIXFc initiation, median ABR and joint ABR in the pre-study prophylaxis group (n = 55) were 2.0 (IQR 0.0-4.0) and 0.0 (IQR 0.0-2.0), respectively. The median number of injections on conventional FIX prophylaxis was two per week.

Conclusions: Baseline data from the ongoing B-SURE study provides insights into demographics and clinical characteristics, and the rationale for initiating prophylaxis with rFIXFc, in HB patients. The study population appears well suited to evaluate real-world use of rFIXFc in France.

PB0890 | Extended Half-Life of the Double-Chain Recombinant Factor VIII-Fc Fusion Protein (FRSW107) in Previous Treated Hemophilia A Patients

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Background: Fusion of Fc part of IgG to another protein is commonly used to extend the half- life of fused protein. Here we introduce a double- chain recombinant FVIII Fc fusion protein exhibit extended in vivo half- lives.

Aims: To determine the pharmacokinetics (PK), safety and tolerability of double chain recombinant human FVIII-Fc fusion protein (FRSW107) in previously treated patients (PTPs) with age > 12 in a phase I clinical trial (clinicaltrials.gov: NCT03747653) which was approved by ethical committee of Hospital of Blood Diseases, CAMS&PUMC.

Methods: PTPs (12 to 60 years old) with FVIII:C < 2% and without history of FVIII inhibitors were enrolled and received a single dose injection of rFVIII (Advate) at either 25 or 65 IU/kg followed by an equal dose of FRSW107. Blood samples for coagulation analysis were collected at scheduled time- points up to 240 h after administration of FRSW107. Adverse events were recorded from obtaining of ICF to 28 days post administration of FRSW107. All subjects must sign the Informed Consent Form before screening.

Results: Seven subjects were enrolled in 25 IU/kg group of FRSW107 and completed the ending visit. Seven subjects were enrolled in 65 IU/kg group of FRSW107 and six completed the ending visit to date. Table1 shows the PK parameters of FRSW107. The Half-lives of FRSW107 are 20.2 h and 22.98 h derived from 25 IU/kg and 65 IU/kg cohort respectively, nearly 1.57 times of Advate. All adverse events were unrelated to study drug. None of the study subjects developed FVIII inhibitors.

Conclusions: In this phase I clinical study, FRSW107 shows no safety issues and is well tolerated in PTPs. The half- life of FRSW 107 is significantly extended compared with conventional rFVIII. The PK and pharmacodynamics of FRSW107 will be further determined in future phase III clinical trial.

TABLE 1

Cohort	25 IU/kg Cohort n = 7			65 IU/kg cohort (n = 6)		
Pharmacokinetics	Control(Advate) Mean	Trial(FRSW107) Mean	Ratio(T/R)(%)	Control(Advate) Mean	Trial(FRSW107) Mean	Ratio(T/R)(%)
Cmax(IU/dL)	68.41	53.4	78.05	134.36	95.17	70.83
λz(1/h)	0.0559	0.0390	39.77	0.0518	0.0352	68.04
t1/2(h)	12.9	20.20	156.65	14.84	22.95	154.62
AUC0-∞ (h*IU/dL)	935.02	922.54	98.67	2521.82	1988.78	78.86
CL/F(dL/h)	2.04	2.08	102.3	2.47	3.44	139.39
IVR([IU/dL]/[IU/kg])	2.73	2.14	78.23	2.06	1.47	71.1

PB0891 | The Current State of Biochemical Marker Research in Hemophilic Arthropathy: A Review

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Background: Hemophilic arthropathy (HA) is a major cause of morbidity in hemophilia patients. Biochemical markers of joint tissue turnover are non-invasive, sensitive markers that might be useful for the early detection of HA. In contrast to imaging, they may reflect dynamic changes. Moreover, biomarkers with the capacity to predict long-term clinical outcomes have the potential to improve efficiency in clinical trials, especially nowadays with the numerous breakthroughs in the hemophilia treatment landscape. While there are many biomarker studies in osteoarthritis, there is less information readily available for HA.

Aims: To give an overview about the state-of-the-art of biochemical marker research in HA and create a solid basis for future research.

Methods: A systematic search in PubMed/EMBASE was performed on September 9, 2019. Studies published in English/Dutch were included when reporting about biochemical markers and HA. All publications (including abstracts from 2018/2019) were independently assessed by two researchers for eligibility.

Results: An initial 1307 publications were found. After strict analysis 20 publications were eligible for inclusion. The majority was cross-sectional in design (15 out 20), including relatively small numbers of patients (range 10-117) or focusing on differences in hemophilia patients versus healthy persons. Several studies consisted of biomarker research in tissues other than blood/urine (e.g. synovial biopsy) or studied biomarkers in animals. Fifteen studies correlated biochemical markers to radiographic joint damage or showed the change of

these markers in reaction to hemarthroses. The most common markers studied are uCTX-II, sCOMP, sC1,2C, sCS846 and VEGF.

Conclusions: Although promising in theory, none of the investigated markers is currently sufficient for use in clinical practice/trials and not all type of markers studied are clinically feasible in HA. Clearly, larger longitudinal studies in well-defined populations should be designed to study the prognostic value of these markers for joint damage progression in an individual patient.

PB0892 | Promising Prospective Data on the Effect of Ankle Joint Distraction in Hemophilic Arthropathy

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Background: Hemophilic ankle arthropathy (HAA) is a major cause of morbidity in hemophilia patients. If conservative treatment fails, major surgical interventions like arthrodesis are indicated. An alternative treatment to postpone these interventions and maintain joint mobility is desired. In osteoarthritis, joint distraction turned out to be a good joint-preserving alternative. Retrospective evaluation of three patients with HAA treated with ankle joint distraction (AJD) showed evident clinical and structural improvements.

Aims: To gather prospective data on clinical efficacy and tissue structure changes of AJD in HAA.

Methods: This ongoing first prospective study included patients ≥ 18 and ≤ 55 years with severe complaints of HAA, insufficiently responding to conservative treatment. AJD was performed during ten weeks by use of an external frame applying 5 mm distraction. Clinical efficacy (questionnaires and performance tests) and tissue structure changes (MRI) were evaluated. The study was approved by the UMCU Medical Ethical Review Board and informed consent was obtained.

Results: One-, two- and three-year follow-up data are available for eight, seven and five patients respectively. Pain, evaluated by the Ankle Osteoarthritis Scale (AOS), decreased significantly. Functional limitations, assessed by the Hemophilia Activities List (HAL) and performance tests, improved over time, clearly adding to improved daily functioning (figure 1). Notably, the full arc of mobility was retained. MRI demonstrated improved osteochondral changes, whereas soft tissue changes remained unchanged (figure 2). The main complication during treatment was pin tract infection (n = 6), commonly seen with external frames and effectively treated with a short course of antibiotics. There were no adverse bleeding events.

Conclusions: This explorative study shows that AJD in HAA results in decreased pain, improved function and tissue repair for a prolonged time. This joint-preserving treatment may have a great impact,

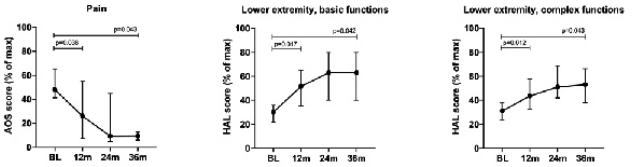

FIGURE 1 AOS pain; 0 = no pain, 100 = worst pain imaginable. HAL; 0 = poor functional status,100 = best functional status. Median values with IQR

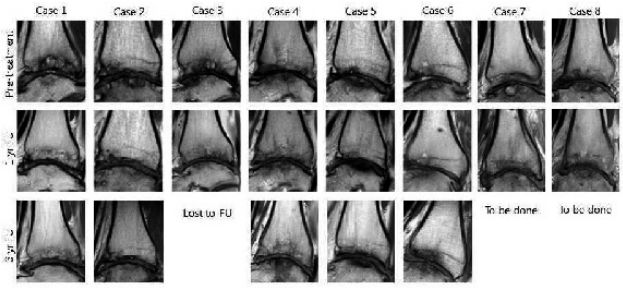

FIGURE 2 Structural changes on MRI (sagittal images at baseline and one and three year after AJD)

especially in the relatively young hemophilia population. Prolonged follow-up is needed to determine the duration of efficacy in HAA.

PB0893 | Detectability and Clinical Significance of Calcium Exchange and 25 (OH) D in the Development of Osteoporosis in Patients with Hemophilia

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Background: To diagnose osteoporosis and osteopenia, the content of total Ca in the blood was studied.

Aims: To determine the relationship between calcium and vitamin D metabolism in the development of osteoporosis in patients with hemophilia.

Methods: 43 patients with hemophilia were examined. Phased laboratory coagulological studies were carried out. Vitamin D deficiency was determined at a level of 25 (OH) D of less than 100 μg / ml.

Results: Congenital deficiency of coagulation factor VIII was detected in 35 patients (81.4%), and in 8 (18.6%) patients congenital deficiency of coagulation factor IX. Severe hemophilia (VIII, IX - < 1%) - in 14 patients (32.5%), hemarthrosis (92.8%) and soft tissue hematomas made up group I. 24 patients (55.8%) with moderate form (VIII, IX - 1-5%) - group II and 5 patients (11.6%) - were diagnosed with mild hemophilia (> 5%) - group III, bleeding occurred after injuries. The content of total Ca + and vitamin D in the blood, depending on the form of the disease, was significantly lower in group I patients than in group III patients. ($p < 0.001$). Moreover, the lowest values of total Ca + in the blood (less than 1.17 mmol / L) and 25 (OH) D (less than 100 μg / L) were found in patients with severe form, which

amounted to 78.5 ± 10.9%, then as in patients of group III, the titer of total Ca + and vitamin D determines the lower limit of normal.

Conclusions: The direct correlations of the deficiency of blood coagulation factors with the exchange of calcium and vitamin D were determined, which indicate a risk of developing osteoporosis of the musculoskeletal system depending on the severity of hemophilia. These data emphasize the importance of timely diagnosis of total Ca + and vitamin D in patients with hemophilia.

PB0896 | A Randomized Controlled Trial: Pharmacokinetic-Guided Dosing of Factor VIII Concentrate Versus Standard Replacement Therapy in Perioperative Hemophilia A Patients (OPTI-CLOT Trial)

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Background: Pharmacokinetic (PK)-guided dosing of factor VIII (FVIII) concentrates is an important tool for hemophilia A patients undergoing surgery to prevent underdosing with increased risk of surgical bleeding as well as overdosing associated with unnecessary treatment costs and risk of trombosis. Despite the fact that several PK studies have been performed, no randomized controlled trial has been performed which compares perioperative PK-guided dosing with routine dosing based on bodyweight.

Aims: Compare PK-guided iterative dosing versus standard FVIII replacement therapy in perioperative hemophilia A patients to establish effect on FVIII concentrate consumption.

Methods: In a multicenter, open-label, randomized controlled trial, hemophilia A patients (FVIII < 0.05 IU/mL) were randomly assigned to treatment arms in 1:1 ratio, stratified for mode of administration and severity of surgical procedure. Primary endpoint was FVIII concentrate consumption during total perioperative period (linear regression). Secondary endpoints included: efficacy of FVIII range targeting (linear mixed effects modeling), safety e.g. bleeding (Chi-square test), and duration of hospitalization (linear regression). The study was approved by Medical Ethical Committees. All patients gave written informed consent.

Results: Sixty-five severe or moderately affected hemophilia A patients have been included. Total perioperative FVIII consumption was similar between PK-guided (mean: 329 ± 199 IU/kg) and standard treatment arm (mean: 375 ± 205 IU/kg) (P = 0.83, Table 1). However, PK-guided dosing significantly improved FVIII range targeting (Figure 1). The relative quadratic difference in the ratio between FVIII-measured and FVIII-target was 0.186 (95% CI 0.119 - 0.290) adjusted for blood type, age, weight, severity of surgery, mode of infusion, perioperative time point. Both perioperative bleeding events and hospitalization period did not differ between treatment arms.

Conclusions: This first RCT shows that PK-guided iterative dosing does not decrease perioperative FVIII concentrate consumption in hemophilia A patients. However, PK-guided dosing leads to more optimal targeting of pre-specified FVIII levels and more precise perioperative dosing.

TABLE 1 Multivariate linear regression model of FVIII concentrate consumption (IU/kg) in perioperative hemophilia A (FVIII < 0.05 IU/ml) patients

	Regression Coefficient (B)	Standard Error	95% Confidence interval		P value
			Lower	Upper	
Constant	262	145	−30	553	0.077
Randomization result (PK-guided dosing)	−10	49	−108	88	0.834
Blood type (Blood type O)	50	49	−48	149	0.309
Age (years)	−3	2	−7	0	0.035
Weight (kilogram)	−1	1	−4	2	0.473
Planned type of FVIII administration (continuous infusion)	118	60	−3	239	0.055
Planned type of surgical procedure (medium risk)	105	62	−20	230	0.099

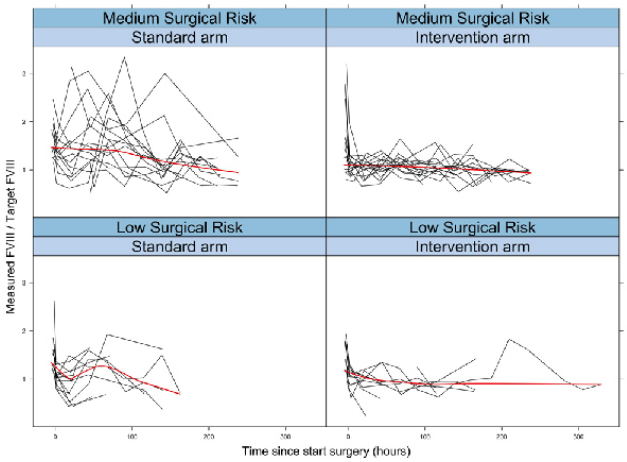

FIGURE 1 Spaghetti plot of calculated ratio of FVIII-measured divided by FVIII-targeted in a perioperative period of 14 days from day of surgery

PB0897 | Assessing the Bleeding Phenotype in Non-Severe hemophilia by use of the ISTH Bleeding Assessment Tool (BAT)

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Background: Patients with non-severe hemophilia experience an increased bleeding tendency predominantly after provocative events such as trauma or surgery. Few studies have quantified the bleeding phenotype in this population. The International Society of Thrombosis and Haemostasis Bleeding Assessment Tool (ISTH-BAT) has been developed to objectively quantify bleeding symptoms and could provide insight into the extent of bleeding reported by non-severe hemophilia patients.

Aims: The aim of this study was to describe the phenotype of patients with non-severe hemophilia A and B using the patient reported ISTH-BAT.

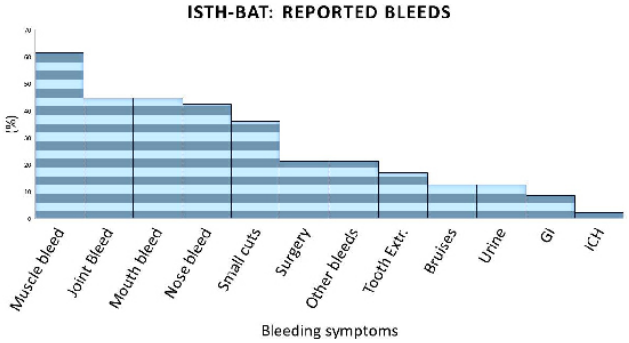

FIGURE 1 Reported bleeding symptoms. Abbreviations; GI = gastro-intestinal, ICH = intracranial hemorrhage, Tooth Extr. = tooth extractions

Methods: The DYNAMO study is a cohort study that includes male congenital hemophilia A and B patients aged 12-55 years with a baseline clotting factor level of 2-35 IU/dL. ISTH-BAT scores were prospectively collected through an online questionnaire omitting gender-specific sections after written consent was obtained. The study was approved by the medical ethics committee (ClinicalTrials.gov Identifier: NCT03623295).

Results: In this preliminary analysis, 47/101 (47%) patients (37 HA and 10 HB) completed the questionnaire. The median age was 39 years (IQR 27-49) and the median FVIII/IX level was 12 IU/dL (IQR 6-17). We found a median ISTH-BAT score of 7 (IQR 3-13), with 74% reporting abnormal bleeding scores (≥4). Joint, muscle and mouth bleeds were reported most frequently (62, 45 and 45%) (Figure 1). Patients with moderate hemophilia (2-5 IU/dL) had higher ISTH-BAT scores compared to patients with mild hemophilia (5-35 IU/dL) (median ISTH-BAT score 10 vs 7). ISTH-BAT scores were similar between hemophilia A and B.

Conclusions: Patients with non-severe hemophilia A and B had a median ISTH-BAT of 7 with 74% of patients reporting abnormal bleeding scores. Patients with moderate hemophilia had higher ISTH-BAT-scores when compared to patients with mild hemophilia.

PB0898 | Comparison of FVIII Prophylaxis Treatment Regimen and Associated Clinical Outcomes between rVIII-SingleChain and Other rFVIII Products Commonly Used in Italian Hemophilia A Patients

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Background: Several recombinant factor VIII (rFVIII) products are available in Italy to treat hemophilia A, including long-acting products with extended dosing intervals. It is interesting to evaluate the real-world utilization of these products to optimize treatment choice.

Aims: To determine factor consumption and annual bleed rates (ABR) associated with prophylactic use of rVIII-SingleChain compared to other commonly used rFVIII products.

TABLE 1 Patient characteristics, and bleeding rates, infusion frequency and factor consumption with selected rFVIII products

Product (patients, n)	Age (years), mean (SD)	Disease severity	Treatment duration (weeks), median (IQR)	ABR, median (IQR)	ABR, mean (SD)	Frequency of infusion	Consumption (IU/kg/week), median (IQR)	Consumption (IU/kg/week), mean (SD)
Long-acting								
rVIII-SingleChain (60)	33.1 (18.5)	severe (81.7%); mild/moderate (18.3%)	40.6 (17.6-52)	0.0 (0.0-0.5)	0.7 (1.4)	>2x/week (43.3%); ≤2x/week (56.7%)	91.6 (62.7-108.9)	92.7 (36.0)
rFVIIIFc (73)	28.4 (19.9)	severe (89.0%); mild/moderate (11.0%)	52 (52-52)	0.0 (0.0-1.0)	0.4 (0.8)	>2x/week (23.3%); ≤2x/week (76.7%)	92.3 (80.0-109.9)	103.8 (70.5)
Standard-acting								
Octocog alfa (83)	27.1 (17.2)	severe (85.5%); mild/moderate (14.5%)	52 (52-52)	1.0 (0.0-2.0)	1.2 (1.6)	>2x/week (83.1%); ≤2x/week (16.9%)	113.2 (78.9-140.0)	122.4 (66.3)
BAY 81-8973 (74)	27.5 (19.2)	severe (82.4%); mild/moderate (17.6%)	52 (52-52)	0.0 (0.0-1.0)	0.8 (1.3)	>2x/week (63.5%); ≤2x/week (36.5%)	100.0 (80.0-121.7)	112.7 (68.6)

Methods: De-identified patient chart information was obtained from Hemophilia Treatment Centers and hematologists for 290 patients currently treated with any of the following four rFVIII products for at least 8 weeks: rVIII-SingleChain (n = 60), rFVIIIFc (n = 73), octocog alfa (n = 83) and BAY 81-8973 (n = 74). Patient selection took into account age and disease severity in order to match the four patient groups as closely as possible. The number of bleeds was annualized to calculate mean ABR. The dosing and infusion frequency on the patient's last prescription were used to calculate prophylactic factor consumption.

Results: A data summary is shown in Table 1. In patients treated with rVIII-SingleChain and rFVIIIFc, 56.7% and 76.7%, respectively, were dosed ≤ 2 times per week, while most patients on octocog alfa (83.1%) or BAY 81-8973 (63.5%) infused more frequently than twice per week. Mean ABR was 0.4 with rFVIIIFc, 0.7 with rVIII-SingleChain, 0.8 with BAY 81-8973 and 1.2 with octocog alfa. Median annual spontaneous bleed rate (AsBR) was 0.0 for all products with 88.3%, 71.1%, 82.4% and 87.7% of patients with zero spontaneous bleeds for rVIII-SingleChain, octocog alfa, BAY 81-8973 and rFVIIIFc, respectively. Mean consumption was lowest with rVIII-SingleChain (92.7 IU/kg/week), 10.7%, 17.7% and 24.3% below rFVIIIFc, BAY 81-8973 and octocog alfa, respectively.

Conclusions: In a patient-chart review including four rFVIII products rVIII-SingleChain shows convincing efficacy, real-life ABR (median = 0) similar to that observed in clinical studies with a reduced factor consumption, thereby achieving cost-saving while providing excellent patient outcomes.

PB0899 | Prophylactic Factor VIII Consumption and Clinical Outcomes in Italian Hemophilia A Patients who Switched to Extended Dosing Intervals with Long-Acting rVIII-SingleChain: A Retrospective Patient Chart Review

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Background: Long-acting recombinant factor VIII (FVIII) therapies provide additional therapeutic options to the previous armamentarium of standard-acting FVIII therapies. One such long-acting FVIII is rVIII-SingleChain. When evaluating different FVIII products in clinical practice, real-world utilization might be useful to optimize treatment outcomes.

Aims: To determine real-world dosing intervals, factor utilization and bleed rates in a group of Italian hemophilia A patients who switched from a prior FVIII product to rVIII-SingleChain.

Methods: Hemophilia Treatment Centers and hematologists provided de-identified patient chart information for 60 patients currently treated with rVIII-SingleChain. Data collected included age, sex, weight, current and prior treatment regimen (prophylaxis/on demand, infusion frequency, dosing), and number of reported bleeds. The dosing and infusion frequency on the patient's most recent prescription were used to calculate prophylactic factor consumption with rVIII-SingleChain and the prior drug. The number of bleeds was annualized to calculate mean annual bleed rate (ABR) for rVIII-SingleChain, and over a period of up to 1 year on the prior drug.

Results: Of the 60 rVIII-SingleChain prophylaxis patients included in the study, 50.9% were dosed 2 or fewer times per week. Fifty-three (88%) patients were also treated prophylactically with their prior drug, with 54.7% of patients dosing at least 3-times per week. In these 53 prophylaxis-to-prophylaxis patients, mean prophylactic factor consumption (IU/kg/week) was similar with prior drug or rVIII-SingleChain (94.6 ± 38.5 and 95.0 ± 37.1, respectively). Mean ± standard deviation ABR decreased in this group of patients from 1.8 ± 1.7 (median, 1.0) with the prior drug to 0.5 ± 1.2 (median, 0.0) with rVIII-SingleChain. The percentage of patients with zero spontaneous bleeds went from 45.3% to 90.6% of patients.

Conclusions: This study shows that rVIII-SingleChain allows patients to use extended treatment intervals without increase in factor utilization and with effective bleed prevention consistent with clinical trial results.

TABLE 1 Dosing, consumption and bleeding rates with rVIII-SingleChain and prior FVIII product for all prophylaxis-to-prophylaxis patients

	All patients (N = 53)		Severe disease only (N = 45)	
	rVIII-SingleChain (n = 53)	Prior FVIII (n = 53)	rVIII-SingleChain (n = 45)	Prior FVIII (n = 45)
Age (years), mean (SD)	32.5 ± 18.8*		33.2 ± 18.3*	
Dose, IU/kg/week ± SD	95.0 ± 37.1	94.6 ± 38.5	96.6 ± 38.1	98.3 ± 40.1
Prophylaxis dosing interval, %				
>2x/week	49.1	56.6	51.1	62.2
≤2x/week	50.9	43.4	48.9	37.8
ABR, mean ± SD	0.5 ± 1.2	1.8 ± 1.7	0.6 ± 1.2	1.8 ± 1.5
AsBR, mean ± SD (zero spontaneous bleeds, %)	0.3 ± 1.1 (90.6)	1.1 ± 1.6 (45.3)	0.3 ± 1.1 (91.1)	1.1 ± 1.3 (42.2)

*Age only available for patients on current product; date of birth information not collected to ensure patient anonymity

PB0900 | Joint Health Outcomes in Patients with Hemophilia A Receiving Antihemophilic Factor (Recombinant) in a Real-World Setting: Results of a 6-Year Interim Analysis of the AHEAD International Study

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Background: The Antihemophilic factor (recombinant) (rAHF) Hemophilia A outcome Database (AHEAD) study evaluates long-term effectiveness and safety outcomes in patients with hemophilia A receiving rAHF in routine clinical practice.

Aims: This 6-year interim analysis assessed age-stratified joint health in AHEAD study patients receiving either prophylactic (PRO) or on-demand (OD) rAHF.

Methods: AHEAD is an international, non-interventional, prospective, multicenter study (NCT02078427, started in 2011) including patients with moderate (factor VIII activity 1-5%) or severe (factor VIII < 1%) hemophilia A. Ethics committee approval and patients' informed consent were obtained. The primary objective for AHEAD is to describe joint health outcomes. In this analysis, joint health was assessed using the pain (score 0-3), bleeding (score 0-3), and physical exam (score 0-12) Gilbert scale parameters; higher scores for each category represent worsening conditions. Here we report results of the 6-year interim descriptive analysis in patients aged 2 to < 12, 12 to < 18, and ≥ 18 years with available Gilbert score data (data cutoff: 15 July 2019).

Results: At year 1, Gilbert score data from 52 children (2 to < 12 years; all PRO), 19 adolescents (12 to < 18 years; all PRO), and 116 adults (≥

TABLE 1 Age-categorized average Gilbert score* across all joints over 6 years in patients with hemophilia A receiving rAHF PRO or OD

Age group (treatment), n	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
2 to < 12 years (PRO), n	52	46	28	16	6	2
Mean ± SD / median (range)	0.8 ± 1.4/0.3 (0.0-8.0)	0.6 ± 1.3/0.1 (0.0-8.0)	0.5 ± 0.7/0.1 (0.0-2.0)	0.3 ± 0.6/0.0 (0.0-2.0)	0.4 ± 0.8/0.0 (0.0-2.0)	0.2 ± 0.2/0.2 (0.0-0.3)
12 to < 18 years (PRO), n	19	19	11	6	1	2
Mean ± SD/median (range)	0.8 ± 1.3/0.2 (0.0-5.0)	0.9 ± 1.4/0.0 (0.0-4.0)	0.9 ± 0.9/0.7 (0.0-3.0)	1.8 ± 2.8/0.5 (0.0-7.0)	0.0/0.0	0.0 ± 0.0/0.0 (0.0-0.0)
≥18 years (PRO), n	86	78	59	31	16	8
Mean ± SD/median (range)	2.7 ± 3.0/2.0 (0.0-14.5)	2.5 ± 2.6/2.0 (0.0-13.0)	2.9 ± 3.2/2.3 (0.0-17.0)	2.1 ± 2.0/1.0 (0.0-6.3)	1.7 ± 1.9/0.9 (0.0-6.0)	1.4 ± 1.2/1.2 (0.0-3.2)
≥18 years (OD), n	30	28	18	8	5	2
Mean ± SD/median (range)	3.6 ± 2.7/3.0 (0.0-10.0)	3.6 ± 2.9/3.3 (0.0-12.0)	4.9 ± 2.6/4.9 (0.3-9.0)	3.9 ± 2.8/4.2 (0.0-9.0)	2.1 ± 2.2/1.4 (0.0-5.0)	6.3 ± 5.3/6.3 (2.5-10.0)

*3 dimensions: pain, bleeding, physical exam. OD, on demand treatment; PRO, prophylactic treatment; rAHF, antihemophilic factor (recombinant).

18 years; PRO, n = 86; OD, n = 30) were available for analysis. In PRO children, average Gilbert scores remained consistently low throughout the observation period (Table 1); scores were lower in children and adolescents than in adults. Among adults aged ≥ 18 years, means and medians for average Gilbert scores were consistently lower in patients receiving rAHF PRO versus OD; this may represent a clinically meaningful difference. No new safety signals were observed for this 6-year interim analysis versus previous analyses.

Conclusions: These real-world data in patients with moderate and severe hemophilia A support early initiation of PRO rAHF as a treatment strategy to preserve joint health.

PB0901 | Pharmacokinetic Profile of Southeastern Brazilian Hemophilia A Patients on Prophylaxis with Octocog Alfa

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Background: Hemophilia A (HA) is an inherited bleeding disorder characterized by deficiency of procoagulant factor VIII (FVIII). Prophylaxis with FVIII is the most effective treatment in patients with moderate/severe (ms) HA (FVIII:C < 5%), which can prevent spontaneous bleeding episodes. Although standard prophylaxis based on body weight and at least 1% of plasmatic FVIII trough levels is effective, spontaneous bleeding still occurs. The modern individualized therapy then considers patient behaviour and FVIII pharmacokinetic (PK) parameters.

Aims: The aim of this study is to describe the PK profiles of msHA patients who were submitted to PK-individualized therapy with octocog alfa (rFVIII).

Methods: Patients were invited from two Southern Brazilian hemophilia treatment centers, if they were 1 year or older, they weighed at least 12 kg, their inhibitor status was negative for at least 6 months, they had ≥ 50 exposition days and they were bleeding-free for at least 2 weeks. Data were obtained from medical files. PK profile was calculated according to the myPKFiT protocol.

Results: Thirty-three patients on PK-individualized prophylaxis were included. Median age was 12.0 [IQR,8.0-24.0] years. One patient was excluded from the analyses because his FVIII half-life was below the normal value. Patients were divided in two groups: younger than 15 years (n = 21) and 15 years or more (n = 12). Median FVIII half-life was statistically lower (p = 0.001) and median clearance was statistically higher (p = 0.016) in the youngest patient group than in the oldest group, although the estimated doses to keep FVIII trough level of at least 1% with each-other-day infusion were similar between the groups (p = 0.065).

Conclusions: Our results confirm previous studies which described a shorter FVIII half-life among children, but surprisingly the estimated doses to keep a trough level above 1% were similar between the age groups. The significance of these findings will be further elucidated after inclusion and follow-up of new patients.

PB0902 | Plasma Factor VIII Activity Measured by Coagulation One-Stage and Chromogenic Assays in Patients with Hemophilia A Treated with Emicizumab

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Background: Emicizumab is an asymmetrical bispecific antibody that mimics the cofactor function of activated factor VIII (FVIIIa). It has become used for prophylactic treatment of patients with hemophilia A (PWHA) since the clinical use was approved in Japan in 2018. However, it has been known that it was difficult to evaluate coagulation abilities in PWHA treated with emicizumab.

Aims: Our objective is to clarify the coagulation abilities by measuring FVIIIa with the coagulation one-stage (OSA) and the chromogenic assays (CGA) in PWHA treated with emicizumab.

Methods: We obtained plasmas drawn from nine PWHAs (involved 2 with FVIII inhibitor) treated with emicizumab. We divided a sample into two and stored below -80 degrees until measurement. Then, one was directly measured FVIIIa with OSA and CGA, and the other was measured after incubating with anti-emicizumab antibody (AEA), which was provided by Chugai Cooperation.

Results: All samples not incubated with AEA showed outliers more than 180% when measured by OSA. However, their values measured by CGA showed about 20% (from 13.1 to 32.3). When incubated with AEA, both values of FVIIIa measured by OSA and CGA, showed baseline values of the respective patients. There was no difference between the values measured by both assays.

Conclusions: We demonstrated that FVIIIa could be measured correctly using the plasma incubated with AEA even in the presence of emicizumab. Furthermore, the value measured by CGA in the sample not incubated with AEA seemed to be equivalent to the FVIIIa as a coagulation ability.

PB0903 | Thrombin Generation is a Better Predictor for Factor VIII Replacement Therapy Effect Than Factor VIII Activity Levels

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Background: Pharmacokinetic monitoring of factor VIII (FVIII) replacement therapy in hemophilia A (HA) patients mainly consists of measuring plasma FVIII activity levels and subsequent dose adjustment. The Nijmegen Hemostasis Assay (NHA) measures thrombin generation (TG), thereby providing a novel pharmacodynamic measurement tool relating dose-to-effect.

Aims: To determine dose-effect responses after a FVIII replacement therapy bolus in HA patients by measuring TG profiles.

Methods: Pharmacodynamic studies with a single bolus of 25-50 IE/kg standard half-life FVIII were performed in 26 HA patients with simultaneous determination of plasma FVIII activity levels and TG using the NHA at 10 time points. The study was approved by the Radboudumc ethics-committee. Patients gave written informed consent. TG parameters (lag time (LT), time to thrombin peak (TTP), thrombin peak height (TPH) and thrombin potential (TP)) were calculated and are presented as absolute values as medians (interquartile range).

Results: Median baseline FVIII concentration was 1.0% (< 1.0-6.0), with a LT 15 of minutes (6.8-23.3), TTP 28.5 minutes (15.5-36), TPH 22 nM (15-35), and TP 404 nM/min (undetectable-876). After FVIII replacement therapy, FVIII concentration increased to 72% (62-82) at 15 minutes and TG parameters normalized. LT was restored to 3 minutes (2.8-3.5), TTP to 8.5 minutes (7.8-9.3), TPH increased to 222 nM (159-255) and TP to 1834 nM/min (1546-2353), figure 1. Twenty-four hours after infusion, FVIII concentration was 15% (10-26), while increased TG parameters remained high [LT 4.7 minutes (3.5-5.3), TTP 12.3 minutes (10.8-14.6), THP 73 nM (58.5-126.3), TP 1394 nM/min (1066-1677)].

Conclusions: HA patients with equal FVIII activity levels show clear differences in TG (TPH, TTP, TP and LT). After FVIII replacement, TG normalizes (TPH, TTP and LT) and thrombin potential remains normal up to 24 hours. We suggest that thrombin generation measurements better predict the dose effect relation in HA patients compared to solely FVIII activity levels.

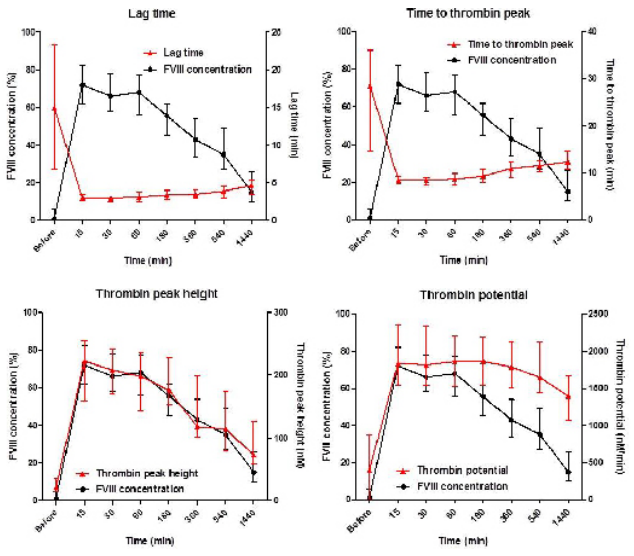

FIGURE 1 Thrombin generation parameters (right y-axis) and FVIII concentration (left y-axis) after a bolus of standard half-life FVIII replacement therapy

PB0905 | A Canadian, Multi-Center, Retrospective, Non-Interventional Study of Clinical Outcomes from Early Use of N9-GP Compared with Previous Treatment in Patients with Hemophilia B in a Real-World Setting

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Background: The Canadian Blood Disorders Registry (CBDR) captures data collected from hemophilia treatment centers and directly from patients. With the recent availability of nonacog beta pegol (N9-GP) in Canada, the CBDR provides a unique opportunity.

Aims: To assess real-world outcomes following N9-GP use in a clinical setting.

Methods: This non-interventional, retrospective study used CBDR data to describe and analyze real-world outcomes in Canadian hemophilia B patients receiving N9-GP for ≥ 3 months in any setting (prophylaxis, on-demand treatment, treatment of breakthrough bleeding [BTB]). For comparison with previously-used products, only patients for whom data existed in CBDR for the 6-month period pre-switch to N9-GP were included.

Results: At the data cut-off (September 30, 2019), 40 patients were included in the analysis, with a median age of 44 years. Distribution of disease severity was 2.5% mild, 40% moderate, 55% severe, and

TABLE 1 Comparative annualized factor consumption for N9-GP versus previous product

Total annualized consumption [kIU/year]*	Number of patients†	Mean	Median	Min, Max	Number of patients†	Mean	Median	Min, Max
For prophylaxis	32	228.9	185.0	36.0, 684.0	38	150.5	141.9	51.1, 308.8
For treatment of BTBs	19	64.9	48.0	8.0, 48.0	22	23.3	9.3	1.7, 107.4
For on-demand treatment	6	62.3	43.0	8.0, 200.0	2‡			

BTBs, breakthrough bleeds. *Annualized consumption estimate for each patient based on the data available (6 months for pre-N9-GP); †Number of patients where information is available; ‡Insufficient data.

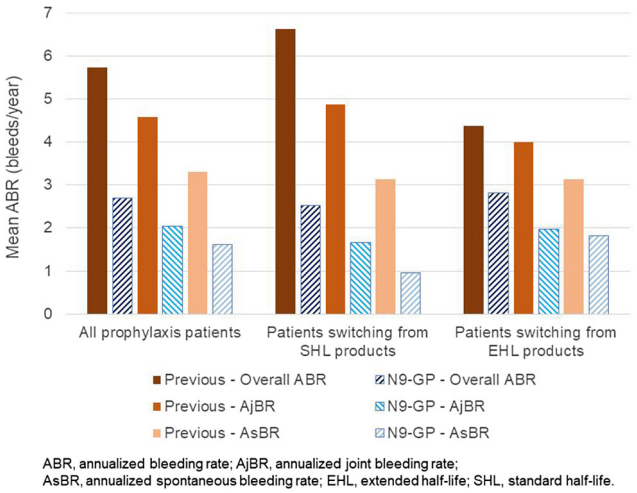

FIGURE 1 Comparative effectiveness of N9-GP prophylaxis versus previous product by type of bleed and previous product

2.5% unknown. At study start, 10 target joints were present in 5 patients. Most patients had previously received rFIXFc (55% versus 40% rFIX), with most previously receiving prophylactic treatment (85% versus 15% on-demand). No patients had present or previous inhibitor development.

During a median treatment period of 11.11 months on N9-GP, 106 BTBs were reported in 22 patients; 42% of patients reported zero bleeds. Median time from last recorded prophylactic injection to start of bleeding was 7.1 days and the mean number of injections required to treat a bleed was 1.23.

Figure 1 shows the comparative effectiveness of N9-GP prophylaxis versus previous product by type of bleed and previous product.

Conclusions: This is the first study to investigate treatment patterns and clinical outcomes with N9-GP in hemophilia B patients in a real-world setting. Initial data suggest improved bleeding outcomes with lower factor consumption after switching to N9-GP, regardless of whether patients previously received standard or extended half-life products.

PB0906 | Baropodometric Barefoot Analysis for Musculoskeletal Health Assessment in Congenital Hemophilia

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Background: Hemophilic arthropathy and muscular hematomas are frequent complications of hemophilia that may impact the postural system. Baropodometric barefoot reliably measures the postural system energy consumption.

Aims: We aimed to investigate the correlation of baropodometric parameters with the hemophilia joint health score (HJHS) and the EQ-5D-5L, a health-related quality of life (HRQoL) tool in patients with hemophilia.

Methods: We evaluated 114 consecutive adult congenital hemophilia A and B patients referring to our center. In order to avoid confounding by incomplete motor-skill development or health conditions other than hemophilia, we investigated patients aged 18-65 and divided them into age classes (18-29/30-41/42-53/54-65) to better characterize musculoskeletal health variables, which are often dependent on age. Parameters obtained with baropodometric barefoot (Biopostural system Pod Base) over 51.2" in a standing position were correlated with HJHS and EQ-5D-5L by Spearman's rho ($p < 0.05$ = * and $p < 0.001$ = **).

Results: Median age of patients was 41 years (interquartile range - IQR 28-50), 90% had hemophilia A, of whom 75% severe, 63% were regularly applying sport activities. The median HJHS was 9 (IQR 3-15).

All barefoot parameters except acceleration showed a slight but significant positive correlation with HJHS (rho 0.22-0.25). HJHS was strongly correlated with the age (0.47**). After dividing patients in age classes, none of the barefoot parameters correlated with HJHS or age, although they correlated with the physical domains of EQ-5D-5L (motility, self-care and usual activities). Patients aged 42-53 showed the strongest correlations between EQ-5D-5L motility, self-care and usual activities and variance of speed (0.47**; 0.53**; 0.61** respectively) and length function of surface (0.55**; 0.56**; 0.59** respectively).

Conclusions: Baropodometric barefoot provides useful information compared to HJHS and HRQoL tools in hemophilia patients. The EQ-5D-5L motility, self-care and usual activities domains, correlated strongly with barefoot parameters in patients aged 42-53.

PB0907 | Favourable Outcomes in Two Young Patients with Severe Haemophilia B After Switching to rIX-FP

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Background: Frequent infusions and bleeding can substantially affect the quality of life of hemophilia B (HB) patients. PROLONG 9FP clinical trials demonstrated that rIX-FP, a recombinant long acting FIX, allows a prophylaxis with extended dosing intervals that is effective in bleeding control and prevention.
Aims: Here we report two cases of young patients with severe HB switching from standard- rFIX to rIX-FP.
Methods: In August 2017, two brothers were switched from rFIX (40 IU/kg twice weekly) to rIX-FP. Doses were adjusted to extend the dosing interval while maintaining FIX levels > 5%. FIX activity was measured at defined time points after a single infusion of 50 IU/kg rIX-FP following a 7-day washout period. Breakthrough bleeding, joint bleeding and inhibitors development were monitored. Physical activity was assessed before and after the switch.
Results: Fifteen minutes after the infusion, FIX activity levels were 110% in patient 1 (11 years old) and 89.4% in patient 2 (8 years old). At day 7, FIX levels were > 10% for both patients. On day 10, patients 1 and 2 maintained the FIX activity at 6.13% and 5.46%, respectively. Consequently, they were prescribed 43 IU/kg and 50 IU/kg rIX-FP every 10 days, respectively. Since switching to rIX-FP prophylaxis, in any case neither breakthrough bleeding, nor inhibitors, nor joint involvements have been observed. Patients considerably increased their physical activity at school, becoming regularly involved in gym (2-3 times/week) plus motor activities (2 times/week). No inhibitors have been observed in either case, and patient 1 learned to self-infuse.
Conclusions: In two young patients with severe HB, an extended 10-day rIX-FP dosing was well tolerated, allowed FIX levels > 5%, provided effective bleeding prevention and a substantial reduction in FIX consumption. Moreover, switching to rIX-FP increased the treatment satisfaction and improved the compliance both in patients and in their caregivers.

PB0908 | Evaluation of Factors Associated with Extended Half-Life Product Utilization for Men with Hemophilia B Participating in the Community Counts Registry from 2014-2018

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Background: Extended half-life (EHL) products were first approved for men with Hemophilia B (MWHB) in 2014. The Community Counts Registry (Registry) provides a unique opportunity to evaluate EHL product utilization.
Aims: To examine factors associated with EHL product usage among MWHB and whether the type of treatment product use varied over time and according to the US Hemophilia Treatment Centers (HTCs) where care was received.
Methods: We used marginal models to examine demographic and clinical factors associated with use of an EHL compared to a standard half-life (SHL) product among MWHB on continuous prophylaxis, enrolled in the Registry between 2014 to 2018. Parameters were estimated with generalized estimating equations to adjust the standard errors, in order to account for correlated data within HTCs.
Results: Of 655 MWHB 39.1% used EHL products. The proportion of severe MWHB on EHL varied significantly by HTC (Figure 1) and the intraclass correlation was 14.9%. In multivariable analyses, adjusting for other variables in the model, those enrolled in 2018 had markedly higher odds of EHL product use than those enrolled in 2014 (aOR 9.4) (Table 1). Severe MWHB had significantly lower odds of EHL product use than non-severe MWHB (aOR 0.6). MWHB treated in HTCs in regions A, F, and H had higher odds of EHL product use than those treated in region E (aOR 4.6, aOR 3.3, and aOR 3.8 respectively). Finally, there was an inverse linear association between use of an EHL product and percentage of missed doses.

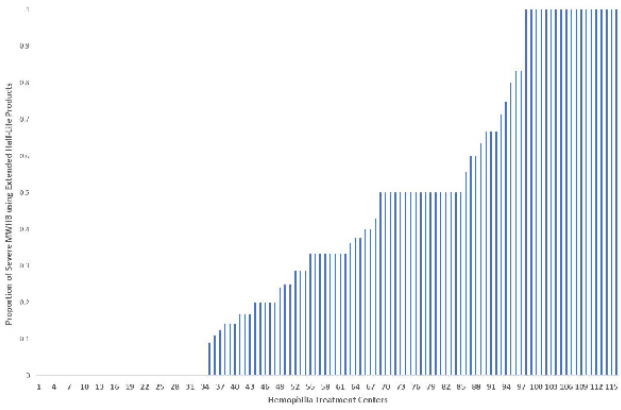

FIGURE 1 Overall Proportion of Men with Severe Hemophilia B using Extended Half-life Treatment Products in the Registry by HTC

TABLE 1 Multivariable Factors Associated with Extended Half-Life Recombinant Product Utilization for Continuous Prophylaxis with GEE Marginal Mode

Characteristics	aOR (95% CI)	P-value	Characteristics (continued)	aOR (95% CI)	p-value
Disease Severity: Severe	0.6 (0.4-0.9)	0.0097	Region A	4.6 (1.7-12.5)	0.0031
Non-severe	reference	-	Region B	2.0 (0.8-4.9)	0.1186
Year of Enrollment 2014	reference	-	Region C	2.1 (0.9-4.9)	0.0994
Year of Enrollment 2015	2.7 (1.4-5.6)	0.0054	Region D	2.1 (0.8-5.4)	0.1141
Year of Enrollment 2016	3.3 (1.5-7.1)	0.0028	Region E	reference	-
Year of Enrollment 2017	7.7 (3.6-16.3)	<0.0001	Region F	3.3 (1.4-7.8)	0.0078
Year of Enrollment 2018	9.4 (3.9-22.6)	<0.0001	Region G	2.5 (0.9-6.8)	0.0743
<10% Missed Dose (reference > 50% missed dose)	4.3 (1.4-13.1)	0.0115	Region H	3.8 (1.5-9.5)	0.0053
10-50% missed dose (reference > 50% missed dose)	2.4 (0.7-8.0)	0.1548			

Conclusions: There was a marked increase in EHL product use between 2014 and 2018. Surprisingly, EHL product use was more common among non-severe MWHB then severe MWHB. Missed prophylaxis doses were less using EHL products than with SHL products. These data provide strong evidence that among MWHB, product type for continuous prophylaxis varies depending on the patients' HTC and region.

PB0909 | Hip Bleeds in Boys with Mild Haemophilia A: A Single Centre Experience

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Background: Patients with mild haemophilia tend not to develop spontaneous haemarthrosis and usually only need factor replacement at times of trauma or surgery. Haemarthrosis commonly occurs in knee and ankle joints; hip bleeds are rare.

We have a cohort of patients with mild haemophilia A who have sustained hip bleeds.

Aims: To describe and quantify hip bleeds in children with mild haemophilia A in a single paediatric comprehnsive care centre.

Methods: A retrospective case note review was carried out of all boys with mild haemophilia A who had reported hip bleeds in the preceding 8 years at a single paediatric comprehensive care centre. The hip bleeds were either diagnosed clinically, by ultrasound or aspiration. Demographics, mechanism of injury, diagnostics, treatment and joint status were explored.

Results: Of a current cohort of 58 patients with mild haemophilia A, seven hip bleeds were identified, including two for whom this was their diagnostic event. Mean age was 7.1 years (3.7 years-11.2 years) and mean baseline factor VIII level was 15 IU/dl (8 IU/dl-25 IU/dL). See table1 for full baseline demographics and diagnostics.

TABLE 1 Summary of patient demographics and diagnostics

Characteristic	N = 7
Baseline factor (IU/dL) mean (range)	15 (8 - 25)
Age at which hip bleed occurred (range)	7 years 1 month (3 years 8 months - 11 years 2 months)
Weight (Centile) mean (range)	56th (3rd-86th)
Mechanism of injury	
Spontaneous - no obvious trauma	4
Minimal trauma - recent sport activity but no obvious trauma	2
Trauma - clear mechanism of injury	1
Diagnosis of bleed	
Clinical suspicion - Experience paediatric haemophilia physiotherapist	1
Ultrasound - Joint effusion	4
Aspiration - Haemarthrosis	2
Delay in presentation (days) mean (range) *excluding 2 patients for who this was their diagnostic event	1.8 (0 - 5)*

Treatment was variable: two patients received no specific haemostatic therapy; two were initially treated with DDAVP followed by recombinant factor VIII; three received recombinant factor VIII only. Treated patients received a mean 4.5 days (range 2-7 days) of factor replacement.

Follow up from bleed to most recent physio review was an average of 27 months (range 1- 79). To date no pain, persistent restriction or joint impairment was identified in any patient.

Conclusions: In this review boys with mild haemophilia A between 3 to 11 years appear at risk of developing spontaneous hip bleeds. To date there appears to be no impact on joint health or function in our cohort and suveillance continues. This has led to a change in our

counselling practice around hip bleed recognition for families with mild haemophilia.

PB0910 | Rationale and Study Design for a Prospective, Low-Interventional Study to Investigate whether Systematic Joint Examination Impacts Haemophilia Treatment Management Decisions in Patients with Haemophilia A in France: The A-MOVE Study

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Background: Management of haemophilia A (HA) aims to avoid bleeds and preserve joint function. Changes in patient joint health may influence physicians' decisions to adjust haemophilia treatment management. Ultrasound is a sensitive tool for assessment of joint health, but is currently not routinely used in France.
Aims: To describe the study design of A-MOVE (NCT04133883), a low-interventional study evaluating if and how systematic joint examination with the haemophilia joint health score (HJHS) or Haemophilia Early Arthropathy Detection with UltraSound (HEAD-US) impacts treatment management decisions in patients with HA.
Methods: A-MOVE is a 12-month prospective, descriptive, low-interventional multicentre study. Included patients will be aged ≥ 6 to ≤ 40 years, have HA treated with plasma-derived factor VIII (FVIII) or recombinant (conventional or extended half-life) FVIII, and ≥ 1 prior joint bleeding episode. The main objective is to evaluate whether functional (HJHS) or structural (HEAD-US) joint evaluation impacts HA treatment management decisions. Secondary objectives include evaluation of joint health status, pain and functional activities. The following will be evaluated at study visits (baseline, 6 and 12 months): HJHS, HEAD-US, changes to care (prescribed product, treatment regimen, dosing), findings impacting the management decision, and whether any specific items of the HJHS/HEAD-US affected the decision. No formal statistical hypothesis testing is planned.
Results: The study aims to recruit approximately 100 patients from 15-20 sites in France. The primary endpoint is change in haemophilia management (yes/no) based on systematic joint examination using HJHS and HEAD-US. Secondary endpoints include specific joint findings leading to the haemophilia management decision, prescribed FVIII dose and regimen, and other changes to haemophilia and pain management (e.g. physical therapy, pain/anti-inflammatory medication, surgery).
Conclusions: A-MOVE will be the first prospective study to evaluate the impact of systematic joint examination (functional and structural) on HA management decisions in France.

PB0911 | Evaluation of Bleeding in Haemophilia Carriers by ISTH-BAT: A Single Center Experience

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Background: Haemophilia A and B are haemorrhagic disorders characterised by coagulant factors VIII/IX deficiency or dysfunction. Due to X-linked recessive inheritance, males are affected, while female are carriers. Haemophilia carriers may have bleeding symptoms and wide range of clotting factor levels: approximately 30% of carriers express low clotting factor level.
Aims: To evaluate bleeding symptoms by quantitative bleeding assessment tools from ISTH (BAT score) in haemophilia A and B carriers and to correlate BAT score with coagulant factor level.
Methods: Haemophilia carriers, referring to Haemophilia and Thrombosis Center of Milan, with low factor level (≤50 IU/dL) were interviewed by a single physician. BAT score ≥ 6 has been considered abnormal in adult females.
Results: BAT score was assigned to 65 female carriers. Bleeding symptoms are summerized in the table. Haemarthrosis and muscle haematomas, the most frequent symptoms in haemophilic patients, were reported in 11% and 17% of women, respectively. Carriers with these type of bleeds had a lower coagulant activity than other carriers without these symptoms. The median factor level in carriers with haemarthrosis was 4 IU/dL (IQR: 3-7) versus 37 IU/dL (IQR: 28-44) in carriers without haemarthrosis ($p < .001$); while in carriers with muscle hematomas, the median factor level was 14 IU/dL (IQR:7-28) versus 38 IU/dL (IQR: 29-44) in carriers without haematomas ($p < .001$). Carriers with high BAT score ≥ 6 showed a median factor level of 28 IU/dL (IQR: 12-37), while haemophilia carriers with BAT score < 6 had a median factor level of 39 IU/dL (IQR: 30-44) ($p = .003$).

TABLE 1 Bleeding symptoms of haemophilia A and B carriers

Menorrhagia	46/62 (74%)
Cutaneous	45/65 (69%)
Tooth extraction	26/46 (54%)
Epistaxis	28/65 (43%)
Bleeding post surgery	20/48 (42%)
Post-partum hemorrhage	10/41 (24%)
Muscle hematomas	11/64 (17%)
Hemarthrosis	7/65 (11%)
Gastrointestinal bleeding	3/64 (5%)
Hematuria	5/65 (3%)

Conclusions: In our study, a significant difference in terms of factor level between the group of carriers with high and low BAT score was found. These findings support the evidence that factor coagulant level, even at the lower end of the normal range in haemophilia carriers, may lead to increased bleeding manifestations.

PB0912 | Current Challenges in Diagnosis and Management of Hemophilia Afghanistan

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Background: Hemophilia is one of the most common challenging congenital bleeding disorder, worldwide. Diagnosis and management of the disorder can be affected by several issues, including economic properties of countries.

Aims: In the present study we assessed current hemophilia situation in Afghanistan.

Methods: All the information on Afghanistan′s hemophilia has been provided by several organization and hospitals since 2011. Esteqlal Hospital, Sehat Tefl (Indera Gandi), Central Blood Bank, Afghan Hemophilia Patients Association and Afghanistan National Charity Organization for Specific Diseases.

Results: To date, few hemophilia patients have been identified in Afghanistan due to lack of health facilities in all provinces of Afghanistan. More than 300 hemophilia patients in Afghanistan, most of them hemophilia A patients and some hemophilia B patients, have been identified. Many of them have migrated to other countries due to numerous problems, especially Iran and Pakistan. In spite of all the problems, Afghanistan National Charity Organization for Special Diseases (ANCOSD) has provided services to these patients in different parts:

Study of genotype of these patients,Identification of Hemophilia Carrier Women and Girls, Identification of patients with RBD, Evaluation of quality of life of these patients, Diagnosis of viral infections in these patients, Inhibitor study in patients with hemophilia, Genetic counseling for the families of these patients.

Conclusions: The World Health Organization and the World Hemophilia Federation should pay particular attention to the hemophilia situation in Afghanistan. Sufficient access to coagulation factors, specialist medical training, equipping and commissioning of laboratories, physiotherapy and specialized health centers, etc. are essential requirements for these patients.

PB0913 | Inhibitors in Hemophilia: Treatment Challenges in Afghanestan

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Background: Hemophilia A (HA) is the most common severe congenital severe bleeding disorders wit high rate of morbidity and mortality. About 30% of the patients develop inhibitor against exogenous factor VIII (FVIII). Inhibitor development is the most challenging complication of the replacement therapy.

Aims: To assess prevalence of FVIII inhibitor in Afghan patients with HA.

Methods: A total of 107 patients with HA were evaluated for inhibitors. The Nijmegen modification of the Bethesda assay was used for inhibitor assay.

Results: Of 107 patients, 11 (~10.3%) were positive for FVIII inhibitor. All patients with inhibitor had severe HA with on-demand therapy.

Conclusions: It seems that low rate of replacement therapy is the main cause of relatively low rate of inhibitor formation in Afghan patients with severe HA.

PB0914 | Elbow Embolization for Recurrent Massive Hemarthrosis: A Case Series with Successful Outcome

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Background: Rare bleeding disorders such as hemophilia A and type-3 von Willebrand Disease (VWD) are caused by a deficiency

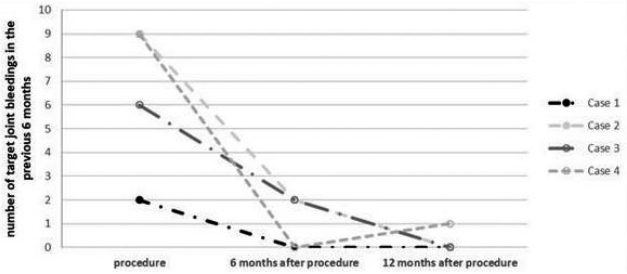

FIGURE 1 Number of target joint bleeding events 6 months before the procedure and 6 and 12 months after the procedure

of clotting factors VIII (FVIII), and von Willebrand factor (VWF) with variable deficiency of FVIII. Recurrent bleeding (hemarthrosis) in target joints (joint with more than 4 bleeding events over 6 months) may lead to chronic arthropathy. Treatment options of acute events include replacement therapy and anti-inflammatory drugs, whereas treatment of compromised joints is mainly major orthopedic surgery. However, some patients may experience arthropathy refractory to any treatment.

Angiographic embolization of target joints is a minimally invasive technique associated with limited bleeding and infection risk. It does not require general or local anesthesia and can be applied earlier than the traditional surgical approach.

Aims: To describe the Milan centre experience on the clinical and radiological outcomes of a series of patients undergoing selective angiographic embolization of target joints.

Methods: Four patients with target joint (elbow) were evaluated for the procedure: three patients affected by severe hemophilia A on prophylaxis with factor VIII concentrate and 1 patient affected by type 3 VWF (basal FVIII:C < 1%) with inhibitor.

Clinical follow-up (hemarthrosis episodes of the target joint, therapeutic regimen) every 3 months and radiological (MRI) follow-up after 12 months was performed.

Results: All four patients underwent the embolization procedure. The procedure was successful in all patients, with an observed reduction of target joint bleeding over the 12-month follow-up. Only one procedure was complicated with transient arterial vasospasm, with fast recovery. The same patient experienced local soft tissue edema one week after the procedure, but then no recurrent bleeding.

Conclusions: Joint vascular embolization is a non-invasive procedure that may be an effective alternative treatment in recurrent hemarthrosis refractory to traditional approaches.

PB0915 | Emicizumab Concentration in Children: A Single Centre Real World Experience

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[1]*Great Ormond Street Hospital, Haemophilia Comprehensive Care Centre, London, United Kingdom;* [2]*Great Ormond Street Hospital, Specialist Coagulation Laboratory, London, United Kingdom*

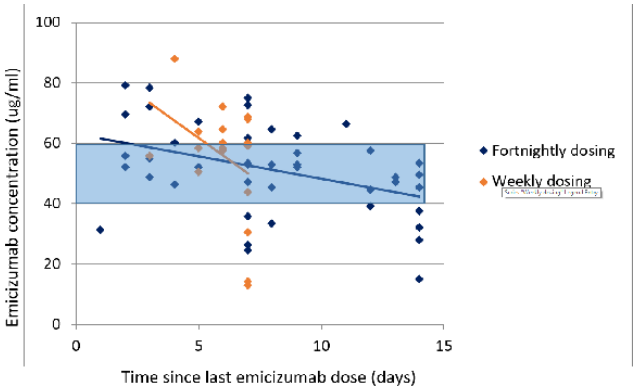

FIGURE 1 Emicizumab concentration compared to time since last emicizumab dose

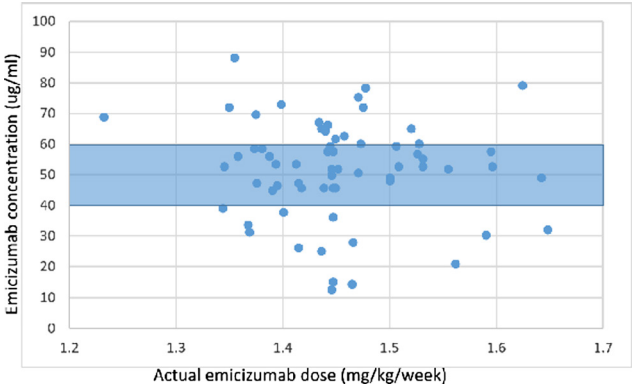

FIGURE 2 Emicizumab concentration compared to actual emicizumab dose

Background: Emicizumab is a bispecific monoclonal antibody which mimics the function of factor VIIIa. It is licensed for patients of any age with haemophilia A with and without inhibitors. The HAVEN trial data has shown that at steady state (1.5 mg/kg/week) emicizumab concentration is 40-60ug/ml. In real world paediatric care the actual dose may vary due to vial sizes and inter-current growth between prescriptions.

Aims: Does the timing since last emicizumab dose affect emicizumab concentration?

Does real world variation in emicizumab dose correlate with emicizumab concentration?

Methods: All patients in a single paediatric comprehensive care center on an equivalent dose of 1.5 mg/kg/week of emicizumab were reviewed. Emicizumab concentrations were analyzed on a CS2000i (Sysmex) using a modified one stage clotting assay comprising an Emicizumab specific calibrator and controls (R2 diagnostics, Enzyme Research Laboratories), Actin FS APTT reagent and Factor VIII deplete plasma (Siemens) in accordance with best practice guidelines.

Results: Twenty-one patients met the criteria, with a mean age 7.3 years (range 11 months - 15.6 years) at time of starting emicizumab and emicizumab for a mean 22 months (range 3 months - 41 months).

The time since last dose and emicizumab concentration was 6 and 8 days for weekly and fortnightly dosing respectively. Although

there is a downward trend the emicizumab concentration remains within the expected range (figure 1). The mean actual emicizumab dose was 1.45 mg/kg/week (range 1.23 - 1.65 mg/kg/week). The mean emicizumab concentration was 51.8ug/ml (range 12.8 - 88.0ug/ml) (figure 2).

There were no bleeding episodes that required factor replacement.

Conclusions: Once in steady state, timing of venepuncture and slight variation in dosage do not significantly affect emicizumab concentration.

There was no correlation/linearity between emicizumab dose and concentration. This raises the question of whether emicizumab dosing should be based on concentrations as well as patient weight, and if so, what target is optimal.

PB0916 | Efficacy and Safety of BAY 94-9027 Prophylaxis for ≥ 5 Years: Outcomes in 36 Patients from the PROTECT VIII Extension Study

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Background: BAY 94-9027 is a B-domain-deleted, extended-half-life, recombinant factor VIII (FVIII) site-specifically PEGylated with a 60 kDa polyethylene glycol. In PROTECT VIII (NCT01580293), efficacy and safety of BAY 94-9027 as treatment for previously treated severe hemophilia A patients, aged 12-65 years, were evaluated.

Aims: To examine efficacy and safety of BAY 94-9027 in patients treated with regular prophylaxis for ≥ 5 years from PROTECT VIII.

Methods: In PROTECT VIII, 114 patients received BAY 94-9027 prophylaxis (25 IU/kg twice weekly [2 × W]) for a 10-week run-in period. Patients with ≤ 1 breakthrough bleed during the run-in period were randomized to receive BAY 94-9027 every 5 days (45-60 IU/kg) or every 7 days (60 IU/kg) for the 26-week study period. Patients with > 1 bleed, and those eligible for randomization but enrolled after randomized arms were full, were assigned to 30-40 IU/kg 2 × W. In the extension, prophylaxis patients could receive BAY 94-9027 on any PROTECT VIII regimen. Patients who switched regimen following the first seven days of the extension were assessed as a variable frequency group.

Results: At extension completion, 36 patients completed ≥ 5 years (median [range] 6.2 [5.0-7.0]) of BAY 94-9027 prophylaxis and a median (range) of 428.5 (293-698) exposure days. Twenty-two patients remained on the same regimen throughout the extension period. Median (Q1; Q3) annualized bleeding rate for the total prophylaxis group during extension was 1.1 (0.4; 2.1) (Figure 1). Median FVIII consumption (IU/kg/year) for prophylaxis patients was 3332 (Table 1). Five (13.9%) patients experienced non-serious treatment-emergent drug-related adverse events. No patients developed FVIII inhibitors. No deaths or thrombotic events were reported.

Conclusions: Efficacy of BAY 94-9027 was maintained over ≥ 5 years' prophylaxis using infusion schedules best fitting individual patients' needs. These data support the favorable risk-benefit profile of BAY 94-9027 and its use as a long-term treatment option for patients with hemophilia A.

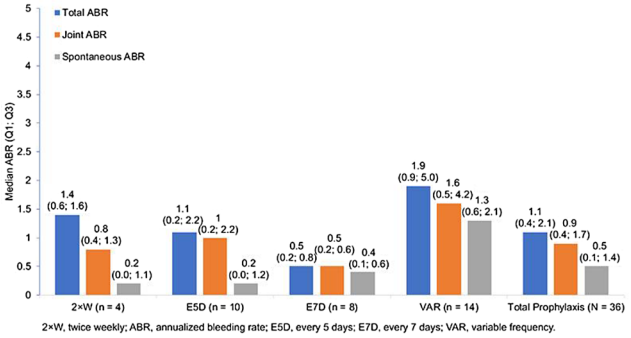

FIGURE 1 ABRs during the extension in patients with ≥ 5 years' BAY 94-9027 treatment

TABLE 1 FVIII consumption in patients with ≥ 5 years BAY 94-9027 treatment

	2 × W (n = 4)	E5D (n = 10)	E7D (n = 8)	VAR (n = 14)	Total (N = 36)
Total dose per infusion, IU/kg, median (Q1; Q3)	35 (30; 41)	44 (43; 48)	59 (57; 62)	55 (49; 57)	51 (44; 58)
Total dose per year, IU/kg/year, median (Q1; Q3)	3725 (3011; 4262)	3179 (3122; 3695)	3083 (3015; 3286)	3826 (3547; 4269)	3332 (3144; 3991)

2 × W, twice weekly; E5D, every 5 days; E7D, every 7 days; FVIII, factor VIII; VAR, variable frequency.

PB0917 | Joint Bleeding Outcomes in Patients with Hemophilia A Receiving Long-Term Prophylaxis with BAY 94-9027 in the PROTECT VIII Extension Study

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Background: BAY 94-9027 (damoctocog alfa pegol; Jivi®) is a B-domain-deleted recombinant factor VIII (FVIII), site-specifically PEGylated to extend its half-life. The phase 2/3 PROTECT VIII study (NCT01580293) demonstrated the efficacy and safety of BAY 94-9027 as prophylactic and on-demand therapy for adults with severe hemophilia A. Reducing the frequency of joint bleeds is expected to reduce arthropathy and improve quality of life in hemophilia A.

Aims: To investigate the long-term efficacy of BAY 94-9027 prophylaxis, particularly on joint bleeding outcomes, in patients with hemophilia A.

Methods: Prophylaxis patients who completed the PROTECT VIII study could enter the optional extension study. Prophylactic treatment regimens included twice per week, every five days (E5D) and every seven days (E7D). Patients who switched regimens during the extension study were assessed in the variable frequency group (VAR). Annualized bleeding rates (ABR) and joint ABR were assessed in this final analysis.

Results: Median (range) time in extension for patients on prophylaxis during the extension (n = 107) was 3.2 (0.1-6.3) years. Median (Q1; Q3) ABR was 8.0 (2.0; 16.0) prestudy (76.6% received prophylaxis prior to enrollment) and joint ABR was 5.0 (1.0; 12.0). Median (Q1; Q3) total ABR in the extension was 1.5 (0.4; 4.8) and joint ABR was 0.9 (0.0; 3.4) (Figure 1). The overall spontaneous median ABR during the extension was 0.8 (0.0; 2.9). Throughout the extension, 20.6% of prophylaxis patients had 0 total bleeds; 29.9% had 0 joint bleeds. In the last six months of the extension (n = 96), 50.0% and 58.3% of patients had 0 total and joint bleeds, respectively (Figure 2).

Conclusions: Data from this completed phase 2/3 study and its extension show that BAY 94-9027 prophylaxis is efficacious in adults with severe hemophilia A at dose intervals of up to E7D and results in a low incidence of joint bleeds with long-term use for several years.

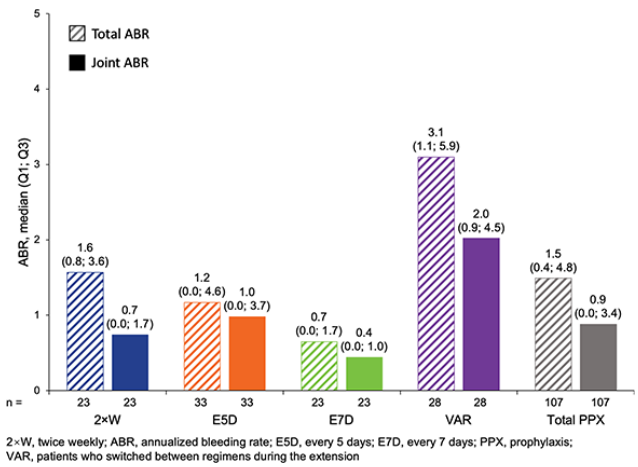

FIGURE 1 Total and joint ABR for prophylaxis patients in the PROTECT VIII extension study

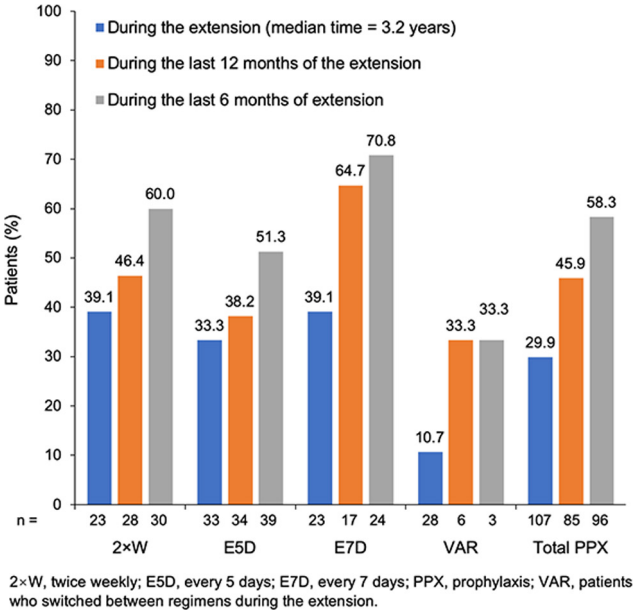

FIGURE 2 Percentage of patients with 0 joint bleeds in the PROTECT VIII extension study

PB0918 | Final Safety Outcomes Following ≥ 5 Years' Observation of BAY 94-9027 Treatment in Patients with Hemophilia A in the PROTECT VIII Extension

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Background: BAY 94-9027 (Jivi®) is a B-domain-deleted recombinant factor VIII product, site-specifically PEGylated to extend half-life, approved in the US, EU, Japan and Canada for previously treated patients with severe hemophilia A, aged ≥ 12 years. The phase 2/3 PROTECT VIII main study and its extension demonstrated safety and efficacy of BAY 94-9027.

TABLE 1 Summary of TEAEs at the end of PROTECT VIII extension

	On demand (n = 14)	Total prophylaxis (n = 107)	Total (N = 121)
Time in extension, years, median (range)	3.2 (0.6-4.1)	3.2 (0.1-6.3)	3.2 (0.1-6.3)
Exposure days in extension, median (range)	101.5 (13-176)	211.0 (9-621)	175.0 (9-621)
Number of patients (%)			
Any TEAE	10 (71.4)	86 (80.4)	96 (79.3)
Any study drug-related TEAE	0	10 (9.3)	10 (8.3)
Any SAE	2 (14.3)	34 (31.8)	36 (29.8)
Any study drug-related SAE	0	2 (1.9)	2 (1.7)
Discontinuation of study drug due to AE	0	2 (1.9)	2 (1.7)
Deaths	0	0	0

Aims: To report final safety data, including treatment-emergent adverse events (TEAEs) and markers of renal and hepatic function, in patients who participated in the PROTECT VIII extension.

Methods: In the PROTECT VIII extension, 121 patients received BAY 94-9027 on demand (n = 14) or prophylaxis (n = 107; 30-40 IU/kg twice-weekly, 45-60 IU/kg every 5 days or 60 IU/kg every 7 days). Safety endpoints included TEAEs, laboratory markers, and FVIII inhibitor development.

Results: At extension completion, median (range) time in study was 3.9 (0.8-7.0) years, of which time in extension was 3.2 (0.1; 6.3) years with a median (range) of 175 (9-621) exposure days; 36 patients had > 5 years of BAY 94-9027 treatment in total. During extension, 10 (71.4%) on-demand and 86 (80.4%) prophylaxis patients reported TEAEs (Table 1). Ten (8.3%) patients experienced study drug-related TEAEs (most common types were musculoskeletal disorders [n = 4] and laboratory abnormal results [n = 3]). Study drug-related serious adverse events occurred in two (1.7%) patients (elevated liver function tests and migratory back pain, respectively), both leading to study drug discontinuation. At the final study visit, patients had similar levels of markers of renal and hepatic function to baseline (Table 2). No patients developed FVIII inhibitors. No deaths or

TABLE 2 Markers of renal or hepatic function at the end of PROTECT VIII extension. Not all patients had biomarker data available

	On demand		Total prophylaxis		Total	
Serum creatinine level at final study visit, median (Q1; Q3), mg/dL	n = 14	0.718 (0.679; 0.973)	n = 98	0.837 (0.746; 0.961)	n = 112	0.820 (0.724; 0.961)
Serum creatinine, median (Q1; Q3) change from baseline, mg/dL	n = 14	−0.051 (−0.079; 0.045)	n = 98	−0.040 (−0.102; 0.034)	n = 112	0.040 (−0.102; 0.034)
Serum creatinine clearance at final study visit, median (Q1; Q3), mL/min (calculated)	n = 14	134.4 (98.3; 174.7)	n = 98	128.1 (104.6; 149.1)	n = 112	128.1 (104.1; 151.0)
Serum creatinine clearance, median (Q1; Q3) change from baseline, mL/min (calculated)	n = 14	8.92 (−4.80; 16.31)	n = 98	5.15 (−4.44; 17.92)	n = 112	5.25 (−4.55; 16.77)
Serum ALT level at final study visit, median (Q1: Q3), U/L	n = 14	26.5 (18.0; 37.0)	n = 98	23.0 (17.0; 40.0)	n = 112	24.0 (17.0; 40.0)
Serum ALT, median (Q1; Q3) change from baseline, U/L	n = 13	−4.00 (−24.00; 6.00)	n = 96	−1.00 (−9.00; 4.50)	n = 109	−1.00 (−9.00; 5.00)
Serum AST level at final study visit, median (Q1: Q3), U/L	n = 14	23.5 (18.0; 30.0)	n = 98	22.0 (18.0; 28.0)	n = 112	22.0 (18.0; 28.5)
Serum AST, median (Q1; Q3) change from baseline, U/L	n = 13	−3.00 (−15.00; 0.00)	n = 95	−1.00 (−7.00; 1.00)	n = 108	−1.50 (−7.00; 1.00)

thrombotic events, including thrombotic microangiopathy, were reported.

Conclusions: BAY 94-9027 was well-tolerated during the PROTECT VIII extension and no patients developed FVIII inhibitors. Low levels of study drug-related TEAEs, and no specific changes in markers of renal and liver hepatic function were detected over ≥ 5 years of observation of BAY 94-9027 treatment.

PB0919 | Real-World Outcomes before and after Switching from Standard Half-Life Factor VIII to Rurioctocog Alfa Pegol in the United States

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Background: Prophylactic factor VIII (FVIII) is the standard of care for managing hemophilia A. However, real-world patient experience with extended half-life (EHL) recombinant FVIII is limited.

Aims: To analyze real-world outcomes in patients with hemophilia A in a US retrospective observational study before and after switching from any standard half-life (SHL) FVIII to EHL rurioctocog alfa pegol (TAK-660, SHP660, BAX 855). This is a modified analysis of Milligan S, et al. *RPTH* 2019;3(suppl 1):330.

Methods: Real-world data (patient records, prescriptions, and patient-reported bleed logs) were from US specialty pharmacies. Informed consent was obtained for all analyzed patient data. Patients receiving TAK-660 prophylaxis between Nov 2015 and Sep 2018 and ≥ 90 days' FVIII before switching to TAK-660 were eligible for inclusion. Main exclusion criteria: previous or current participation in TAK-660 clinical trials, use of on-demand treatment only before switching to TAK-660, or presence of active FVIII inhibitors requiring treatment and/or immune tolerance induction during the study. In contrast to Milligan *RPTH* 2019, on-label dosing was not required, which resulted in more evaluable patients. Assessments included dosing frequency, factor consumption, and annualized bleeding rate (ABR) before and after switching to TAK-660. Analyses were performed on data from patients with sufficient bleed log information and > 90 days' treatment for both prior FVIII and TAK-660 after switching.

TABLE 1 FVIII consumption before/after switching from SHL FVIII to TAK-660 in patients with available ABR data (values shown are rounded)

Parameter	Disease severity	Patients, n	Prior SHL FVIII consumption	TAK-660 consumption	Percentage difference: FVIII consumption
Mean FVIII dose per administration, IU/kg	Severe	41	38.5	50.0	+29.7
	Mild-moderate	5	38.0	42.0	+10.5
	Total	46	38.5	49.1	+27.7
Mean number of FVIII administrations per week	Severe	41	2.9	2.1	-26.9
	Mild-moderate	5	2.7	1.8	-33.3
	Total	46	2.9	2.1	-27.6
Mean FVIII dose per week, IU/kg	Severe	41	129.0	107.0	-17.1
	Mild-moderate	5	93.0	72.0	-22.6
	Total	46	125.0	103.0	-17.5

TABLE 2 Mean ABRs before/after switching from SHL FVIII to TAK-660 in patients with available ABR data (values shown are rounded)

Disease severity	Patients, n	Prior SHL FVIII mean ABR	TAK-660 mean ABR	Percentage difference: mean ABR
Severe	41	3.5	1.7	-52.9
Mild-moderate	5	3.1	2.5	-18.3
Total	46	3.5	1.7	-49.5

Results: Of 54 patients with available ABR data, 85% (46/54) had received prior SHL FVIII treatment. FVIII consumption decreased in all patients after switching from SHL FVIII to TAK-660, with higher dose per administration but fewer administrations per week (Table 1). Mean ABR decreased by ~50% after switching to TAK-660, with greater decreases in patients with severe disease (Table 2).

Conclusions: Switching from SHL FVIII to TAK-660, given at lower administration frequency and overall weekly consumption, resulted in reduced ABRs in patients with hemophilia A.

PB0920 | Personalizing Prophylaxis with Rurioctocog Alfa Pegol in Previously Treated Patients with Severe Hemophilia A: Outcomes from the Phase 3b CONTINUATION and Phase 3 PROPEL Studies

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Background: Prophylaxis with rurioctocog alfa pegol (TAK-660, SHP660, BAX 855) using fixed dose (FD) 40-50 IU/kg twice-weekly to achieve > 1% factor VIII (FVIII) troughs is effective and well

TABLE 1 Point estimates (95% CI)* of mean ABRs by baseline FVIII $t_{1/2}$ in PROPEL (second 6-month study period; PPAS†)

Parameter	FVIII trough level 1-3% (n = 52)			FVIII trough level 8-12% (n = 43)		
FVIII $t_{1/2}$‡	6 to < 12 h	12 to < 18 h	18 to < 36 h	6 to < 12 h	12 to < 18 h	18 to < 36 h
Patients, n	12	31	9	13	23	7§
Total ABR	3.53 (2.25-5.53)	2.48 (1.59-3.86)	1.48 (0.59-3.68)	0.66 (0.26-1.64)	1.04 (0.54-2.01)	1.65 (0.58-4.74)
Spontaneous ABR	2.79 (1.63-4.77)	0.95 (0.43-2.07)	1.20 (0.49-2.94)	0.28 (0.07-1.14)	0.58 (0.21-1.59)	0.49 (0.11-2.22)
Spontaneous joint ABR	1.97 (1.11-3.50)	0.54 (0.18-1.60)	0.73 (0.23-2.26)	0.15 (0.02-1.07)	0.30 (0.07-1.26)	0.18 (0.02-1.93)
Injury-related ABR	0.95 (0.35-2.57)	1.29 (0.75-2.22)	0.13 (0.01-3.88)	0.46 (0.13-1.61)	0.41 (0.16-1.08)	2.13 (0.30-14.97)

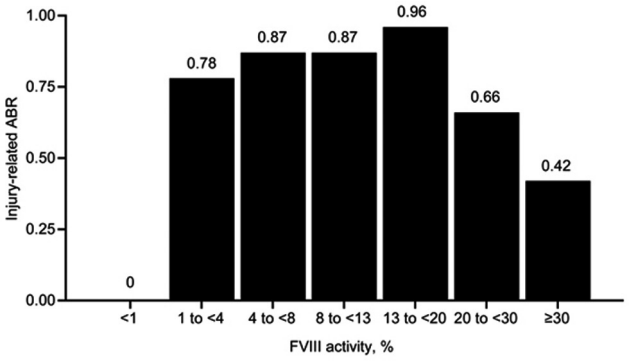
ABR, annualized bleeding rate; FVIII, factor VIII; PPAS, per-protocol analysis set; $t_{1/2}$, half-life. Values are point estimates (95% CI) unless otherwise indicated. *Point estimates and 95% CIs were obtained from a generalized linear model fitting a negative binomial distribution. †Patients in the PPAS completed second 6 months of prophylaxis with no significant protocol deviations. ‡FVIII $t_{1/2}$ category determined by one-stage clotting assay. §Includes 1 patient who experienced 5 injury-related knee bleeds in 6 months, which had a pronounced effect on total ABR.

tolerated in previously treated patients with hemophilia A. Recent data suggest personalizing dosing can better control/prevent bleeds without compromising TAK-660 efficacy or safety.

Aims: To explore potential benefits of personalized TAK-660 prophylaxis by evaluating data on extended FD or pharmacokinetic (PK)-tailored dosing from 2 studies.

Methods: Design and primary outcomes of CONTINUATION (NCT01945593) and PROPEL (NCT0285960) were previously reported; ethics committees' approval and patient informed consent obtained. CONTINUATION patients aged ≥ 12 years with a spontaneous annualized bleeding rate (ABR) = 0 during 6 months' TAK-660 prophylaxis could switch from twice-weekly to extended FD prophylaxis (every 5 days [q5d], subsequently q7d). PROPEL patients aged 12-65 years were randomized to 12 months' PK-guided prophylaxis targeting 1-3% or 8-12% FVIII troughs.

Results: In CONTINUATION, point estimates of mean ABRs were low across all FD regimens of twice-weekly, q5d and q7d (total ABR: 2.2 [n = 186], 2.1 [n = 56], 2.7 [n = 15]; spontaneous ABR: 1.2 [n = 186], 1.3 [n = 56], 1.8 [n = 15]). In PROPEL, lower ABRs and higher ABR = 0 rates were achieved and maintained with 8-12% versus 1-3% FVIII troughs; TAK-660 consumption varied but overlapping ranges between arms reflect variability in patient FVIII half-life ($t_{½}$) and dosing regimens. PROPEL patients with shorter FVIII $t_{½}$ had better bleed prevention with 8-12% versus 1-3% troughs (Table). Lower injury-related ABRs in PROPEL were observed during periods of FVIII activity ≥ 20% (Figure). TAK-660 safety profiles in CONTINUATION and PROPEL were consistent with earlier studies.



ABR, annualized bleeding rate; FAS, full analysis set; FVIII, factor VIII.
*Bleeding episodes that occurred during the extended observation period (12 months; excluding surgery periods) in the FAS (randomized patients who received ≥1 prophylactic dose; N=115). FVIII activity levels were determined by one-stage clotting assay. Similar trends for reduced bleeding rates in periods with FVIII activity ≥20% were observed for total (all bleeds), spontaneous, spontaneous joint, and joint ABRs.

FIGURE 1 Injury-related ABR by FVIII activity level in PROPEL (12-month study period; FAS).*

Conclusions: These results support the feasibility and efficacy of personalized TAK-660 prophylaxis by extending the FD interval or targeting elevated FVIII levels with PK-tailored dosing. These benefits may be especially important for patients requiring higher FVIII activity levels because of active lifestyles and increased risk of injury-related bleeds.

PB0921 | Efficacy and Target Joint Resolution with Pharmacokinetic-Guided Rurioctocog Alfa Pegol Prophylaxis Targeting Two Factor VIII trough Levels in Patients with Severe Hemophilia A: Post-Hoc Analysis of the Phase 3 PROPEL Trial

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Background: Primary results of PROPEL (NCT02585960) demonstrated clinical benefits of targeting elevated factor VIII (FVIII) troughs with rurioctocog alfa pegol (TAK-660, SHP660, BAX 855) prophylaxis with no change in safety profile.

Aims: Assess efficacy and target joint (TJ) resolution with pharmacokinetic (PK)-guided TAK-660 prophylaxis in a post-hoc analysis.

Methods: PROPEL study design was previously reported (Klamroth et al., *RPTH* 2019); ethics committees' approval and patient informed consent obtained. Patients were randomized to PK-guided TAK-660 prophylaxis targeting FVIII troughs of 1-3% (reference) or 8-12% (elevated). TJ was defined as a joint with ≥4 bleeds during any consecutive 6 months. Outcomes evaluated: total annualized bleeding rate (ABR; all bleeds) according to patients' TJ status at screening and TJ ABR (per-protocol analysis set; second 6 months completed, no significant protocol deviations); course of TJs in treated patients with ≥6-months' observation (full analysis set; randomized, ≥1 prophylactic dose received).

Results: In patients with 0 (n=26) and ≥1 (n=69) TJs, respectively, mean total ABR point estimates (95% CI) were 2.6 (1.5-4.6) and 2.4 (1.6-3.5) in 1-3% arm and 1.5 (0.6-3.5) and 0.9 (0.5-1.6) in 8-12% arm (Table 1). Spontaneous and spontaneous joint ABRs were also lower in 8-12% versus 1-3% arm irrespective of TJ status; injury-related ABR was slightly higher in 8-12% arm with no TJs and lower with ≥1 TJ at screening. Mean TJ ABRs (both arms) decreased during 12-months' treatment (Table 2). In 112/115 evaluable patients, TJs resolved in all but 1 patient per arm after 6 months' prophylaxis.

Conclusions: Targeting 8-12% versus 1-3% FVIII troughs was associated with reduced bleeding into TJs, reduced ABR irrespective of baseline TJ status, and TJ resolution within the first 6 months in all but 1 patient per arm. Results show the clinical benefit of elevating FVIII troughs with TAK-660 for TJ resolution, an important consideration for joint health in hemophilia A.

TABLE 1 ABR by TJ status at screening: second 6-month study period in the per-protocol analysis set

TJ status	Point estimate (95% CI)* of mean ABR	FVIII trough level 1-3%	FVIII trough level 8-12%
No TJs (1-3% arm, n=16; 8-12% arm, n=10)	Total ABR	2.6 (1.5-4.6)	1.5 (0.6-3.5)
	Spontaneous ABR	1.3 (0.6-2.5)	0.1 (0.0-1.1)
	Spontaneous joint ABR	1.2 (0.7-2.3)	0.0 (NC)
	Injury-related ABR	0.8 (0.3-2.2)	1.0 (0.3-3.5)
≥1 TJ (1-3% arm, n=36; 8-12% arm, n=33)	Total ABR	2.4 (1.6-3.5)	0.9 (0.5-1.6)
	Spontaneous ABR	1.3 (0.7-2.3)	0.5 (0.2-1.1)
	Spontaneous joint ABR	1.2 (0.6-2.4)	0.5 (0.2-1.3)
	Injury-related ABR	1.1 (0.6-1.9)	0.4 (0.2-1.0)

ABR, annualized bleeding rate; FVIII, factor VIII; NC, not calculable due to no events in the 8-12% arm; TJ, target joint. *Point estimates and 95% CIs were obtained from a generalized linear model fitting a negative binomial distribution.

TABLE 2 ABR of TJ bleeds: first and second 6-month study periods in the per-protocol analysis set

Study period	Parameter		FVIII trough level 1-3% (n=52)	FVIII trough level 8-12% (n=43)
First 6 months	TJ ABR	Mean (SD)	0.5 (1.4)	0.7 (2.3)
		Median (Q1-Q3)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Second 6 months	TJ ABR	Mean (SD)	0.1 (0.6)	0.2 (1.3)
		Median (Q1-Q3)	0.0 (0.0-0.0)	0.0 (0.0-0.0)

ABR, annualized bleeding rate; FVIII, factor VIII; Q, quartile; TJ, target joint.

PB0923 | Safety and Efficacy of Albutrepenonacog Alfa ih Haemophilia Patients: The Greek Experience

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Background: Albutrepenonacog alfa (IDELVION, CSL Behring) is an EHL concentrate, presenting with enhanced systemic exposure using genetic fusion with albumin.
Aims: To report on the Greek experience with regards to the use of albutrepenonacog alfa in patients with haemophilia B.
Methods: Data of patients who switched to albutrepenonacog alfa when it became available in May 2018 were retrospectively reviewed.
Results: Nine male patients were enrolled: 5/9 (55.5%) children and 4/9 (44.5%) adults. Pediatric age range was 5-16years and adult age range 34-68 years. Pediatric patients and 2/4 adults were on prophylaxis with standard half-life rFIX before switch, and remained on prophylaxis. The other 2 adults switched to prophylaxis from on demand therapy. Prior switch dose was 24-50 IU/kg and infusion was given twice weekly to 5/7 (71.4%) patients, every 3rd day to 1 (14.2%) and once weekly to 1 (14.2%) patient. Prior switch troughs were 2-4% and number of annual bleeding episodes 1-12. The HJHS assessed in pediatric patients ranged between 0 and 8. Post switch, albutrepenonacog alfa was given for 3-19 months and dose ranged from 27 to 54 IU/kg. Four patients (44.5%) were infused once weekly, 2 (22.2%) every ten days and 3 (33.3) fortnightly. In total, infusions were reduced from 4-10 to 2-4 infusions per month, new treatment achieving a more than half reduction in monthly infusions. Trough levels increased in all patients. Bleeding episodes decreased: 6 out of 9 (66.7%) patients experienced zero bleeds, 2 presented with two and 1 with one episode during follow up. HJHS was improved in all patients presenting with an abnormal score prior switch. No adverse events were reported.
Conclusions: Altrepenonacog alfa is proving to be an effective prophylactic treatment, leading to higher troughs and achieving zero bleeds in many haemophilia patients.

PB0924 | Screening of Inhibitor Development among Haemophilia a Patients Being Treated with Recombinant Factor VIII Concentrates in South-East Nigeria

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Background: Development of inhibitory antibodies in hemophilia patients receiving replacement therapy has became the most important complication of hemophilia management.
Aims: This research aims at screening for inhibitors among hemophilia A patients being treated with recombinant FVIII concentrates in southeast Nigeria.
Methods: Twenty patients between 3 - 16 years on recombinant factor VIII concentrates for a minimum of one year participated in the study. University of Nigeria Teaching Hospital Research Ethics Committee (NHREC/05/01/200B-FWA00002458-1 RB00002323) approved the study. Each of the participants also gave their consent. Five (5) ml of blood was collected from the subjects; 3.6mls was transferred to a citrate bottle for coagulation studies and 1.4mls transferred to an EDTA bottle for full blood count. Twenty age and sex matched individuals served as control. The full blood count, prothrombin time, activated partial thromboplastin time and mixing studies were performed. Descriptive statistics and Students t-test were performed on the data using Graphpad prism (version 5). Patients with mixing studies result higher than 40 seconds were classified as being positive for inhibitors.
Results: The mean platelet count of the hemophilia A subjects was 126% (Mean ± 2SD 432.3 ± 116.60 ($x10^9$/l)) higher and the mean hemoglobin level was 10.36% (11.34 ± 1.27 (g/l).) lower than that of the control population (Mean ± 2SD:191 ± 68.76 ($x10^9$/l) and 12.65 ± 1.741 (g/l)). Thirty percent (30%) of the hemophilia A subjects may have inhibitors.
Conclusions: On - demand (episodic) treatment is associated with increased risk of inhibitor development. In Nigeria, caretakers of hemophilia A patients opt for episodic treatments instead of prophylactic treatments because they consider it cheaper for their patients. This might have contributed to high probability of inhibitor prevalence among our study subjects

PB0925 | Prediction Markers for Development of Persistent Inhibitors in Previously Untreated Patients with Severe Hemophilia A

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TABLE 1 Risk of inhibitor persistence according to the number of IgG subclass combinations

Number of IgG subclass combinations	Persistent anti-FVIII inhibitors (N=31)	Transient anti-FVIII inhibitors (N=12)	RR (95% CI)°
1 (only IgG1 - taken as reference)	1 (3%)	2 (17%)	1
2 (combinations of 2 different IgG subclasses)	8 (26%)	7 (58%)	1.71 (0.25 to 2.94)
3 (combinations of 3 different IgG subclasses)	11 (35%)	2 (17%)	2.57 (0.73 to 3.01)
4 (all 4 IgG subclasses)	11 (35%)	1 (8%)	2.81 (1.15 to 3.02)

RR (95% CI): Relative Risk with 95% Confidence Interval.

TABLE 2 Anti-FVIII IgG subclasses over 6 months post inhibitor in 14 PUPs* with inhibitors

	IgG1 positive/total (%)		IgG2 positive/total (%)		IgG3 positive/total (%)		IgG4 positive/total (%)	
Time point	Persistent	Transient	Persistent	Transient	Persistent	Transient	Persistent	Transient
T0	9/9 (100%)	5/5 (100%)	2/9 (22%)	1/5 (20%)	9/9 (100%)	5/5 (100%)	7/9 (78%)	3/5 (60%)
T1	9/9 (100%)	5/5 (100%)	5/9 (56%)	1/5 (20%)	9/9 (100%)	3/5 (60%)	8/9 (89%)	4/5 (80%)
T2	9/9 (100%)	4/5 (80%)	6/9 (67%)	1/5 (20%)	9/9 (100%)	4/5 (80%)	9/9 (100%)	4/5 (80%)
T3	8/8 (100%)	5/5 (100%)	7/8 (88%)	1/5 (20%)	6/8 (75%)	4/5 (80%)	8/8 (100%)	5/5 (100%)
T4	8/8 (100%)	4/5 (80%)	4/8 (50%)	0/5 (0%)	5/8 (63%)	2/5 (40%)	8/8 (100%)	5/5 (100%)

*14 PUPs: The 14 patients analyzed became 13 at time points T3 and T4, as subjects with persistent inhibitors reduced to 8 PUPs based on plasma availability.

Background: Neutralizing antibodies (inhibitors) are the main complication of replacement therapy in hemophilia A. Some of these inhibitors disappear shortly after development (transient), while others remain (persistent), through a mechanism still unknown.

Aims: To investigate the predictive value of anti-FVIII IgG subclasses (IgG1, IgG2, IgG3, IgG4) within 6 months from inhibitor development on inhibitor persistence in previously untreated patients (PUPs) with severe hemophilia A.

Methods: Patients: Initially, we analyzed 43 PUPs from the SIPPET study who developed inhibitors, using samples taken within the first 60 days from inhibitor development. Subsequently, from the 43 PUPs we studied 14 (5 transient inhibitors and 9 persistent), at 5 time points over a course of 6 months post inhibitor development: T0: at inhibitor detection, T1: between 1-30 days following first inhibitor, T2: between 31-60 days, T3: between 61-120 days, T4: between 121-180 days.

Laboratory methods: Anti-FVIII IgG subclasses were measured by ELISA assay as reported previously (Cannavò et al. Blood, 2017) with minor modifications.

Statistical analysis: Logistic regression was applied to assess the association of "number of IgG subclasses" with "inhibitor persistence".

Results: Relative Risks showed that within 60 days, the risk of inhibitor persistence increased with the rising number of IgG subclasses (Table 1). The analysis of IgG subclasses profiles over 6 months, showed a predominant presence of IgG1 and IgG4 in all inhibitor patients, regardless of transience/persistence. IgG3 antibodies were present in the early response and progressively reduced in both groups. IgG2 was the only subclass that had a differential tendency; disappeared in the transient group and increased in the persistent (Table 2).

Conclusions: Within 60 days from inhibitor formation, the presence of more than one anti-FVIII IgG subclass was associated with an increased risk of inhibitor persistence. Over a course of 6 months, IgG2 resulted as the only IgG subclass harboring predictive hallmarks of inhibitor persistence.

PB0926 | The Experience of Orthopedic Surgeries in Ukrainian Hemophilia Patients with Inhibitor

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Background: The development of inhibitors (antibodies against FVIII/FIX concentrates) is the main complication in the treatment of hemophilia. Hemophilia patients with inhibitors are more vulnerable to arthropathy and orthopedic and musculoskeletal complications leading to a prolonged hospital stay and uncontrolled bleeding.

Aims: In this study, we evaluated our hemophilic patients with inhibitor during orthopedic surgery.

Methods: We reviewed a series of orthopedic surgery procedures in hemophilia patients with inhibitors who underwent between 2019 and 2018 at our hospital.

In total, 5 surgeries were conducted in 4 hemophilia patients with inhibitors. The median age of the patients was 10.5 (range, 6-16) years. Our clinical series of patients comprised 3 cases of hemophilia A (75%) and 1 cases of hemophilia B (25%). All of the patients had severe hemophilia, with factor activity in blood less than 1%. The

inhibitor titer at procedures was range - 3,5 - 562 BU. Elective surgical procedures were performed 1 achilloplasty in patient with equinus position of the left foot, 1 arthroscopic debridement of left knee, 1 internal fixation for femur fracture and 1 removing metal fixation, 1chemical synovectomy with rifampicin of left knee in patient with inhibitory hemophilia B. In total, 2 patients received rFVIIa, 2 patients - rFVIIa and APCC.

Results: In most cases, postoperative bleeding control was successful. However, 1 patient with inhibitory hemophilia B during the last procedure of rifampicin injection had acute hemarthrosis of left knee, which was controlled by 3-day infusions of rFVIIa; 1 patients after arthroscopic debridement without appropriate rehabilitation has progression of hemophilic arthropathy of left knee.

Conclusions: Hemophilia patients with inhibitor present difficult management challenges when they need surgery. Surgery in patients with inhibitors requires more careful preparation and efficiency depend on not only hemostatic agents, but on organisation of multidisciplinary team (surgeons, hematologist and physiotherapist) in the comprehensive hemophilia treatment centers.

PB0928 | Real-World Experience with Emicizumab in Persons with Hemophilia A (HA) with or without Inhibitors

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Background: Emicizumab, a recombinant humanized bispecific antibody that binds FIXa/FX and mimics FVIII cofactor function, is a novel agent FDA-approved for prophylaxis in hemophilia A (HA) with/without inhibitors. Despite reassuring safety/efficacy clinical trial data, real world data is limited.

Aims: We report single-center, post-licensure experience in 122 moderate/severe HA patients treated with emicizumab for ≥6 months; 25 surgeries were performed in 22 patients.

Methods: A post-licensure retrospective observational analysis was performed to assess safety/efficacy in HA on emicizumab between 10/08/2018-12/31/2019; patients in HAVEN studies were excluded. The EMR was queried for moderate/severe HA on emicizumab for ≥6 months and HA on emicizumab for any duration who underwent surgery.

Results: 122 patients [1-66yrs, median 16] received emicizumab for ≥6 months; 35 patients[28.7%] were < 12yrs

[1-11yrs; median 5]; 88[72.1%] were ≥12yrs [12-66yrs; median 21]; 7[5.7%] with inhibitors [3-23yrs; median 6], 115[94.3%] without inhibitors [1-66yrs; median 17]. Twenty-five surgeries were performed in 22 patients

[2-66yrs;median 15] with 5 procedures in 4 inhibitor patients [18.2%;median 5.5yrs].

During emicizumab prophylaxis, 128 bleeding events occurred in 60/122(49.2%) patients [13/35(37.1%) < 12 yrs and 47/87(54%) patients ≥12 yrs]; 62(50.8%) patients had 0 treated bleeds. Perioperative bleeding occurred in 8/25 (32%) surgeries. Additional perioperative hemostatic agents were used in 20(80%) surgeries; fewer bleeds occurred with pre/intraoperative hemostatic therapy (3/15; 20%) vs. emicizumab as sole hemostatic agent (5/10; 50%).

Conclusions: These data represent real world use of emicizumab in a large population with HA. Confounding factors affecting bleeding rates include: differentiating bleeds from arthropathy, pre-emptive treatment after injury and prior to hemostatic challenges. Patients may not report treating suspected bleeds/injuries.

No thrombosis/thrombotic microangiopathy or deaths occurred. We observed effective bleed control on emicizumab and safety with additional hemostatic agent use in surgery. With further experience, we expect a decrease in postoperative bleeding. These results add to reported study data on safety/efficacy of emicizumab.

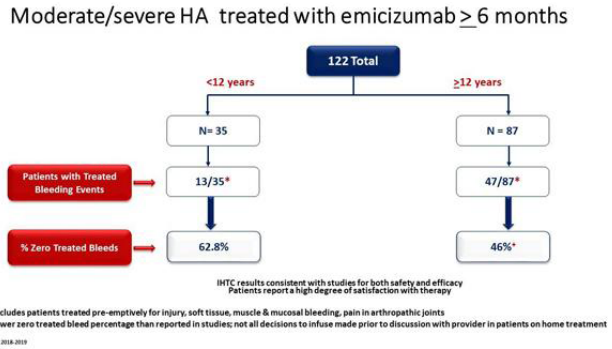

FIGURE 1 Moderate/severe HA treated with emicizumab > 6 months

TABLE 1 Moderate/severe HA treated with emicizumab > 6 months

ID	Surgery/Procedure	Inhibitor	Hemlibra Dosing	Pre/intra-op hemostatic treatment	Post-Op hemostatic treatment	Bleeding
1*	Port removal	Yes	QW	rFVIIa 90 ug/kg x2	rFVIIa Q2h x2; Q4 h x2; Q6h x1	Intra-op; No post-op
2		No	QW	None	None	No
3		Historical	QW	None	None	No
4		Historical	Q2W	None	rFVIII 50 IU/kg x3	Chest wall hematoma
5		Yes	QW	None	None	No
6		-	Q4W	None	None	Delayed (minor on POD 7)
7		No	QW	None	None	No
8		No	Q2W	None	rFVIIIa 50 IU/kg QD x 5	Swelling at port removal site (seroma on ultrasound)
9*		No	Q2W	None	None	No
10	R medial patellofemoral ligament reconstruction	No	Q2W	rFVIII 50 IU/kg	rFVIII 40 IU/kg Q8h x3; 40 IU/kg Q12h x2; 40 IU/kg QD x2	No
11	R ankle foreign body removal	No	Q4W	rFVIII 50 IU/kg	rFVIII 50 IU/kg QD x 2	No
12	ORIF R 5th phalangeal fx	No	QW	rFVIII 50 IU/kg	rFVIII 50 IU/kg Q12h x 2	No
13	L elbow arthroscopic synovectomy/ nerve transposition	No	Q4W	rFVIII 50 IU/kg	rFVIII 50 IU/kg Q12h x 5, then daily POD 8 - POD 13	Bleeding into soft tissue/ muscles of left forearm after PT/OT
14	Microlumbar discectomy	No	QW	rFVIII 50 IU/kg; then gtt 4 IU/kg/hr	rFVIII gtt 4 IU/kg/hr x 1 day; then rFVIII 50 IU/kg QD x 7	No
15	Screw fixation of right femoral neck fx	No	Q4W	rFVIII 50 IU/kg 1h before surgery	rFVIII 32 IU/kg QD x5; then x 1 on POD 9 before PT	No
16	EGD	No	Q2W	rFVIII 40 IU/kg 2-3h preop	None	No
17	Colonoscopy + polypectomy	No	QW	rFVIII 40 IU/kg	rFVIII 40 IU/kg QD x 3; TA 1300 mg PO TID x 10d	No
18	Endoscopy + colonoscopy with bx	No	Q4W	rFVIII 22 IU/kg	TA 1300 mg PO TID x 5d	No
19	Dental extractions x 5 with abscesses	Yes	QW	rFVIIa 200 mcg/kg	rFVIIa 200 ug/kg x 1; rFVIIa 90 ug/kg x 13 doses total. IV TA 10 mg/kg Q8h. EACA PO x 10d.	Oral bleeding
20*	12 dental crowns, OR with general anesthesia	No	Q2W	VWF/FVIII 50 FVIII IU/kg	EACA 100 mg/kg 5-7d; D/C POD 2 d/t GI upset	No
21	Buccal mucosa biopsy	No	Q4W	None **	rFVIII 20 IU/kg x 2, EACA started x7d	Gums, liver clot
22	Stage 3 sacral ulcer - excision and closure R ischium ulcer/flap	No	Q4W	rFVIII 30 IU/kg	rFVIII 4 IU kg/hour X 1d; then 40 IU/kg QD x4	No
23	Excision of squamous cell carcinoma of L temple (Mohs)	No	QW	None	rFVIII 25 IU/kg; repeat rFVIII 50 IU/kg on POD 9	Oozing from L temple, delayed bleed through steri-strips
24	RHC for removal of central line fragment	No	QW	rFVIII 40 IU/kg	None	No
25	Laparoscopic appendectomy	Yes	Loading	rFVIIa 90 ug/kg	rFVIIa 90ug/kg Q2h x9; Eloctate 200 U/kg QD x2 + TA x 5d	No

** Dual diagnosis of hemophilia A + VWD Type I; ** Plan for rFVIII 20 IU/kg, patient did not infuse; EACA, epsilon aminocaproic acid; TA, tranexamic acid*

PB0929 | Hemlibra Treatment in Pediatric Hemophilia A Patients - Real World Data of Safety and Uptake in the PedNet Cohorts

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Background: Hemlibra® (Roche), the bispecific humanised antibody bridging FIXa and FX, was recently approved as prophylaxis for haemophilia A (HA) patients with and without inhibitors. Data in young children are limited, and moreover even less evidence exists regarding real world use. The risk for adverse events during long-term use and concomitant use with FVIII /bypass agents in children is to be established.

Aims: To evaluate safety and efficacy on PedNet patients using Hemlibra.

Methods: The PedNet Registry is a prospective, multicentre cohort study that includes all children with haemophilia born since January 1st 2000 and diagnosed and treated in one of the 31 participating haemophilia centres in Europe, Canada and Israel. The present study focuses on patients using Hemlibra at the cut-off date of January 1st 2020.

Results: 70 children (n=67 severe HA) are currently treated with Hemlibra, of whom 63 had a FVIII inhibitor at start of treatment. 17 infants started Hemlibra before the age of 2 years (2 before 6 months). The median treatment period was 6.9 (IQR 2.1-14.6) months. 23 patients (22 with inhibitors) reported a total of 40 bleeding episodes; the remaining 47 patients (41 with inhibitors) had zero bleeds. Details of bleedings can be found in the table. Concomitant treatment with FVIII was reported in 12 children (11 with inhibitors, of whom 6 received ITI). Ten children received rFVIIa therapy. No inhibitors were diagnosed in patients treated with Hemlibra; only a local skin reaction was reported so far. No serious adverse events were reported.

Conclusions: Hemlibra was well tolerated both in inhibitor and non-inhibitor patients. No serious adverse events were reported.

TABLE 1 Number of included patients and reported bleeds by age group

Table: Number of included patients and reported bleeds by age group

	Birth- <28 days	29 days- <6 months	6 months- <2 years	2- <6 years	6- <12 years	12- <18 years	Total
	N	N	N	N	N	N	N
All patients	0	2	15	15	23	15	70
Number of patients with inhibitor	X	2	14	15	20	12	63
Number of patients with zero bleeds	X	0	8	14	15	10	47
Number of reported major* bleeds	X	0	2	0	2	1	5
Number of reported minor** bleeds	X	3	12	1	8	11	35

* Major Bleed: bleed characterized by pain, swelling, limitation of motion and failure to respond within 24 hours of treatment

** Minor bleed: Bleed characterized by mild pain, minimal swelling, minimal restriction of motion, resolving within 24 hours of treatment

PB0930 | Efficacy and Safety of a 21-Day Individualized Dosing Interval in Patients with Hemophilia B Treated with rIX-FP

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Background: rIX-FP is a fusion protein genetically linking recombinant human coagulation factor IX with recombinant human albumin. It has an improved pharmacokinetic profile compared with standard FIX products, allowing a prolonged dosing interval in patients with hemophilia B.

Aims: To assess the efficacy and safety of a 21-day individualized dosing interval in patients with hemophilia B.

Methods: As part of the PROLONG-9FP phase 3 clinical trial, patients received routine prophylaxis with rIX-FP; dosing intervals could be changed at any 6-month follow-up. Patients began weekly treatment intervals (35-50 IU/kg) with rIX-FP and after 6 months could extend to every 10 or 14 days (50-75 IU/kg). In the extension study, patients ≥18 years could switch to a 21-day regimen (100 IU/kg) if they were well controlled on a 14-day regimen for ≥6 months, with no bleeds in the 2 months prior to switching.

Results: 59 patients were treated with rIX-FP in the extension study; 11 patients switched to a 21-day regimen, including five patients who were initially treated on-demand (Figure). After 6 months on the 21-day regimen, two patients switched to a 14-day regimen due to the occurrence of breakthrough bleeding. For all patients on the 21-day regimen, the total mean monthly consumption of rIX-FP was 146.9 IU/kg/month for a median of 21.5 months (range 5-37) of treatment. Patients who switched to the 21-day regimen had median annualized spontaneous bleed rate of 0.0 throughout the study (Table); 64% of these patients reported zero spontaneous bleeds.

TABLE 1 Patient characteristics and efficacy of rIX-FP in patients switching to a 21-day regimen

	21-day regimen (n=11)		
Age, years, mean (SD)	40.2 (12.79)		
Weight, kg, mean (SD)	69.4 (9.84)		
	7-day (n=11)	**14-day (n=11)**	**21-day (n=11)**
Duration on regimen, days, mean (SD)	308.1 (145.4)	516.2 (413.5)	654.5 (333.8)
AsBR, mean (SD)	0.14 (0.477)	0.23 (0.596)	0.60 (1.408)
AsBR, median (range)	0.0 (0.0, 1.6)	0.0 (0.0, 2.0)	0.0 (0.0, 4.7)
Patients with zero spontaneous bleeds, n (%)	8 (73)	6 (55)	7 (64)
ABR, mean (SD)	0.52 (0.78)	0.44 (0.79)	1.19 (1.57)
ABR, median (range)	0.00 (0.0, 1.9)	0.00 (0.0, 2.0)	0.32 (0.0, 4.7)

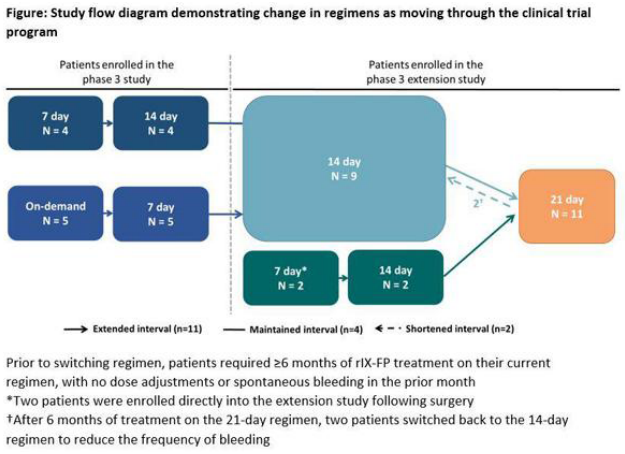

FIGURE 1 Santagostino_ISTH 2020_21-day dosing figure
Santagostino_ISTH 2020_21-day dosing figure

Most reported adverse events with the 21-day regimen were mild/moderate in intensity, one (peritonsillar abscess) was considered severe, and all were unrelated to rIX-FP treatment. No inhibitor, anaphylactic reaction or thromboembolic events were reported.
Conclusions: rIX-FP allows extended treatment intervals of 21 days, in selected adult patients, with good efficacy and safety.

PB0931 | Total Hip Arthroplasty In Avascular Necrosis of the Femoral Head in a Patient with Hemophilia A

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Background: Surgery for patients with hemophilia requires additional planning. Adequate quantities of clotting factor concentrates should be available for the surgery itself and to maintain adequate coverage postoperatively for the length of time required for rehabilitation.
Aims: Describing prophylaxis in a patient with hemophilia A for a major orthopedic surgery.
Methods: Review a clinical case.
Results: A 42-year-old man, weighting 68 kg, with moderate hemophilia A (FVIII: 1.4%) with diagnosis of avascular necrosis of the left femoral head was submitted to a total hip replacement under replenishment with recombinant CFVIII (rCFVIII).
12 hours before surgery the patient received 2000 IU of rCFVIII.
2 hours before surgery he had replenished 90% of FVIII and tranexamic acid.
6 hours after administering the rCVIII we had measured FVIII (FVIII: 60%).
8 hours after surgery, 75 mL of sero-hematic fluid was drained. We administered additional 2000 IU of rCFVIII.
In D1 post-surgery, the patient initiated replacement treatment with rCFVIII 3000 UI every 12 hours. Drained 320 mL of sero-hematic fluid.
In D2, he maintained replacement treatment with rCFVIII 3000 IU in the morning, prior to the removal of the drain, and 2000 IU of rCFVIII at night.
After D3, he maintained replacement treatment at a 60% level of FVIII for 3 days.
In D6 post-surgery the patient was discharged from hospital.
He maintained treatment with rCFVIII until D14 post-surgery, after he restarted prophylactic treatment regimen.
In D38 we did not find inhibitor.
Conclusions: The most common complication of hemophilia is joint disease due to recurrent joint bleeds, resulting in joint replacement surgery as a usual procedure for these patients.
In our case the patient did not have hemophilic arthropathy but had avascular necrosis of femoral head.
The total hip arthroplasty is a major surgery with high bleeding risk. Performing this surgery on a patient with hemophilia requires proper planning.

PB0932 | Prospective, Non-Interventional Study to Assess the Burden in Adolescents and Adults with Haemophilia A (HA) without Inhibitors and their Caregivers in Spain - Hemolife Study

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Background: HA is a disorder characterized by deficiency in clotting FVIII producing bleeding events, primarily musculoskeletal, leading to arthropathy and impacting patients and caregivers` daily lives.

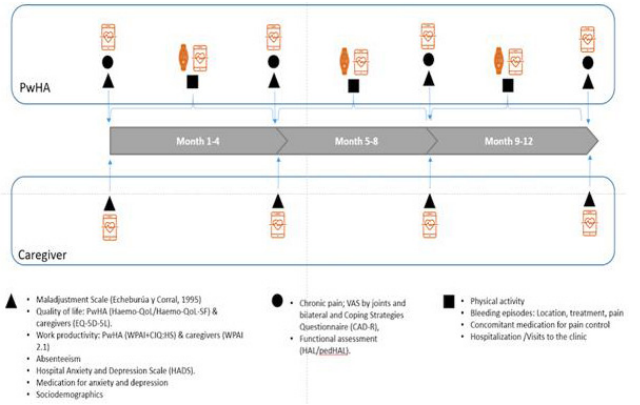

FIGURE 1 Study visit scheme

Aims: To describe the psychosocial impact of HA without inhibitors in patients/caregivers and provide an overview of the variables that most affect quality of life (QoL).

Methods: A multidisciplinary scientific committee of experts in HA, including hematologists, a psychologist and a caregiver designed this study. Those different points of view developed a new and enlightening study design where technology (app & wearable) will collect accurate data from participants, producing valid scientific evidence for the Haemophilia community.

The primary objective will be measured with a maladjustment scale. Secondary objectives will be included to assess the sociodemographic profile, QoL, work/school productivity and absenteeism, functionality, pain and use of analgesia, anxiety/depression and physical activity to assess the impact of the disease.

The study includes the same objectives, when relevant, on the caregivers; understanding caregiver as the person in the environment of patient who shares the burden of the disease.

A sample of 100 patients with Haemophilia A (PwHA) and up to 100 caregivers will be followed for 1 year. The design and schedule of assessment is described in figure 1.

Results: This study is in progress. It has been approved by recognized Ethics Committee and it is being run in approximately 20 Hospitals in Spain to obtain a representative sample of the Spanish population (22 different levels hospital from 17 spanish regions).

Conclusions: Therefore, this is the first study linking cost-utility and social impact of PwHA and their caregivers to provide relevant information to the Haemophilia community and contribute to improving clinical practice and patient care in PwHA in Spain.

PB0933 | Dynamic Changes in Peripheral Blood Transcriptome Profiles May Predict Inhibitor Formation in Previously Untreated Patients with Severe Hemophilia A

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Background: Inhibitor formation is currently the greatest treatment related complication in severe hemophilia A. Inhibitor risk factors are genetic and environmental. Prediction of individuals at risk of developing inhibitors may optimize care.

Aims: To prospectively evaluate changes in blood transcriptome expression prior to and following first FVIII concentrate treatment.

Methods: Severe hemophilia A previously untreated patients (PUPs) were treated using a B-domain deleted FVIII concentrate, simoctocog alfa (ClinicalTrials.gov identifier: NCT01712438). Study cumulative inhibitor incidence (105 PUPs) was: high-titre=17.6% (95%CI:10.0-25.3%); all inhibitors=27.9% (95%CI:19.1-36.7%). Whole blood mRNA was collected prior to treatment and every 3-4 exposure days (EDs) until inhibitor detection or 20ED, with 84 patients (449 samples) recruited. Case-control matching (3:1) was performed, based on ED1 age and timing of subsequent samples. Next-generation RNA sequencing (RNA-Seq) was performed using the Illumina HiSeq.

Results: 16 patients (controls=12, inhibitors=4) with median age (ED1) of 11.5 months on prophylaxis were included. Inhibitors occurred at a median 10ED (7-14ED), with peak titre of 27.4BU (2.3-137.6BU). Serial transcriptome analysis was performed on baseline, early (3-4ED) and inhibitor/matched samples. Greatest variation in transcriptome profiles was seen on principal component analysis (PCA) relating to individual and age, with apparent grouping of inhibitor patients on Kernel PCA. Differential expression was seen comparing baseline profiles in inhibitor to non-inhibitor patients. Adjusting for potential false discovery, upregulation of HLA-DMB and downregulation of LENG8 was seen at all timepoints in inhibitor patients. Comparing baseline to early samples in inhibitor patients, a number of genes were differentially regulated: the most prominent being upregulation of TGM4.

Conclusions: Differential transcriptome profiles were seen in this pilot cohort comparing inhibitor patients to matched controls. Upregulation of the HLA Class II beta chain paralogue, HLA-DMB, involved in MHC Class II peptide loading appeared discriminatory to inhibitor risk. Extension of this analysis within the whole cohort may provide additional insights into inhibitor formation.

PB0934 | Cardiovascular Risk Factor Profile among North-Eastern Brazilian Adults with Haemophilia

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Background: Since the introduction of episodic and prophylaxis treatment with safer products, the life expectancy of people with

haemophilia enhanced considerably. This comes with an increasing number of older patients with hemophilia and with associated diseases, such as cardiovascular disease.

Aims: HemoCardio was a cross-sectional study aimed to describe CVD risk factors among North-eastern Brazilian people with haemophilia.

Methods: Male patients 30 years or older were interviewed, had physical examinations and provided blood samples.

Results: Eighty-four patients were included, of whom 83.3% had haemophilia A and half had severe disease. Median age at study entry was 42.0 years [interquartile range IQR, 36.0-51.0]. Obesity, systemic arterial hypertension and diabetes frequencies were 15.7%, 58.2% and 15.9%, respectively. Hypertriglyceridaemia, hypercholesterolaemia and hypoHDLaemia were present in 17.5%, 43.1% and 31.0%, respectively. Metabolic syndrome was considered in 36.8%. Major electrocardiogram changes were described in 29.5%. There were 46.7% patients with high risk of developing cardiovascular events in the next 10 years, according to the Framingham risk score. Several reports from general Brazilian men described a prevalence of high-risk Framingham risk score ranging from 4.7% to 12.7%.

Conclusions: In conclusion, our findings suggest a relatively high prevalence of CVD risk factors and associated CVR risk score among men with haemophilia compared with the general male population.

PB0935 | Real World Efficacy of Emicizumab in Haemophilia A with Inhibitors: A Report from the UK National Haemophilia Database

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Background: Emicizumab was introduced in the UK for haemophilia A with inhibitors (HA-I) in February 2018. Although clinical trials demonstrate significant reduction in bleeding rates, real-world studies are limited.

Aims: To describe and compare the use and efficacy of Emicizumab with prior treatment modalities in UK HA-I patients.

Methods: Haemtrack (HT) and National Haemophilia Database (NHD) treatment data provided demographics, ABR, AJBR and inhibitor titres for descriptive comparison between switchers with non-switchers, (01/02/2018-30/09/19). A within-patient before and after switching comparison was conducted in the subset with ≥6 months pre and post-switching HT data, analysed using Wilcoxon signed-rank testing. Bleed-free proportion was analysed using the X^2 McNemar test.

Results: Of 172 patients with HA-I, 81 (47%) switched to Emicizumab, median (IQR) age 26 (11-51) years. Dosing schedules, available for 75/81 patients, showed weekly dosing in 53/75, fortnightly in 19/75 and variable in 3/75. Immune tolerance induction (ITI) occurred concomitantly in three. Emicizumab uptake increased steadily across the follow-up period and was associated with an overall reduction of 92% and 68.5% in the use of FEIBA and rFVIIa for HA-I, respectively. The 61/81 patients with ≥6 months HT compliant Emicizumab treatment data had a median (IQR) follow-up 57 (42;78) weeks, ABR 0 (0;0) and AJBR 0 (0;0) and 89% (n=54) were bleed-free. Within-patient comparison of 30 patients with ≥6 months pre-switch data showed a fall in ABR and AJBR ($p < 0.001$) (Table). The 58/172 patients not switched to Emicizumab (with ≥6 months HT compliant data) were generally younger and with lower inhibitor titres, bleed rates and use of bypassing agents (Table). Emicizumab was discontinued in two mild haemophilia patients whose inhibitors remitted and in one patient with recurrent reactions.

Conclusions: Emicizumab prophylaxis in HA-I is associated with dramatic reduction in bleeding rates in the real-world. Further data are required to determine long-term clinical outcomes.

TABLE 1 Comparison of patient characteristics and within-patient analysis of Emicizumab treated patients

	Non-switchers, n=67	Pre-switch, n=30	Post-switch, n=30	Within-patient comparison p value
Severity	severe 58, moderate 9	severe 29, moderate 1		-
Inhibitor titre at presentation (BU) med. (IQR)	5.25$(1.33-14.28), n=52	9.04$(3.95-45.6), n=13		-
Age med. (IQR)	6 (2;15.5)	30 (12;47)		-
Follow-up (weeks) med. (IQR)	78 (59;78)	48 (41;71)	49 (39;80)	-
ABR med. (IQR)[range]	0.7 (0;1.9) [0;18]	12 (2.5;16.8)[0;61.2]	0 (0;0) [0;41.1]	p<0.001
AJBR med. (IQR) [range]	0 (0;0.7) [0;18]	4.4 (1.3;10.0) [0;23.8]	0 (0;0) [0;32.8]	p<0.001
Bleed-free number (%)	32 (48%)	3 (10%)	26 (87%)	p<0.001#

$p=0.11 #$X^2$ goodness-of-fit test.

PB0936 | No Relapse in Patients with Previous Inhibitors Switched to rFVIIIFc in Ongoing Observational Phase 4 Studies

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Background: Relapse after successful immune tolerance induction (ITI) in haemophilia A (HA) has been reported to occur in up to about 30% of cases. Switch between factor VIII (FVIII) products has not been shown to increase the risk of inhibitors. The Fc domain fused to recombinant FVIII (rFVIII) in rFVIIIFc has been ascribed potential immunomodulatory features, which is supported both by pre-clinical and early clinical data. No inhibitors occurred in previously treated patients (PTP) during the rFVIIIFc clinical development programme (phase 1-3). However, patients with inhibitor history were excluded from these studies. Therefore, we here evaluate tolerance status of previous inhibitor patients after switch to rFVIIIFc in the ongoing prospective observational phase 4 studies A-SURE and PREVENT, which did not exclude these patients.

TABLE 1 Patients with inhibitor history and subsequent negative inhibitor prior to rFVIIIFc switch

	PREVENT N=18		A-SURE N=18		All N=36	
Feature	n[a]		n[a]		n[a]	
Historical peak titre (BU/mL) mean median (min-max)	11	548.7 8.0 (1-5500)	13	215.5 3.2 (1-2500)	24	368 4.1 (1-5500)
High responder (>5 BU/mL), n (%)	11	6 (55%)	13	5 (38%)	24	11 (46%)
Received ITI: Yes/No, n	16	14/2	15	11/4	31	25/6
Years since inhibitor <0.6 BU/mL mean median (min-max)	8	6.9 6.0 (1.0-16.0)	11	10.5 11.0 (1.0-20.0)	19	9.0 10.5 (1.0-20.0)

rFVIIIFc, recombinant factor VIII Fc; BU, Bethesda unit; ITI, immune tolerance induction. [a]Patients with available data for the respective features.

TABLE 2 Data after switch to rFVIIIFc

	PREVENT N=18	A-SURE N=18	All N=36
Age at rFVIIIFc initiation (years) mean median (min-max)	17.8 15.0 (4-51)	17.7 13.5 (4-55)	17.8 14.0 (4-55)
EDs on rFVIIIFc, mean median (min-max)	249 236 (59-569)	278 249 (118-475)	263 245 (59-569)
Inhibitor development on rFVIIIFc, n (%)	0 (0%)	0 (0%)	0 (0%)

rFVIIIFc, recombinant factor VIII Fc; ED, exposure day.

Aims: To evaluate if patients with a history of inhibitors who became inhibitor free on a previous FVIII product maintain tolerance after switch to rFVIIIFc.

Methods: PTPs with documented inhibitor history and subsequent negative inhibitor prior to switch to rFVIIIFc and a minimum of 50 exposure days on rFVIIIFc were identified from the ongoing real-world studies PREVENT (Germany, NCT03055611) and A-SURE (EU, NCT02976753). Previous ITI and current tolerance status were analysed. Cut-off date for data extraction was December 9th 2019.

Results: Eighteen patients in PREVENT and 18 from the rFVIIIFc arm in A-SURE had previous inhibitors and became inhibitor free before switch to rFVIIIFc (Table 1). No inhibitors were detected in these 36 patients on rFVIIIFc treatment (Table 2), with a mean duration of rFVIIIFc exposure of 21 and 26 months in PREVENT and A-SURE, respectively.

Conclusions: No inhibitor recurrence was detected after switch to rFVIIIFc in patients with previous inhibitor on another FVIII product and subsequent negative inhibitor prior to switch to rFVIIIFc, indicating that the risk of recurrent inhibitors is very low when switching to rFVIIIFc.

PB0937 | Improved Prophylaxis Adherence After Switching to Emicizumab: Real-World Data from a Single Center

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Background: Emicizumab prophylaxis in persons with hemophilia A appears to be less burdensome than factor VIII (FVIII) prophylaxis, with subcutaneous and less frequent dosing. It is not known whether switching to emicizumab results in improved prophylaxis adherence.

Aims: We aimed to describe patients' adherence to prophylaxis regimens and compare medication adherence before and after switching to emicizumab.

Methods: We included all individuals enrolled in an IRB-approved, consented single-center cohort study (HemoPICS) who had pharmacy dispensing data available and started emicizumab prophylaxis at least 90 days prior to 12/31/2019. We restricted analysis of factor prophylaxis to the year prior to switching to emicizumab. Adherence was defined as >80% proportion of days covered (PDC) by either emicizumab or factor prescriptions. We compared adherence and PDC before and after switching to emicizumab using a chi-square test and paired samples t-test, respectively.

Results: Thirty-four patients were eligible for inclusion in the analysis (mean #days on emicizumab 321). Eight were using FVIII on-demand and 1 patient had an active FVIII inhibitor and used bypass agent prophylaxis prior to switching to emicizumab. Dispensing records for factor prophylaxis prior to switching to emicizumab were available for 18 (mean #days on factor 364). PDC and adherence rates for factor and emicizumab are shown (Table 1). Adherence was significantly more frequent in the emicizumab group (82% for emicizumab vs. 44% for factor, p=0.02). PDC increased significantly after switching to emicizumab (93% for emicizumab vs. 71% for FVIII, p< 0.001). No patients who were adherent on factor became non-adherent after switching to emicizumab (Figure 1).

Conclusions: Patients appear to be more adherent to their medication regimens when they are on prophylaxis with emicizumab compared to FVIII. Improved adherence may be a compelling justification for switching to this medication, particularly if improved adherence leads to better outcomes.

TABLE 1 Adherence and Proportion of Days Covered (PDC) on Factor and Emicizumab.

	Emicizumab (all subjects), n=34	Emicizumab (subj. w/ factor data available), n=18	Factor prophylaxis, n=18
Adherent, n (%)	28 (82%)	13 (72%)	8 (44%)
Chi-square*	5.538 (p=0.02)		
PDC			
Mean (SD)	92.4% (10.6)	89.0% (12.6)	71.4% (22.9)
Median (IQR)	97.8% (86.4 - 100)	96.4% (76.0 - 100)	76.4% (51.4 - 92.1)
T-statistic for paired comparison	n/a	4.545 (p<0.001)	

*all subjects included in comparison
PDC=proportion of days covered, SD=standard deviation, IQR=interquartile range

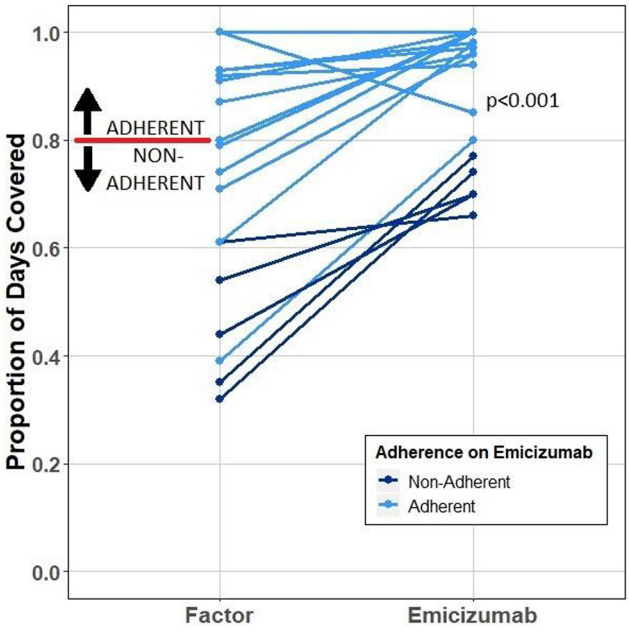

FIGURE 1 Paired Comparison of Proportion of Days Covered on Factor and Emicizumab

PB0938 | Does Variation between Label and Actual Potency of Factor VIII Concentrate Vials Affect Results of Pharmacokinetic (PK)-Guided Dosing of Replacement Therapy in Hemophilia A?

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Background: Inter-individual differences in pharmacokinetic (PK) response influence coagulation factor VIII (FVIII) activity levels in hemophilia A patients and should be accounted for. Dosing can be individualized by PK-guidance of treatment. To date, it is unknown if factor concentrate vial variation between label and actual potency, as allowed by legislation, impacts individual PK parameters and therefore predicted FVIII activity levels.

Aims: Explore effects of vial variation between label and actual potency to determine if FVIII antigen/activity (Ag/Act) ratio influences analyses of individual FVIII PK parameters and therefore predicted FVIII activity levels.

Methods: We analyzed data of severe and moderate hemophilia A patients from the OPTI-CLOT randomized controlled perioperative trial. FVIII vial label potency was documented. Actual FVIII potency of used vials during preoperative individual PK profiling were provided by pharmaceutical companies. Paired t-testing was performed to analyze differences between PK parameters estimated by Bayesian estimation using nonlinear mixed-effects modelling (NONMEM) using both label and actual potency. Multiple regression was applied to evaluate independent effects of variation in potencies on differences in estimated clearance.

Results: Fifty patients with median age of 47.5 years (range 4.0-76.9) were included. Mean variation between label and actual potency was 6.8% (range 0.0-15.5%). Actual potency was higher in 45 patients (mean difference 323 IU, standard deviation 33 IU), leading to significant differences between PK parameters (p< 0.001; Figure 1). Difference in clearance was predicted by potency variation for 80%, independent of PK modifying factors age, blood group O and VWF:act (p< 0.001). However, altered PK parameters do not affect FVIII concentration-time curves of patients' PK profiles (Figure 2).

Conclusions: In conclusion, the observed higher actual potency than label potency of FVIII concentrate vials leads to alterations of PK parameters. However, this does not affect FVIII activity-time profile and does not seem to influence PK-guided FVIII dosing advice.

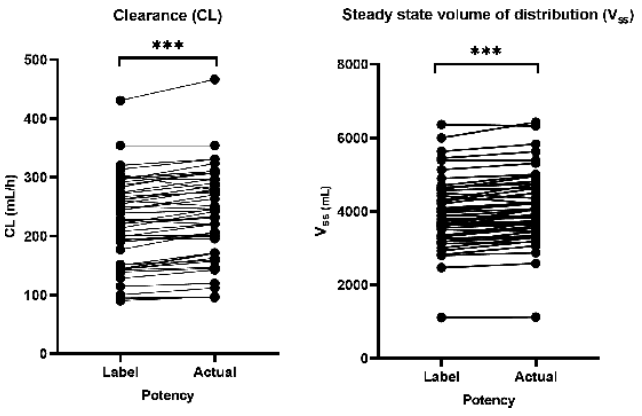

FIGURE 1 Comparison of clearance and steady state volume of distribution estimated using both label and actual potency.***P-value<0.001

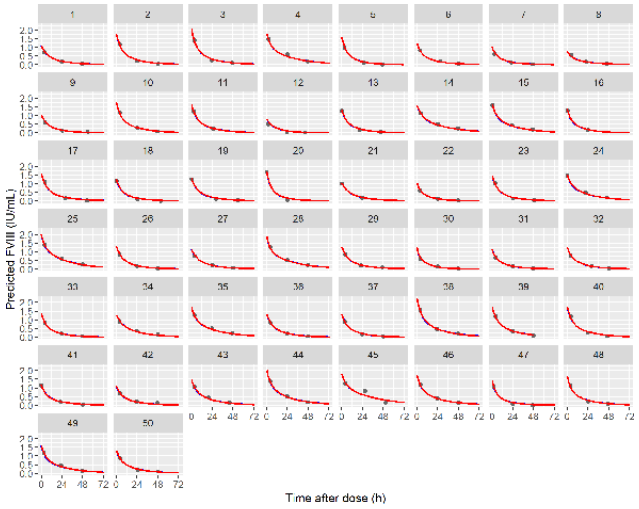

FIGURE 2 FVIII activity-time curves of PK profiles using both label (red) and actual potency (blue). PK profiles overlap for all patients

PB0939 | Surgical Experience from the Phase III STASEY Trial of Emicizumab Prophylaxis in Persons with Hemophilia A (PwHA) with FVIII Inhibitors: Data from the Second Interim Analysis

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Background: Emicizumab, a bispecific monoclonal antibody, bridges activated factor (F) IX and FX replacing the function of missing activated FVIII.

Aims: To present the surgical experiences in the STASEY trial.

Methods: Following informed consent and ethics committee approval, PwHA aged ≥12 years with FVIII inhibitors received emicizumab 3 mg/kg/week for 4 weeks, then 1.5 mg/kg/week for the remaining 2-year treatment period (NCT03191799). Minor and unplanned major surgeries were managed per the

TABLE 1 Case Details of an Arthroscopic Knee Surgery and a Hip Replacement*

Surgery	Patient Characteristics	Emicizumab Dosing	Pre-operative Treatment	Peri- and Post-operative Treatment	Case Details and Outcomes
Semi-acute arthroscopic knee surgery due to infected knee prosthesis (minor)	47-year old male with hemophilia A and long-lasting high titer FVIII inhibitors (292 BU)	Emicizumab 1.5 mg/kg/week for 5 months prior to surgery; emicizumab dosing was continued post-operatively	Bolus rFVIIa 85 µg/kg was administered 1 hour pre-operation	rFVIIa 60 µg/kg every 4 hours for 5 days	The PwHA presented with an active infection of the knee; 2 punctures (intra-articular and in the surrounding tissue) were covered by 2 bolus injections of 60 µg/kg rFVIIa given 3 hours apart Cultures were positive for *Klebsiella Pneumoniae*; PwHA was admitted and treated with antibiotics The arthroscopic knee surgery was uneventful and there was no evidence of TMA On Day 4 after surgery, the PwHA was discharged
Total hip replacement (major)	51-year old† male with hemophilia A with high-responding FVIII inhibitors	Emicizumab 1.5 mg/kg/week for 44 weeks prior to surgery; emicizumab dosing was continued post-operatively	Bolus rFVIIa 90 µg/kg was administered at the start of surgery	During surgery, rFVIIa 80 µg/kg was administered every 3 hours Post-operatively, rFVIIa 80 µg/kg dosing was administered every 4 hours (Day 1-3); every 6 hours (Day 4-7); and every 8 hours (Day 8-11) TA (3 g/24 hours) and prophylactic LMW heparin were administered for the entire post-operative period	The surgeon rated hemostasis as 'good to excellent'; the lowest hemoglobin (99 g/L) was recorded on Day 2 The patient had no signs of TMA and was discharged in good health on Day 11

BU, Bethesda unit; LMW, low molecular weight; PwHA, person with hemophilia A; rFVIIa, activated recombinant activated factor VII; TA, tranexamic acid; TMA, thrombotic microangiopathy.
*These case details are from case narratives provided by two STASEY trial investigators; †Patient was aged 50 years at enrollment on the STASEY trial.

investigator's discretion. Additional peri-operative prophylaxis, if any, and frequency and management of post-operative bleeds, were analyzed.

Results: As at 20 May 2019, 31 minor and 9 major surgeries were performed in 22 and 8 participants, respectively, in the study treatment phase (one minor procedure [skin graft] was combined with a major procedure [arthrodesis] and is not included here). Two-thirds (20/31; 64.5%) of minor surgeries were managed without prophylactic coagulation factor; of these, only 3/20 (15%) required post-operative treatment for bleeds (Figure 1). One of 11 (9%) minor surgeries managed with prophylactic coagulation factor required post-operative treatment for a bleed. Dental interventions with antifibrinolytics were uncomplicated with the exception of one case where an additional dose of FVIIa 90 ug/kg was added; the combined use of antifibrinolytics and FVIIa led to hypertrophic clot formation at the extraction site, which required surgical removal. Overall, 8/9 (89%) major surgeries were managed with prophylactic coagulation factor, resulting in four treated post-operative bleeds, two untreated

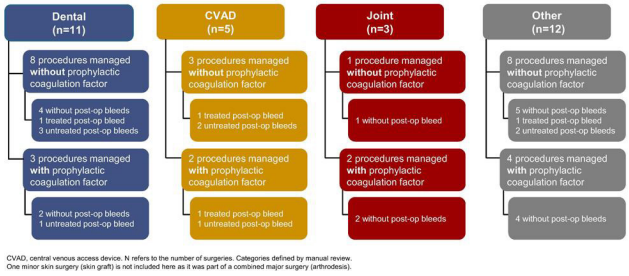

FIGURE 1 Outcomes of Minor Surgeries (n=31)

bleeds, and two resulting in no bleeds. Table 1 shows case details of arthroscopic knee and major hip replacement surgeries.

Conclusions: In PwHA with FVIII inhibitors on emicizumab, most minor procedures were performed without prophylactic coagulation factor and did not result in post-operative treated bleeds. Major surgeries were safely performed with additional coagulation prophylaxis.

PB0940 | Primary Prophylaxis Assessment in Patients with Severe Haemophilia A without Inhibitors Based on Genetic Load and Thrombin Generation Test

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Background: Patients with severe haemophilia A have different haemorrhagic phenotypes regarding their pharmacokinetic profile, genetic load and their haemostatic balance. In fact, discrepancies have been found between the phenotype and genotype, and some polymorphisms in the factor VIII (FVIII) gene have been linked to a higher annual bleeding rate (ABR), even though all severe patients have a baseline FVIII < 1%. In addition, global haemostatic tests have also contributed to a more accurate understanding of the haemorrhagic versus thrombotic equilibrium in patients with haemophilia. The thrombin generation test provides valuable information about the haemostatic status that can be related to bleeding complications.

Aims: To stratify the individual bleeding risk associated to their genetics and thrombin generation capacity.

Methods: This is a non-interventional observational study including patients with severe haemophilia A over 14 years old. We included the following parameters: demographic data, ABR, FVIII genetics (by full gene sequencing), prothrombotic genes (Thrombo inCode, US), Thrombin generation (CAT Thermofisher, US) and FVIII by one-stage coagulative method (IL,US). We measured thrombin generation and FVIII activity just before FVIII replacement therapy and half an hour after infusion. A certified normal plasma is used as reference in thrombin generation parameters.

Results: We have included 8 severe haemophilic patients recruited in our center. We detected C46T factor XII mutation in 2 patients and 1 of them also presented factor V Leyden mutation. We observed different ABR depending on the genetic load (see table 1). Higher trough and peak FVIII levels are not associated with increased thrombin peak and ETP (see table 2).

Conclusions: Trough and peak FVIII activity are not associated with increased thrombin generation. In addition, the interaction between prothrombotic genes and FVIII genetics could condition the ABR.

TABLE 1 Genetics and annual bleeding rates in patients with severe haemophilia A

Patient	Gene mutation	Prothrombotic genes	Other genes	Annual Bleeding rate (ABR)
1	Intron 22 inversion	Not found	Not found	9
2	Intron 22 inversion	Not found	V34L mutation in FXIII	0
3	Intron 22 inversion	Not found	V34L mutation in FXIII	7
4	Not available	Not found	Not found	6
5	Exon 4 deletion	C46T mutation in FXII, F5Leiden	Not found	3
6	Exon 24 substitution pArg2228(pGly2209) Missense in domain C2	Not found	V34L mutation in FXIII	8
7	Exón 14 substitution pArg814 (pArg795) Nonsense in domain B	Not found	V34L mutation in FXIII	14
8	Intron 1 inversion	C46T mutation in FXII	Not found	0

TABLE 2 FVIII activity (one-stage method) and thrombin generation before and 30 minutes after FVIII infusion

Patient	FVIII (%) trough level	TGT-PRE Peak (%)	TGT-PRE ETP (%)	FVIII (%) 30 MIN after infusion	TGT-POST Peak (%)	TGT-POST ETP (%)
1	6.7	21.56	28.82	71.3	118.4	84.52
2	2.3	21.55	46.05	24.9	42.62	83.64
3	4.6	31.08	49.13	92.5	219.41	152.8
4	6.9	14.16	22.13	36.6	31.28	39.74
5	6.3	26.27	37.87	53	102.31	107.96
6	4.8	6.03	10.89	88.7	30.24	34.03
7	0.5	6.78	17.56	73.1	132.13	132.65
8	7	28.84	45.71	97.7	41.2	51.66

PB0941 | Phase 1 Study to Evaluate the Pharmacokinetics, Pharmacodynamics, and Safety of Ascending Doses of Subcutaneous Marzeptacog Alfa (Activated) in Adult Subjects with Hemophilia

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Background: For hemophilia patients with inhibitors, intravenous (IV) recombinant Factor VIIa (rFVIIa) and activated prothrombin complex concentrates are used to treat acute bleeding.

Aims: MAA-102 is assessing the pharmacokinetics (PK), dose proportionality, pharmacodynamics (PD), and safety of IV and subcutaneously (SQ) administered marzeptacog alfa (activated) (MarzAA), a novel variant of rFVIIa, to evaluate its potential to treat acute bleeding in hemophilia and other bleeding disorders.

Methods: The study is enrolling at least 8 subjects (consented per local Ethics Committee) with hemophilia, with or without inhibitors, in each stage to assess 9 dose levels of MarzAA, IV dose of 18µg/kg with PK over 24 hours; increasing SQ single doses of 30, 45, 60, a split dose of 60 (2 × 30 in different anatomical regions), 90, and 120 µg/kg; multiple doses of 60 µg/kg MarzAA, dosed at 3 hour intervals for 2 doses, and 3 doses respectively with PK over 48 to 72 hours. Pharmacodynamics, thrombogenicity, and bioavailability will be assessed. Safety and anti-drug antibodies (ADA) to MarzAA will be assessed weekly and at 30 days post dosing.

Results: Subcutaneous single dose level dosing is complete. Enrollment and dosing in multiple dose stages is ongoing. After SQ dosing, MarzAA mean Tmax =7.5 hours and C_{max} = range 19 to 77 ng/ml (Table 1). Mean residence time for MarzAA, SQ =24.3 hours, IV =3.8 hours. Peak thrombin generation occurred within 6 hours at all dose levels and did not exceed the normal range. No safety concerns (3 Injection Site reactions/ 90 SQ injections), ADAs or thrombotic events occurred.

Conclusions: Interim PK and PD data show that MarzAA has the potential to quickly achieve and maintain prolonged therapeutic levels with an acceptable safety profile. This data supports the potential for MarzAA to treat acute bleeding events with SQ injections in hemophilia patients with and without inhibitors.

TABLE 1 Pharmacokinetic parameters

PK parameters (units)	IV Mean ± SD 18 ug/kg n=8	SQ Mean ± SD 30ug/kg n=8	45ug/kg n=8	60ug/kg n=8	60ug/kg (split) n=8	90ug/kg n=8	120ug/kg n=8
C_{max} (ng/ml)	389 ± 100	18.7 ± 10.3	33.8 ± 18.9	41.9 ± 15.1	40.7 ± 16.2	55.3 ± 24.9	78.8 ± 34.7
AUC 0-inf (ng/ml * hr)	1375 ± 424	493 ± 171	864 ± 338	1064 ± 176	1025 ± 340	1486 ± 436	2095 ± 656
AUC 0-t (ng/ml * hr)	1368 ± 420	417 ± 179	707 ± 268	924 ± 178	880 ± 316	1323 ± 447	1874 ± 669
$T_{1/2}$ (hr)	3.4 ± 0.4	16.1 ± 5.3					
T_{max} (hr)	0.20 ± 0.33	7.5 ± 1.7					
MRT (hr)	3.8 ± 0.43	24.3 ± 7.3					
Vol of dist. (mL/kg)	54.6 ± 16.9	1079 ± 747					
Clearance (mL/kg/hr)	14.4 ± 4.9	83.5 ± 20.2					

PB0942 | Pharmacokinetics and Coagulation Biomarkers in Persons with Hemophilia A (PwHA) and FVIII Inhibitors Receiving Emicizumab in the Phase IIIb STASEY Study

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Background: Emicizumab, a recombinant, humanized, bispecific antibody, restores the function of missing activated factor (F)VIII in PwHA by bridging activated FIX and FX. It showed a favorable safety profile and clinically meaningful bleed prevention in PwHA with FVIII inhibitors in the STASEY study (NCT03191799; Jimenez-Yuste, et al. ISTH 2019).

Aims: To present the pharmacokinetics (PK) and pharmacodynamics (PD) of emicizumab prophylaxis in PwHA with FVIII inhibitors in STASEY.

Methods: Following informed consent and ethics committee approval, PwHA with FVIII inhibitors aged ≥12 years received 3 mg/kg once-weekly (QW) emicizumab for 4 weeks followed by 1.5 mg/kg QW maintenance dosing (data cut-off: 20 May 2019; N=193). PK and PD were assessed throughout emicizumab exposure (median 51 weeks). Emicizumab levels were measured with a validated ELISA assay. PD was assessed using a chromogenic FVIII activity assay (FVIII:C) containing human reagents, and activated partial thromboplastin time (aPTT). Changes from baseline in prothrombin time (PT/INR) and D-dimer were determined. FVIII inhibitor titers were measured using a chromogenic Bethesda assay containing bovine reagents.

Results: Mean steady-state emicizumab trough plasma concentrations reached >50 µg/mL by Week 5 and were sustained throughout emicizumab maintenance dosing, consistent with a half-life of ~30 days. PD markers demonstrated on-target activity of emicizumab. Mean reported FVIII:C activity increased to ~19 U/dL by Week 5 and was maintained throughout the study. Reported FVIII:C activity correlated with emicizumab concentration. aPTT was normalized after the first emicizumab dose and subsequently remained at or just below the normal range. Emicizumab did not affect concentrations of safety markers of activated coagulation (PT and D-dimer). Participants' FVIII inhibitor titers remained stable or declined slightly over time.

Conclusions: The PK and PD profiles, and PK/PD relationship of emicizumab in STASEY were consistent with those from other emicizumab studies (Adamkewicz, et al. ISTH 2017; Kiialainen, et al. EAHAD 2019).

PB0943 | Associations between Physical Activity Levels and Bleeding Frequency in People with Mild, Moderate, and Severe Hemophilia A (HA): A Preliminary Analysis of the CHESS II Study

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Background: HA is a congenital bleeding disorder caused by a deficiency in clotting factor (F) VIII. Bleeding frequency and progressive joint damage correlates with increasing disease severity, however the relationship between bleeding tendency and level of engagement in activities is not currently well understood.

Aims: To examine physical activity and bleeding frequency in adults with HA without current FVIII inhibitors.

Methods: This analysis draws on 12 months' retrospective data from adults with mild, moderate, and severe HA (defined according to endogenous FVIII [IU/dL]) enrolled in the CHESS II (Cost of Haemophilia in Europe: a Socioeconomic Survey-II) burden-of-illness study, across 8 European countries. Data on bleed frequency reported by hemophilia specialists was analyzed along with physical activity levels reported on patient-completed forms. Patient-reported activity level was classified as 'none' or 'any', based on participation in any recreation and fitness-based activity such as jogging, cycling, or team sport, among others, but excluding activities such as walking and gardening. Limitations include the variability of the endpoint and cross-sectional design. Informed consent was obtained and the study was approved by University of Chester ethical committee.

Results: Of 787 patients profiled in the interim CHESS dataset, approximately 41% (n=258) of the 628 patients with HA returned patient-completed forms. Of this cohort 16% (n=41), 28% (n=72), and 56% (n=145) had mild, moderate, and severe HA, respectively (Table 1). Mean (SD) age was 38.66 (14.86), 39.96 (15.66) and 37.42 (14.57) years, and mean (SD) BMI was 24.55 (2.25), 24.94 (2.94) and 24.66 (2.57), in the mild, moderate, and severe cohorts, respectively. Higher levels of physical activity were predominantly associated with more frequent bleeding episodes in the moderate and severe HA groups (Table 2).

Conclusions: In people with moderate and severe HA, the data suggest a trend towards increased participation in sports and recreational activities and bleeding frequency.

TABLE 1 Baseline Characteristics of People with Mild, Moderate, and Severe HA, by Activity Level

	Mild HA (n=41)		Moderate HA (n=72)		Severe HA (n=145)	
	No Activity	Any Activity	No Activity	Any Activity	No activity	Any activity
Treatment strategy, n						
No treatment	9	16	20	25	0	0
On-demand	8	6	11	11	29	29
Prophylaxis	0	2	4	1	51	36
All, n (%)	17 (41)	24 (59)	35 (49)	37 (51)	80 (55)	65 (45)
Problem joint, n (%)	4 (24)	4 (17)	14 (40)	14 (38)	36 (45)	32 (49)
Target joint, n (%)	1 (6)	1 (4)	6 (17)	5 (14)	32 (40)	35 (54)

HA, hemophilia A.

TABLE 2 Activity Levels and Bleeding Frequency in People with Mild, Moderate, and Severe HA

	Mild HA (n=41)	Moderate HA (n=72)	Severe HA (n=145)		All HA (n=258)	
Treatment strategy, n	Any	Any	Any	Prophylaxis (n=87)	Any	Prophylaxis (n=94)
Activity level						
None* n ABR, mean (SD)	17 1.41 (0.87)	35 2.31 (2.00)	80 3.53 (2.78)	51 3.73 (2.82)	132 2.93 (2.53)	55 3.62 (2.75)
Any* n ABR, mean (SD)	24 1.29 (0.95)	37 2.92 (4.62)	65 4.46 (3.07)	6 5.14 (3.33)	126 3.40 (3.56)	39 4.90 (3.31)

*Activity level was classified as either 'none' or 'any' activity.
ABR, annual bleed rate; HA, hemophilia A; SD, standard deviation.

PB0944 | Coinfection by Hepatitis B, C and HIV in Patients with Haemophilia

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Background: Haemophilia patients are under high risk of many transfusion related infections, especially in the era that plasma-derived products were used.

Aims: To identify coinfection by Hepatitis B (HBC), Hepatitis C (HCV) and Human immunodeficiency virus (HIV) in haemophiliacs.

Methods: 107 haemophiliacs have been followed at Fundação Hemominas (FH) Juiz de Fora - Brazil from 2008 to 2018. For the current analysis, age, type and severity of hemophilia and serum profile for hepatitis and HIV were included. Laboratory tests evaluated were: HBsAg, anti-HBc, anti-HCV, anti-HIV by the enzyme-linked immunosorbent assay method. HIV+ samples were confirmed by the Western Blot and HCV+ exams by the Polymerase Chain Reaction (PCR). Positive and negative groups were compared with the Mann-Whitney test and associations were evaluated by Chi-Square test. Research approved by the FH ethics committee.

Results: The average of age was 35.95 years. Predominance of Haemophilia A patients (77.6%) was observed, with ages ranging between 10 and 87 years old (mean 35.95±17.58). The severe form of Haemophilia A ratio was 44%, Haemophilia B medium form was 52.2%, the most common. Laboratory results demonstrated that 24.3% of the patients were HBC+ and 40.2% were HCV+, with 21.42% positive by PCR. Prevalence of HIV+ was 11.2%. In general analysis, 44.82% had at least one type of viral infection and 23.4% presented coinfections. HIV+ patients were all positive for HCV (11.2%) and 7.5% of patients were infected with HIV, HCV and HBC. Considering the year of birth, most patients with HCV or HIV were born before 1980s ($p < 0.005$).

Conclusions: Infections by HBC, HCV and HIV are late complications in patients that received blood products before 1990s. Infection by a viral agent and the year of birth has a direct association, due to the standardization and implantation of tests for HIV and HCV in the 80s and 90s.

PB0946 | Haemostatic Efficacy of rVIII-SingleChain in a Case of Surgical Prophylaxis for Hip Replacement

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Background: rVIII-SingleChain (single chain Factor VIII) is a B-domain truncated recombinant factor comprised of covalently bonded FVIII heavy and light chains. The AFFINITY development program confirmed efficacy and safety of rVIII-SingleChain for the treatment and prophylaxis of bleeding in severe haemophilia A patients, including in the surgical setting.
Aims: To report a real-world experience of the use of rVIII-SingleChain in a case of orthopedic major surgery.
Methods: rVIII-SingleChain dosing regimen was adjusted in perioperative setting (pre-surgery until 14 day after surgical procedure) based on type of surgery and clinical status of patient. The haemostatic efficacy was rated by using a 4-point scale (excellent, good, moderate, poor/no response) adapted from WFH guidelines for the assessment of haemostasis. A haemostatic efficacy rating of Excellent was assigned when haemostasis was not clinically significantly different from normal and blood loss was not > 20% higher than the predicted for the intended surgery.
Results: In July 2018, a 66 years old man affected by severe haemophilia A and comorbities, such as hypertension and hepatitis C, switched to rVIII-SingleChain from a previous rFVIII. An on-demand regimen was maintained until surgery. In October 2019, the patient underwent a major surgery for hip replacement. rVIII-SingleChain was administered as a bolus in surgical prophylaxis. The pre/intraoperative consumption was 93 IU/kg (62 IU/kg pre- and 31 IU/kg 12 hrs post-surgery). Total rVIII-SingleChain perioperative consumption was 690 IU/kg with a total of 18 infusions. Optimal FVIII trough levels were maintained. No unplanned transfusions and no adverse events were reported. Overall, haemostatic efficacy of rVIII-SingleChain was rated as Excellent. After this surgery a prophylaxis regimen was introduced.
Conclusions: In line with results obtained during the AFFINITY trial, this case report reinforced that rVIII-SingleChain is safe and effective to achieve and maintain haemostatic control during orthopedic surgical prophylaxis in haemophilia A patients.

PB0947 | UK EHL Registry: Patient Reported Outcomes Measured by Haemoprefer Patient Treatment Preference and Perception Tool

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Background: The haemoprefer questionnaire is newly developed tool introduced to potentially measure the ease of use and preference for treatment of people with haemophilia (PWH). Haemoprefer was part of a battery of Patient reported outcomes (PRO) used in the UK EHL registry.
Aims: To describe levels of satisfaction for patients on the UK EHL registry.
Methods: Informed consent and ethics committee approval were obtained. Haemoprefer assesses the following;
1) Global Score - scored 0 to 140 - Higher scores indicating greater patient preference and treatment satisfaction.
2) Ease of Using CFC - scored 0 to 40 - Higher scores indicate greater ease of use.
3) Burden of clotting factor treatment - scores range from 0 to 20 - Higher scores indicate lower burden of treatment.
4) Impact of clotting factor treatment - scored 0 to 30 - Higher scores indicate less negative impact on daily life.
5) Risk associated with clotting factor treatment - scored 0 to 30 - Higher scores indicate lower levels of worry associated with treatment.

TABLE 1 Baseline Scores at Study Entry Pre EHL Switch (n=136)

Score	Scoring Range	EHL Arm - Median	EHL Arm - IQR	EHL Arm - Min	EHL Arm - Max	Standard CFC Arm - Median	Standard CFC Arm - IQR	Standard CFC Arm - Min	Standard CFC Arm - Max
Global Score	0-140	104	92-114	59	133	100	88-112	40	124
Ease of Using CFC	0-40	37	32-40	15	40	34	29-38	11	40
Burden of clotting factor treatment	0-20	12	10-16	0	20	14	11-17	0	20
Impact of clotting factor treatment	0-30	25	19-28	7	30	22	17-27	0	30
Risk associated with clotting factor treatment	0-30	26	21-30	4	30	24	20-29	0	30
Influence of others on treatment choices	0-20	7	2-10	0	20	5	1-9	0	20

TABLE 2 Follow up scores-EHL CFC arm only (n=159)

Score	Scoring Range	Median	IQR	Min	Max
Global Score	0-140	110	97-119	60	140
Ease of Using CFC	0-40	38	34-40	13	40
Burden of clotting factor treatment	0-20	15	13-18	2	20
Impact of clotting factor treatment	0-30	25	21-28	7	30
Risk associated with clotting factor treatment	0-30	27	24-30	0	30
Influence of others on treatment choices	0-20	7	2-10	0	20

6) Influence of others on treatment choices - scored 0 to 20 - Higher scores indicate greater importance of the opinions or behaviour of others.

Results: Haemoprefer was evaluable at study entry for 136 patients with 77 on the EHL CFC arm pre EHL switch and 59 on Standard CFC.

At follow up 77 patients on the EHL arm returned 159 questionnaires evaluable for analysis and with a median follow up of 12 months (range 3 - 30 months).

Conclusions: Haemoprefer has been used for the first time in prospective study and its sensitivity and specificity for identifying items of interest for both patients and clinicians in routine clinical practice need to be determined.

PB0948 | Real-World Data of First-Time and Rescue Immune Tolerance Induction with Recombinant Factor VIII Fc Fusion Protein in Patients with Hemophilia A with Inhibitors in Japan: "Fc Adolescent and Children Treatment" Study (FACTs) Part 2 Interim Analysis

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Background: Inhibitor development is a serious complication of factor VIII (FVIII) replacement therapy in patients with hemophilia A (HA). Immune tolerance induction (ITI) is the standard-of-care for eradication of inhibitors and restoration of response to FVIII; however, studies assessing ITI with recombinant FVIII Fc fusion protein (rFVIIIFc) are limited (Carcao et al, *Haemophilia* 2018). The prospective, multicenter, observational FACTs study is designed to evaluate the effectiveness of rFVIIIFc in pediatric patients with HA in Japan (Part 1) and summarize the experience of ITI (Part 2).

Aims: To present the second interim analysis from Part 2 of the FACTs study.

TABLE 1 Patient demographics and disease characteristics

	First-time rFVIIIFc ITI (N=13)	Rescue rFVIIIFc ITI (N=7)
Median (min-max) age at rFVIIIFc ITI start, years	2 (0–63)	3 (1–51)
Hemophilia A severity, n (%)	9 (69.2)	3 (42.8)
Severe	1 (7.7)	2 (28.6)
Moderate	2 (15.4)	1 (14.3)
Mild	1 (7.7)	1 (14.3)
Missing		
Inhibitor family history, n (%)	2 (15.4)	1 (14.3)
Yes	8 (61.5)	4 (57.1)
No	3 (23.1)	2 (28.6)
Unknown		
Inhibitor responder type, n (%)	12 (92.3)	7 (100.0)
High responding (titer ≥5 BU)	1 (7.7)	0 (0)
Low responding (titer <5 BU)		
Median (min-max) historical peak inhibitor titer, BU/mL	31.9 (3.0–1659.0)	160.3 (108.7–1450.0)
Median (min-max) inhibitor titer at start of rFVIIIFc ITI, BU/mL	6.0 (0.4–214.2)	77.0 (22.0–353.0)
Median (min-max) time from inhibitor diagnosis to rFVIIIFc ITI, weeks	51.0 (0.1–240.4)	81.3 (15.0–423.6)

BU, Bethesda unit; ITI, immune tolerance induction; max, maximum; min, minimum; rFVIIIFc, recombinant factor VIII Fc fusion protein.

TABLE 2 rFVIIIFc ITI interim data

	First-time rFVIIIFc ITI (N=13)	Rescue rFVIIIFc ITI (N=7)
Median (min-max) duration of ITI, weeks	52 (17–137)	52 (15–131)
Median (min–max) dose, IU/kg	74.0 (48.0–227.0) n=11[a]	52.0 (36.0–133.0) n=7
Median (min–max) infusion frequency per week	2.3 (2.0–7.0) n=11[a]	3.0 (2.3–7.0) n=7
ITI outcome, n (%)		
Success[b]	1 (7.7)	0 (0)
Inhibitor negative[c]	5 (38.5)	0 (0)
Treatment ongoing	5 (38.5)	4 (57.1)
Terminated ITI[d]	2 (15.4)	3 (42.3)

ITI, immune tolerance induction; IU, International unit; max, maximum; min, minimum; rFVIIIFc, recombinant factor VIII Fc fusion protein.
[a] Data missing for 2 patients.
[b] ITI success was defined as repeated negative inhibitor status and incremental recovery ≥1.32 IU/dL (66%).
[c] Four inhibitor-negative patients are receiving ongoing rFVIIIFc ITI.
[d] Reasons for termination of ITI: First-time ITI — physician's decision and re-increase of inhibitor titer; rescue ITI — physician's decision, no decrease in inhibitor titer.

Methods: Study participants provided written informed consent, and study approval was provided by local Research Ethics Committees for all study sites. Baseline data were collected for patients with HA with inhibitors who received rFVIIIFc as first-time or rescue ITI. ITI regimen was at the discretion of the investigator. Follow-up data will be collected on treatment regimen, immune modulation, and use of bypass treatment. ITI success was defined as >1 negative inhibitor status and incremental recovery ≥1.32 IU/dL (66%).

Results: As of July 2019, data were available for 20 patients who received ITI with rFVIIIFc (13 first-time and 7 rescue ITI [≥1 prior ITI failure]; Table 1). Most patients had received prior rFVIII treatment; 1 patient had received prior rFVIIIFc treatment.

rFVIIIFc ITI regimens varied from 36 IU/kg three times/week to 227.3 IU/kg daily. One first-time ITI patient achieved ITI success and 5 first-time ITI patients were confirmed twice as having negative inhibitor status. Two first-time and 3 rescue ITI patients terminated ITI; 5 remaining first-time and 4 rescue ITI patients are receiving ongoing treatment (Table 2). No severe adverse events were reported.

Conclusions: Based on interim results from this real-world study in Japan, ITI with rFVIIIFc may offer beneficial outcomes for patients with HA with inhibitors.

PB0949 | Population Pharmacokinetic (PK) Analysis of BIVV001 (Rfviiifc-VWF-XTEN), a New Class of Factor VIII (FVIII) Replacement

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Background: While hemophilia A treatment goals are evolving towards functional cures and health equity (Skinner, *Haemophilia*. 2019), FVIII products that achieve optimal bleed protection with weekly dosing remain an unmet need. BIVV001, a new class of FVIII replacement, circulates independently of endogenous von Willebrand factor (VWF) and provides high sustained FVIII activity (Konkle, *Blood*. 2018; Lissitchkov, *Blood*. 2019).

Aims: To develop a population model that characterizes BIVV001 PK and to identify factors that determine BIVV001 PK variability in adults with severe hemophilia A.

Methods: FVIII activity measured via a one-stage clotting assay during two clinical trials (NCT03205163, N=15; EudraCT: 2018-001535-51, N=24) was used to assess BIVV001 PK. Population PK was evaluated by maximum likelihood nonlinear mixed-effects modeling. Forward inclusion and backward elimination processes evaluated covariate effects. Diagnostic plots and objective function evaluation guided model building and goodness-of-fit assessment.

Results: BIVV001 population PK was best fitted with a one-compartment model with two covariates: body weight on clearance and body weight and hematocrit on central volume. VWF concentration was not a covariate, consistent with BIVV001 clearance being endogenous VWF-independent. Error models included interindividual variability in clearance and central volume, correlation between both, and proportional and additive residual errors (Table 1).

TABLE 1 Parameter estimates for the BIVV001 population PK model[a]

Parameter	Estimate (95% CI)
Clearance, dL/hour	0.434 (0.396, 0.476)
Volume (central compartment), dL	27.6 (26.0, 29.3)
Allometric exponent of body weight on clearance	0.703 (0.416, 0.990)
Allometric exponent of body weight on central volume	0.513 (0.322, 0.704)
Exponent of hematocrit (fraction) on central volume	-0.568 (-0.974, -0.162)
Interindividual variability	
Interindividual variability clearance (%)	21.3 (15.1, 27.7)
Interindividual variability on central volume (%)	15.1 (8.81, 21.5)
Correlation of inter-individual variability clearance on central volume	0.741 (0.541, 0.942)
Residual variability	
Proportional error (fraction)	0.152 (0.140, 0.164)
Additive error, IU/dL	0.196 (0.115, 0.277)

aPTT, activated partial thromboplastin time; CI, confidence interval; PK, pharmacokinetic.
[a] Based on the one-stage (aPTT) clotting assay.

TABLE 2 Estimated time to FVIII activity levels at 50 IU/kg BIVV001 steady state in adult subjects with severe hemophilia A (n=9)[a]

FVIII activity	Time (hours)[b]	Time (days)[b]
10%	162.75 (148.43, 178.46)	6.78 (6.18, 7.44)
15%	137.48 (124.97, 151.25)	5.73 (5.21, 6.30)
20%	119.54 (108.22, 132.04)	4.98 (4.51, 5.50)
40%	76.18 (67.22, 86.35)	3.17 (2.80, 3.60)

aPTT, activated partial thromboplastin time; FVIII, factor VIII.
[a] Based on the one-stage (aPTT) clotting assay.
[b] Values are geometric mean (95% confidence interval).

Clinical trial simulations were conducted for various BIVV001 regimens to support Phase 3 prophylactic BIVV001 dose selection (once-weekly 50 IU/kg). BIVV001 PK modeling demonstrated high sustained geometric mean FVIII activity in the normal to near-normal range (≥40%) for 3.2 days, ≥20% for 5.0 days, and ~10% for 7 days post dose (Table 2).

Conclusions: Population PK analyses provided comprehensive quantitative characterization of BIVV001 activity-time profiles in adults with severe hemophilia A. Population PK modeling predicts high sustained FVIII activity in the normal to near-normal range for the first 3-4 days post dose to ~10% for one week after 50 IU/kg BIVV001.

PB0950 | Long-Term Safety and Efficacy of Emicizumab for up to >5 Years in a Phase 1/2 Study in Patients with Severe Hemophilia A

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Background: Safety and efficacy of emicizumab for up to 2.8 years were previously reported in a phase 1/2 study in patients with severe hemophilia A.[1]

Aims: To evaluate further long-term safety and efficacy of emicizumab.

Methods: Emicizumab was administered subcutaneously once weekly (QW) at maintenance doses of 0.3, 1, or 3 mg/kg with potential up-titration. Finally, all patients changed their doses to an approval maintenance dose of 1.5 mg/kg QW after Japanese regulatory approval. Bleeding episodes that occurred after dose modification were summarized to the group of modified dose when calculating annualized bleeding rates (ABRs).

Results: Patients (N=18: 7 patients without inhibitors and 11 patients with inhibitors) aged 12 to 58 years received emicizumab for a median (range) of 4.8 years (0.079 to 5.8 years). No new significant

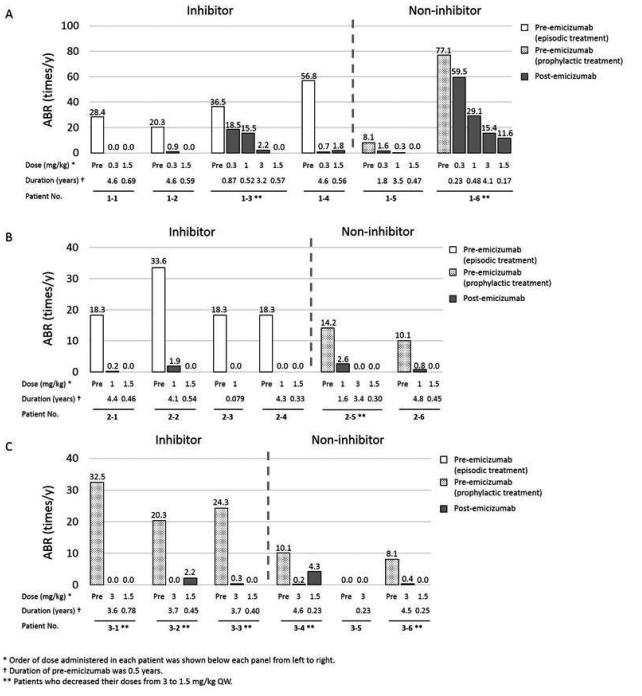

FIGURE 1 Individual ABRs during pre- and post-emicizumab for each patient with or without inhibitors. (A) 0.3 mg QW. (B) 1 mg/kg QW. (C) 3 mg/kg QW

safety-related findings were identified. No adverse events (AEs) which increased due to long-term treatment were identified. The majority of AEs were mild or moderate in intensity. The medians of ABRs for bleeding events treated with coagulation factors were low at 0.87, 0.41, and 0 during the 0.3, 1, and 3 mg/kg QW dosing periods, respectively. Out of 8 patients who decreased their doses from 3 to 1.5 mg/kg QW, ABRs decreased in 4 patients, remained at zero in 2 patients, and increased in 2 patients (*Figure*, *Patient No. with* **). The 2 patients with increased ABRs had one traumatic treated bleeding event during the 1.5 mg/kg QW dosing period. Breakthrough bleeding events (> 150 events) during emicizumab prophylaxis were managed with episodic treatment of either factor VIII or bypassing agents without thromboembolic events or thrombotic microangiopathy.

Conclusions: Favorable safety and efficacy of emicizumab were maintained for up to >5 years in patients with severe hemophilia A.

1. Shima et al., Blood Adv. 2017.

PB0951 | Females with Haemophilia in Nordic Countries Have a Higher Risk of Developing Pain, Anxiety and Depression Based on Treatment Patterns as Compared to Matched Controls: Data from a Registry Study over a Period of 11 Years

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Background: Females with haemophilia (FwH) including carriers may have increased risk of bleeds due to low factor levels and may be at risk of pain due to joint bleeds. Swedish MIND study data of carriers identified increased prescription of pain medication, neuroleptics and anti-depressants suggesting a need for further attention and research.

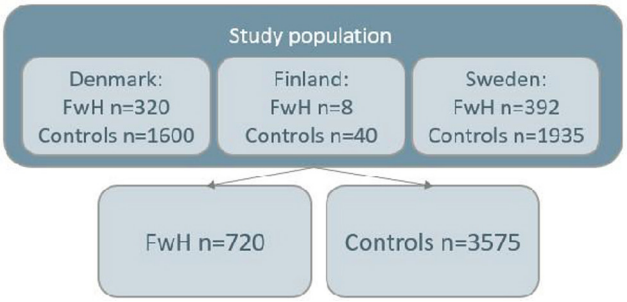

FIGURE 1 Study population

Aims: The MIND study (NCT03276130) aims to identify patterns of prescribed pain, anti-depressive and anti-anxiety medication and management of pain, depression and anxiety in people with haemophilia (PwH) in four Nordic countries, based on patient registry data and surveys. Here we describe registry data of current and retrospective patterns of prescribed medication from Denmark, Finland and Sweden focused on FwH, compared with the general female population.

Methods: This 11-year (2007-2017) retrospective population-based registry study, uses international classification systems for diagnoses, surgeries and medications. The study population includes females of all ages identified by diagnosis of haemophilia A or B, or at least one prescription of any factor VIII, IX, or bypassing agent. For each study subject, five age- and gender-matched controls were included.

Results: Data were extracted for 720 FwH (study population in figure 1). The probability of being prescribed analgesics, neuroleptics and anti-depressants in FwH compared to controls are shown in Table 1.

Conclusions: A substantially higher prescription of analgesics in Nordic FwH as compared to controls suggests a need for increased focus on bleed and pain management in FwH including improved bleed protection and prevention of, e.g. arthropathy. The increased prescription of neuroleptics in Denmark and Sweden and anti-depressants in Sweden indicates that anxiety and depression may be overrepresented in FwH and underscores the importance of identifying and managing the female haemophilia population including non-frequent health care users. The data suggests a need for high medical attention and further research to address knowledge gaps and unmet needs.

TABLE 1 Age-adjusted probability of analgesics, neuroleptics or anti-depressant prescriptions, and difference (%) in annual volume of medication*

	Denmark: FwH (n=320) vs Controls (n=1600)		Finland: FwH (n=8) vs Controls (n=40)		Sweden: FwH (n=392) vs Controls (n=1935)	
	OR (95% CI)	% diff (p-value) if use>0*	OR (95% CI)	% diff (p-value) if use>0*	OR (95% CI)	% diff (p-value) if use>0*
Opioids	**2.58 (1.97 to 3.39)**	**57.8 (0.003)**	**8.68 (2.24 to 33.65)**	85.6 (0.344)	**2.40 (1.93 to 2.99)**	-0.4 (0.981)
Non-opioids	**1.40 (1.10 to 1.80)**	17.9 (0.069)	**4.87 (1.92 to 12.33)**	25.8 (0.338)	**2.13 (1.74 to 2.60)**	**15.0 (0.007)**
NSAID	**0.77 (0.60 to 0.98)**	-3.4 (0.710)	1.86 (0.71 to 4.90)	**70.2 (0.002)**	1.17 (0.96 to 1.41)	**50.0 (<0.001)**
Steroids	**2.26 (1.56 to 3.27)**	**79.1 (<0.001)**	0.48 (0.14 to 1.71)	-69.8 (0.246)	**2.42 (1.86 to 3.16)**	**44.9 (0.004)**
Neuroleptics	**1.61 (1.17 to 2.21)**	18.2 (0.282)	1.95 (0.39 to 9.66)	29.2 (0.706)	**1.56 (1.24 to 1.95)**	-0.7 (0.952)
Anti-depressant	1.29 (0.92 to 1.81)	-9.6 (0.494)	0.60 (0.17 to 2.09)	**-157.3 (0.007)**	**1.41 (1.09 to 1.82)**	8.0 (0.573)
Anti-epileptics	1.24 (0.70 to 2.19)	**-61.3 (0.026)**	0.69 (0.13 to 3.83)	-166.2 (0.096)	1.59 (0.96 to 2.63)	-4.2 (0.855)

Numbers marked in bold shows significant values. *Percentage difference in annual volume of medication compared to controls, for women using the respective medications. OR=Odds ratio; CI=Confidence interval; FwH=Females with haemophilia; NSAID=Nonsteroidal anti-inflammatory drug.

PB0952 | First Interim Analysis of a 24-Month, Prospective, Non-Interventional, Multicentre Study in Germany Evaluating the Real-World Usage and Effectiveness of rFVIIIFc and rFIXFc in Patients with Haemophilia A or B (PREVENT)

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Background: The safety and efficacy of recombinant factor VIII Fc (rFVIIIFc) and recombinant factor IX Fc (rFIXFc) were demonstrated in pivotal Phase 3 trials in patients with severe haemophilia (PwH) A and B, respectively. The non-interventional PREVENT study provides data on the real-world usage and effectiveness of prophylactic treatment with rFVIIIFc and rFIXFc in Germany over a 24-month prospective period.

Aims: To report data from the first interim analysis of patients with at least 9 months prospective observation period from the ongoing PREVENT (NCT03055611) study in Germany.

Methods: PREVENT aims to enrol 200 PwH A and B from 25 centres. Previously treated patients who have been prescribed prophylactic treatment with rFVIIIFc or rFIXFc at enrolment are eligible. The primary endpoints are annualised bleeding rate (ABR), injection frequency and factor consumption. Additionally, secondary endpoints include reason for switch to rFVIIIFc or rFIXFc. Data are analysed employing descriptive statistics. Here, the analysis population includes all patients with at least one follow-up visit after 9 months from enrolment at the interim data cut, September 30th, 2019.

Results: The analysis population comprised 67 PwH A and 21 PwH B with a mean (SD) age of 26.2 (19.5) and 31.6 (20.7) years, respectively. Of these, 95.5% of PwH A and 76.2% of PwH B had severe haemophilia. The rFVIIIFc and rFIXFc treatment regimens remained unchanged in the majority of patients from enrolment until interim data cut (PwH A: 80.6%; PwH B: 57.1%). ABR, injection frequency, factor prescription, and treatment satisfaction are shown in table 1. The most common reason for switch was to reduce injection frequency while maintaining protection from bleeds (table 2).

Conclusions: The interim prospective analysis showed a low injection frequency while maintaining good protection from bleeds in PwH A and B treated with rFVIIIFc and rFIXFc prophylaxis, respectively. Physicians and patients were satisfied with rFVIIIFc/rFIXFc treatment.

TABLE 1 Prospective interim analysis data

	Haemophilia A (n = 67)[a]		Haemophilia B (n = 21)[a]	
	n		n	
ABR, median (Q1;Q3) mean (SD)	67	0.9 (0.0;2.4) 2.3 (6.2)	21	2.0 (0.6;3.1) 2.7 (3.1)
Joint ABR, median (Q1;Q3)	67	0.0 (0.0;1.3)	21	1.0 (0.0;2.1)
Weekly injection frequency, median (Q1; Q3)	66	2.2 (2.0;3.0)	21	1.0 (1.0;1.0)
Weekly dose (IU/kg/week), median (Q1;Q3)	63	85.8 (70.0;114.3)	20	41.8 (31.4;49.6)
Physicians satisfied/highly satisfied with treatment after 1 year, n (%)	53	46 (87)	17	15 (88)
Patients satisfied/highly satisfied with treatment after 1 year, n (%)[b]	24	23 (96)	9	9 (100)

ABR, annualised bleeding rate.
[a] For some of the outcomes data were not available for all patients at interim data cut.
[b] Completion of all patient reported outcomes was optional.

TABLE 2 Reason for switch to rFVIIIFc/rFIXFc

	Haemophilia A (n = 67)	Haemophilia B (n = 21)
Improve protection from bleeds, n (%)	23 (34.3)	2 (9.5)
Improve protection to increase physical activity level, n (%)	2 (3.0)	0 (0.0)
Reduce injection frequency while maintaining protection from bleeds, n (%)	42 (62.7)	19 (90.5)

rFVIIIFc, recombinant factor VIII Fc; rFIXFc, recombinant factor IX Fc

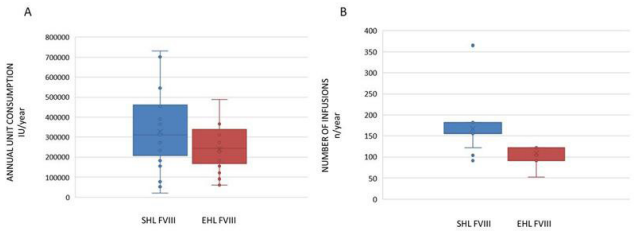

FIGURE 1. Annual unit consumption per patient/year (A) and number of infusions per patient/year (B) before and after switch to EHL (FVIII:Fc)

PB0953 | A Single Center Data on Extended Half-Life Recombinant Factor VIII (FVIII:Fc) in Patients with Hemophilia A

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Background: Hemophilia A (HA) is a congenital Factor VIII (FVIII) deficiency and replacement treatment using recombinant coagulation FVIII is the gold-standard therapeutical approach. Over the last few years, many patients have been switched from standard recombinant products (SHL) to extended half-life recombinant factors (EHL). However, it remains unclear whether this switching approach actually reduces the number of infusions per year without increasing annual bleeding events and subsequent need for supplementary factor infusions.

Aims: to compare the number of infusions, bleeding episodes and annual FVIII IU consumption of HA patients on prophylaxis before and after switching from SHL to EHL.

Methods: we included patients on prophylaxis referring to our hemophilia center with severe/moderate HA who had been switched from SHL to EHL (FVIII:Fc). We excluded all "on demand" patients and previously untreated patients (PUPs) from the analysis. Variables are expressed as median and interquartile range (IQR). Wilcoxon test for paired groups was used ($p < 0.05$ statistically significant).

Results: twenty-nine patients with HA, median age 25 years [IQR 19-37, min-max 6-71], 93% with a severe disease, fulfilled the inclusion criteria and were included in the analysis. The median number of annual infusions was 156 per patient [IQR 156-182] before switching and decreased to 122 infusions per patient [IQR 91-122, $p = 0.0001$] after switching to EHL, with a reduction of 22% of total treatments. Median annual unit consumption before switching was 312.000 IU per patient [IQR 208.000-461.500] and decreased to 244.000 IU per patient [IQR 169.000-339.000, $p < 0.002$] after switch to EHL. The mean annual bleeding rate was reduced from 0.65 to 0.13 after the switch to EHL.

Conclusions: the use of the only available EHL product (FVIII:Fc) led to a reduced number of annual FVIII infusions and unit consumption per patient together with an improvement of the clinical outcomes.

PB0954 | Abdominal Catastrophe in Hemophilic Patients: Towards Wise Decision Making

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Kasr Alainy Faculty of Medicine, Cairo University, Internal Medicine, Cairo, Egypt

Background:

- Hemophilia A is a hereditary hemorrhagic disorder that can cause intra-abdominal bleeding.
- Careful assessment of coagulation must be performed in these patients.
- The administration of the deficient factor is sufficient to resolve the problem, avoiding unnecessary surgeries.

Aims: The aim of the study is to evaluate the efficacy and safety of non-surgical interventions in Hemophilia A patients presenting acute abdominal emergencies.

Methods: This study included 23 Hemophilia A patients (without inhibitors) presenting with acute abdominal pain. All patients were assessed clinically, and unstable patients were admitted in the intensive care unit.

Laboratory workup in the form of CBC, coagulation profile and biochemical profile were done. Imaging workup in the form of Ultrasonography, CT, and MRI were done to evaluate the site, size and extent of the intraabdominal hematomas and to assess the needs for intervention.

Results: Nineteen (82.6%) patients were admitted in the regular medical ward under careful observation, while 4 (17.4%) were admitted to the ICU. Nineteen patients (82.6%) required blood transfusion. Eleven (47.8%) patients had more than one hematoma while 12 (52.2%) patients had single hematoma.

Eighteen (78.2%) iliopsoas hematomas; four (17.3%) retroperitoneal collections: Eight (34.7%) pelvic collections and two (8.7%) perinephric hematomas were encountered.

Ultrasound guided aspiration was done repeatedly in allpatients; total number of procedures were 80. Ultrasound guided pigtail insertion was done in seven patients (30.43%).

Twenty (86.9%) patients underwent complete recovery and were discharged; while three (13.04%) mortalities were encountered due to other comorbidities apart from abdominal catastrophes.

Conclusions: Non-surgical management of Hemophilia A patients presenting with abdominal emergencies could be both safe and effective and it is needed to avoid the problems related to rushed surgical decision or the reluctant surgical behavior specially of limited availability of factor replacementespecially in this high-risk group of patients.

PB0955 | Treatment-Based Risk Assessment of Developing Pain, Anxiety, and Depression as Compared to Matched Controls in People with Haemophilia. A Nordic Registry Study Over a Period of 11 Years

K. Steen Carlsson[1,2]; B. Winding[3]; J. Astermark[4]; F. Baghaei[5]; E. Brodin[5]; E. Funding[6]; M. Holmström[7]; K. Österholm[8]; S. Bergenstråle[3]; E. Andersson[1]; S. Lethagen[3,9]
[1]The Swedish Institute for Health Economics, Lund, Sweden; [2]Lund university, Lund, Sweden; [3]Swedish Orphan Biovitrum AB, Stockholm, Sweden; [4]Skåne University Hospital, Malmö, Sweden; [5]Sahlgrenska University Hospital, Gothenburg, Sweden; [6]Rigshospitalet, Copenhagen, Denmark; [7]Karolinska University Hospital, Stockholm, Sweden; [8]Helsinki University Hospital, Helsinki, Finland; [9]Copenhagen University, Copenhagen, Denmark

Background: People with haemophilia (PwH) have an increased risk of bleeds associated with acute and chronic pain, and long-term disability. In addition, anxiety and depression are other determinants negatively affecting quality of life in PwH. Despite the severity of these co-morbidities, they have not been extensively investigated and associations between them even less.

Aims: The MIND study (NCT03276130) aims to identify patterns of prescribed pain, anti-depressive and anti-anxiety medication and management of pain, depression and anxiety in PwH in four Nordic countries, based on patient registry data and surveys. Here we describe registry data of current and retrospective treatment patterns of prescribed medication in PwH from Denmark, Finland, and Sweden compared with the general population.

Methods: This 11-year (2007-2017) retrospective population-based registry study uses international classification systems for diagnosis, surgeries and medicines. In Denmark, Norway and Sweden, the study populations were identified by diagnosis of haemophilia A or B, and or by one prescription of any factor VIII, IX, or bypassing agent. In Finland, the study population was identified by their eligibility for purchasing factor concentrate. For each study subject, five age- and gender-matched control subjects were included, except for Norway (due to local regulations).

Results: Registry data were extracted for a total of 3388 PwH. The country study populations are shown in figure 1. Treatment patterns of PwH compared to controls are shown in Table 1.

Conclusions: There was a significantly higher prescription of analgesics in all PwH cohorts compared to controls in the Nordic countries, which suggest a need for improved bleed protection to prevent arthropathy. In addition, there was a significantly higher prescription of neuroleptics, anti-depressants and anti-epileptics in the majority of PwH cohorts compared to controls, which underscores the importance of applying a holistic and multidisciplinary approach when managing PwH to also identify and address anxiety and depression.

TABLE 1 Age-adjusted use of different medication types by country and factor consumption

	Denmark:		Finland:		Sweden:	
	High factor consumption[a] (n = 221) vs controls[c] OR (95% CI)	Low factor consumption[b] (n = 554) vs controls[c] OR (95% CI)	High factor consumption[a] (n = 200) vs controls[c] OR (95% CI)	Low factor consumption[b] (n = 239) vs controls[c] OR (95% CI)	High factor consumption[a] (n = 419) vs controls[c] OR (95% CI)	Low factor consumption[b] (n = 1016) vs controls[c] OR (95% CI)
Opioids	**6.57 (4.69 to 9.19)**	**2.33 (1.89 to 2.88)**	**5.64 (3.75 to 8.50)**	**4.33 (3.17 to 5.91)**	**4.70 (3.69 to 6.00)**	**2.51 (2.17 to 2.90)**
Non-opioids	**3.43 (2.45 to 4.79)**	**1.64 (1.34 to 2.02)**	**3.35 (2.35 to 4.79)**	**2.84 (2.11 to 3.82)**	**5.64 (4.44 to 7.17)**	**2.19 (1.89 to 2.54)**
NSAID	1.14 (0.81 to 1.60)	**0.71 (0.59 to 0.87)**	**1.48 (1.10 to 2.00)**	1.06 (0.83 to 1.36)	**3.20 (2.59 to 3.96)**	**1.26 (1.09 to 1.45)**
Steroids	1.26 (0.68 to 2.33)	**1.77 (1.30 to 2.42)**	1.01 (0.52 to 1.97)	1.02 (0.56 to 1.88)	**3.00 (2.28 to 3.96)**	**1.62 (1.32 to 1.97)**
Neuroleptics	1.36 (0.83 to 2.22)	**1.65 (1.29 to 2.12)**	**1.97 (1.11 to 3.50)**	1.27 (0.79 to 2.04)	**2.08 (1.55 to 2.80)**	**1.61 (1.36 to 1.92)**
Anti-depressant	**2.26 (1.34 to 3.81)**	**1.48 (1.09 to 2.01)**	0.78 (0.39 to 1.56)	0.95 (0.55 to 1.63)	1.51 (1.00 to 2.30)	**1.30 (1.03 to 1.62)**
Anti-epileptics	2.01 (0.99 to 4.08)	0.80 (0.48 to 1.34)	**3.08 (1.27 to 7.47)**	0.69 (0.13 to 3.83)	**2.87 (1.65 to 5.00)**	**1.84 (1.31 to 2.58)**

Numbers marked in bold shows significant values. [a]Definition of high factor consumption: Total filled prescriptions during one year corresponding to FVIII ≥ 40 IU/kg/ week and FIX ≥ 10 IU/kg/week, respectively. Not included if factor use > 350 IU/kg/week. [b]Definition of low factor consumption: Men with haemophilia not meeting high factor consumption definition. [c]Each person with haemophilia was matched with five age- and gender-matched controls. OR=Odds Ratio; CI=Confidence Interval; NSAID=Nonsteroidal anti-inflammatory drug.

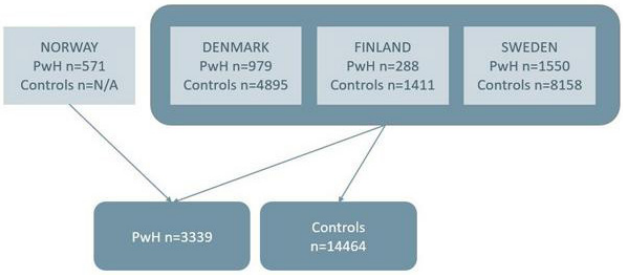

FIGURE 1 Country study populations. Norwegian regulations did not allow for age and gender-matched subjects to be included. PwH; People with haemophilia

PB0956 | Final Results of PUPs B-LONG Study: Evaluating Safety and Efficacy of rFIXFc in Previously Untreated Patients with Haemophilia B

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Background: This is the first study of recombinant factor IX Fc fusion protein (rFIXFc) for prevention and treatment of bleeds in previously untreated patients (PUPs) with haemophilia B.

Aims: To evaluate the safety and efficacy of rFIXFc in PUPs.

Methods: In this open-label, multicentre, multinational, Phase 3 study (NCT02234310), male PUPs aged <18 years with haemophilia B (≤2 IU/dL endogenous FIX) were to receive prophylaxis with rFIXFc. Investigators could treat patients episodically prior to initiating prophylaxis. Primary endpoint was occurrence of inhibitor development. Secondary endpoints included annualized bleeding rate (ABR) and assessment of response to treatment of bleeding episodes with rFIXFc.

Results: Of 33 patients enrolled, 26 (79%) were <1-year old; 6 (18.2%) had a family history of inhibitors, 28 (84.8%) received prophylaxis (17 (51.5%) switched from episodic treatment); 5 (15.2%) received episodic treatment only. Twenty-seven (81.8%) patients completed the study. Twenty-one (63.6%), 26 (78.8%), 28 (84.8%) patients had ≥ 50, ≥20, ≥10 exposure days (EDs) to rFIXFc during the study, respectively. One patient on prophylaxis developed a low-titre inhibitor (<5.00 BU/mL) after 11 EDs; rate of inhibitor development was 3.0% (1/33 patients). Twenty-three (69.7%) patients had 58 treatment-emergent serious adverse events (TESAEs); 2 were assessed as treatment-related (FIX inhibition and hypersensitivity in 1 patient, resulting in withdrawal). Median ABR (prophylaxis) was 1.2 (Table). Median number of rFIXFc injections required to resolve a bleeding episode was 1 (Table). Response to bleeding episode treatment was rated as excellent/good for 100% of 22 injections in the episodic treatment group and 87.7% of 50 injections in the prophylaxis treatment group.

Conclusions: The study population was representative of PUPs with severe haemophilia B. Prophylaxis and treatment of bleeding episodes with rFIXFc was highly effective and generally well tolerated, without unanticipated safety findings; type and incidence of TESAEs were similar to those expected for the paediatric haemophilia population.

TABLE 1 Annualized bleeding rate and number of injections required for resolution of a bleeding episode

Treatment		Episodic (n = 22*)	Prophylactic (n = 28*)
rFIXFc dosing	Median average weekly dose, IU/kg (range)	N/A	57.96 (47.0-233.9)
	Median average dose interval, days (range)	N/A	7 (3.3-14.6)
Annualized bleeding rate (ABR)	Overall, median (IQR)	0.21 (0.00, 5.00)	1.24 (0.00, 2.49)
	Spontaneous, median (IQR)	0.00 (0.00, 2.26)	0.00 (0.00, 0.00)
	Spontaneous joint, median (IQR)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
Number of injections required for resolution of a bleeding episode†	Median (IQR)	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)

ABR, annualized bleeding rate; IQR, interquartile range (25th, 75th percentile). ABR is the total number of bleeding episodes during the efficacy period extrapolated to a 1-year interval of time for all subjects in the efficacy period, regardless of duration in the efficacy period. *Number of patients with an efficacy period. Efficacy period reflects the sum of all intervals of time during which patients are treated with rFIXFc according to the treatment regimens of the study excluding surgical/rehabilitation periods and large injection intervals (>28 days). Patients are included in each treatment regimen they participated in for the duration of time on that regimen and as such may appear in more than one treatment regimen. †The majority of bleeding episodes required ≤ 2 injections to resolve: 88.9% and 96.6% for the episodic and prophylactic regimens, respectively.

PB0957 | A Randomized, Multicenter, Open-label, Phase III Clinical Trial to Evaluate the Efficacy, Safety, and Pharmacokinetics of Prophylactic Emicizumab Versus No Prophylaxis in Persons with Hemophilia A in the Asia-Pacific region (HAVEN 5)

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Background: Emicizumab bridges activated factor IX (FIXa) and FX to restore hemostasis in persons with hemophilia A (PwHA), and is widely approved for prophylaxis in PwHA with/without FVIII inhibitors.

Aims: To present the efficacy, safety and pharmacokinetics of emicizumab from the Phase III HAVEN 5 (NCT03315455) trial in PwHA in the Asia-Pacific region.

Methods: Following informed consent and ethics committee approval, PwHA (≥12 years, with/without FVIII inhibitors) were randomized 2:2:1 to receive subcutaneous (SC) emicizumab prophylaxis: 1.5 mg/kg once-weekly (QW), Arm A; 6 mg/kg once every four weeks (Q4W), Arm B, or; no prophylaxis, Arm C. Loading doses were administered for the first four weeks (3 mg/kg QW SC emicizumab; Arms A and B). The primary endpoint was annualized bleeding rate (ABR) for treated bleeds, calculated using a negative binomial-regression model. Secondary endpoints included additional bleeding-related endpoints, adverse events (AEs) and pharmacokinetic analyses.

Results: At clinical cut-off (06/21/2019), 70 participants aged 12-66 (median 29.0) years were enrolled and had completed 24 weeks of treatment. Significant reductions in treated bleed ABRs of 96% were observed with emicizumab prophylaxis (Arm: A, 1.0; B, 1.0) versus no prophylaxis (Arm C, 27.0; $p < 0.0001$; Table 1). In Arms A and B, 65.5% (19/29) and 55.6% (15/27) of participants had zero treated bleeds respectively, versus 7.1% (1/14) of participants in Arm C. Efficacious mean emicizumab trough plasma concentrations > 30 μg/mL were sustained from Week 5. Emicizumab was well tolerated; the most common AEs were upper respiratory tract infection (Arm: A, 31.0%; B, 18.5%; C, 14.3%) and injection site reaction (Arm: A, 13.8%; B, 18.5%; C, 0.0%). No fatalities, thrombotic microangiopathies or thrombotic events were reported. Eight participants developed treatment-induced anti-drug antibodies; one with transient neutralizing potential.

Conclusions: Emicizumab QW or Q4W prophylaxis offers a highly efficacious, well tolerated, flexible option for PwHA from the Asia-Pacific region.

TABLE 1 Duration of Exposure and ABRs in the HAVEN 5 Study

	Arm A 1.5 mg/kg emicizumab prophylaxis QW (n=29)	Arm B 6 mg/kg emicizumab prophylaxis Q4W (n=27)	Arm C No prophylaxis (n=14)
Median duration of exposure to emicizumab*, weeks (range)	43.1 (28.1–60.1)	44.1 (20.1–56.6)	–
Treated bleeds†			
ABR‡, (95% CI)	1.0 (0.5–1.9)	1.0 (0.5–1.8)	27.0 (13.3–54.9)
ABR reduction, p-value§	96%, p<0.0001	96%, p<0.0001	–
ABR, median (IQR)	0.0 (0.0–1.2)	0.0 (0.0–2.3)	45.3 (21.7–70.5)
Participants with zero bleeds, %	65.5	55.6	7.1
Participants with 0–3 bleeds, %	86.2	92.6	7.1
All bleeds			
ABR‡, (95% CI)	1.9 (1.2–3.0)	2.1 (1.3–3.3)	41.1 (26.4–64.2)
ABR, median (IQR)	1.5 (0.0–4.2)	1.9 (0.0–5.6)	56.7 (26.1–70.8)
Treated spontaneous bleeds			
ABR‡, (95% CI)	0.4 (0.2–1.0)	0.5 (0.2–1.1)	23.6 (9.3–60.0)
ABR, median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–1.0)	21.8 (6.5–52.2)
Treated joint bleeds			
ABR‡, (95% CI)	0.7 (0.4–1.5)	0.6 (0.3–1.2)	17.7 (8.3–37.6)
ABR, median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–1.4)	10.9 (8.7–50.0)
Treated target joint bleeds			
ABR‡, (95% CI)	0.4 (0.2–1.1)	0.3 (0.1–0.9)	8.6 (3.2–23.4)
ABR, median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–1.1)	6.5 (0.0–19.7)

*Duration of exposure is the date of the last dose of study medication minus the date of the first dose plus one day.
†Treated bleeds defined as a bleed followed by treatment for a bleed. Bleeds due to surgery/procedure are excluded.
‡Calculated using a negative binomial regression model.
§ABR reduction ratio for Arm A versus Arm C and Arm B versus Arm C. p-values are obtained via a global model with a 3-level categorical effect for treatment.
ABR, annualized bleed rate; CI, confidence interval; IQR, interquartile range; QW, once weekly; Q4W, once every 4 weeks.

PB0958 | Second Interim Analysis Results from the STASEY Trial: A Single-Arm, Multicenter, Open-Label, Phase III Clinical Trial to Evaluate the Safety and Tolerability of Emicizumab Prophylaxis in People with Hemophilia A (PwHA) with FVIII Inhibitors

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Background: Emicizumab, a subcutaneously administered, bispecific monoclonal antibody, bridges activated factor (F)IX and FX, replacing the function of missing activated FVIII in PwHA, thereby restoring hemostasis.

Aims: To provide updated interim results from STASEY (NCT03191799), a study of emicizumab prophylaxis in PwHA with FVIII inhibitors.

Methods: Following ethics committee approval and informed consent, PwHA aged ≥ 12 years with FVIII inhibitors (N = 195; intent-to-treat population) received emicizumab 3 mg/kg/week for 4 weeks, followed by 1.5 mg/kg/week for the remainder of 2 years. The primary objective was safety (adverse events [AEs], including thromboembolic events [TEs], hypersensitivity); secondary objectives included efficacy (annualized bleed rates [ABRs]).

Results: At cut-off (May 20, 2019), 193 PwHA had received emicizumab and were evaluable for safety; median age (range) was 28.0 (12-80) years; median (range) treatment duration was 50.9 (1.1-88.1) weeks. Emicizumab was well tolerated (Table 1). Two AEs were classed as TEs: ST-elevation myocardial infarction (STEMI) (n = 1); hypertrophic clot following tooth extraction caused by multiple doses of antifibrinolytic combined with rFVIIa (n = 1). The 55-year-old PwHA with STEMI had several risk factors, including a history of smoking, hypertension and family history of coronary heart disease. He did not receive bypassing agents and continued emicizumab without dose adjustment; the treating physician assessed the event as unrelated to emicizumab. Emicizumab-related AEs were reported in 33 PwHA; injection-site reactions were most common (22/193 PwHA; 11.4%). One fatality was reported (polytrauma), assessed as unrelated to emicizumab. Three PwHA received activated prothrombin complex concentrate and 32 received rFVIIa, with no associated thrombotic microangiopathy or arterial/venous TEs. Ten PwHA (5.2%) developed anti-drug antibodies, none with neutralizing potential (by pharmacokinetic/clinical assessment); they continued on 1.5 mg/kg/week emicizumab with no increased bleeding. ABRs remained low (Table 2).

Conclusions: No new safety signals were identified; these data confirm the safety results from the HAVEN clinical program.

TABLE 1 Safety Summary (Safety-Evaluable Population)

Adverse Event (AE), n (%)	Emicizumab 1.5 mg/kg/week n = 193
Total number of AEs	**551**
Number of people with hemophilia A (PwHA) with ≥ 1 event	**145 (75.1)**
Fatal AE	1 (0.5)*
Serious AE	19 (9.8)
AE leading to treatment withdrawal	1 (0.5)
AE leading to dose modification or interruption	3 (1.6)
AE leading to study discontinuation	0
Grade ≥ 3 AE	22 (11.4)
Study treatment-related AE	33 (17.1)
Injection-site reaction	22† (11.4)
AEs of interest	
Systemic hypersensitivity/anaphylactic/anaphylactoid reaction	0
Thromboembolic event (TE)	2 (1.0)‡
TE associated with activated prothrombin complex concentrate (aPCC) and emicizumab	0
Thrombotic microangiopathy (TMA)	0
TMA associated with aPCC and emicizumab	0
Most common AEs (≥10% of PwHA)	
Nasopharyngitis	24 (12.4)
Headache	23 (11.9)
Injection-site reaction	22† (11.4)

*Polytrauma with fatal head injury, unrelated to emicizumab, previously reported in first interim analysis.

† One injection-site reaction was manually added, as the AE page may have been incorrectly completed (currently being queried).

‡ ST-elevation myocardial infarction due to thrombus in a coronary artery in one PwHA, and hypertrophic clot at site of tooth extraction in one PwHA who was receiving anti-fibrinolytics. The latter is a known complication of tooth extractions.

Coded using Medical Dictionary for Regulatory Activities (MedDRA) Version 21.1.

TABLE 2 Efficacy Summary (Intent-to-Treat Population)

Annualized bleed rate (ABR)*	Emicizumab 1.5 mg/kg/week N = 195
Treated bleeds†	
Mean ABR, model based‡ (95% confidence interval [CI])	0.5 (0.27-0.86)
Median ABR, calculated (interquartile range [IQR])	0.0 (0.00-0.00)
People with HA (PwHA) with zero bleeds, n (%)	167 (85.6)
All bleeds	
Mean ABR, model based‡ (95% CI)	1.3 (0.95-1.80)
Median ABR, calculated (IQR)	0.0 (0.00-1.14)
PwHA with zero bleeds, n (%)	121 (62.1)
Treated spontaneous bleeds†	
Mean ABR, model based‡ (95% CI)	0.3 (0.13-0.67)
Median ABR, calculated (IQR)	0.0 (0.00-0.00)
PwHA with zero bleeds, n (%)	179 (91.8)
Treated joint bleeds†§	
Mean ABR, model based‡ (95% CI)	0.3 (0.12-0.84)
Median ABR, calculated (IQR)	0.0 (0.00-0.00)
PwHA with zero bleeds, n (%)	182 (93.3)
Treated target joint bleeds†	
Mean ABR, model based‡ (95% CI)	0.2 (0.06-0.68)
Median ABR, calculated (IQR)	0.0 (0.00-0.00)
PwHA with zero bleeds, n (%)	186 (95.4)

*The Bleed and Medication Questionnaire was completed by participants/caregivers via an electronic handheld device. Bleed definitions were based on International Society on Thrombosis and Haemostasis criteria (Blanchette VS, et al., J Thromb Haemost 2014;12:1935-39).

† Treated bleeds were defined as a bleed directly followed by a hemophilia medication reported as a treatment for bleed, without an intervening bleed and irrespective of the time between the treatment and the preceding bleed. If multiple bleeds occurred on the same calendar day, the subsequent treatment was considered to apply to each of these multiple bleeds. Bleeds due to surgery/procedure are excluded.

‡ Calculated using negative binomial regression method.

§ Joint bleeds are defined as bleeds with type reported at 'joint' in combination with ≥ 1 of the following symptoms: increased swelling or warmth of the skin over the joint; increasing pain; decreased range of motion or difficulty in using the joint compared with baseline.

PB0960 | Safety and Efficacy of FVIII Replacement with Turoctocog Alfa in the UK

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Background: There are limited comparative safety and efficacy data on Turoctocog alfa in routine clinical practice.

Aims: To investigate adverse events (AEs), and within-patient change in clotting factor consumption (CFC), annualised bleed rate (ABR) and annualised joint bleed rate (AJBR) in patients who switched to turoctocog alfa in the UK.

Methods: Treatment between 01/04/2017 and 30/6/2019 of UK males with severe haemophilia A (FVIIII level < 1%) who switched exclusively to turoctocog alfa with ≥ 6 months' post-switch treatment data was examined. A within-patient comparison was made of ≥ 6 months' treatment data before and after switching. ABR and AJBR were analysed in Haemtrack-compliant patients. Exclusions included inhibitors and clinical trial participation. Age was calculated at study midpoint. Within-patient changes were tested using the Wilcoxon signed-rank test. All AEs reported by December 2019 were examined.

Results: The median within-patient post-switch increase in CFC was 7.7% ($p < 0.0005$, Table 1), in line with total FVIII usage in the UK which increased by 5.7% in children and 12.8% in adults in the same time period. The median (IQR) ABR and AJBR in 130 patients post-switch were 2.3 (0.0-6.1) and 1.5 (0.0-3.8) respectively. Within-patient comparison showed that ABR and AJBR were unchanged after switching in adults and children (Table 2). There were five AEs reported: One inhibitor in a PTP (5.8 Bethesda units), one malignancy, one poor efficacy event and two allergy events. In all cases except the malignancy, the patient stopped using turoctocog alfa. There were seven deaths, including three related to bleeding but not caused by a lack of efficacy of turoctocog alfa.

Conclusions: Within-patient comparison showed that switching to turoctocog alfa was associated with increased CFC in line with all FVIII products in the UK. There was no change in ABR or AJBR. Turoctocog alfa has similar efficacy to previous FVIII product in adults and children.

TABLE 1 Within-patient comparison of pre- and post-switch to turoctocog alfa FVIII usage in non-inhibitor haemophilia A patients

Age (years)	Patients (n)		Pre-switch	Post-switch	Within-patient change (%)
Under 18 years	41	Median	89.9	106.2	9.7
		Interquartile range	(64.3, 120.2)	(72.8, 130.3)	(-14.8, 51.9)
		Range	[14.9, 197.5]	[34.7, 290.7]	[-42.3, 408.3]
18 years and above	210	Median	67.2	75.2	6.7
		Interquartile range	(41.8, 93.9)	(50.9, 100.0)	(-15.2, 40.40)
		Range	[2.2, 250.0]	[0.0, 238.7]	[-100.0, 4337.5]
Total	251	Median	69.6	79.6	7.7 ($p < 0.0005$)
		Interquartile range	(44.8, 98.2)	(54.2, 106.6)	(-15.2, 46.3)
		Range	[2.2, 250.0]	[0.0, 290.7]	[-100.0, 4337.5]

TABLE 2 Pre- and post-switch to turoctocog alfa comparison of bleed rates in non-inhibitor haemophilia A patients

Age (years)	Patients (n)		Pre-switch	Post-switch	Within-patient change
Under 18 years	23	Median	1.1	1.1	0.0
		Interquartile range	(0.0, 2.1)	(0.0, 4.0)	(-0.7, 1.3)
		Range	[0.0, 22.2]	[0.0, 20.0]	[-2.1, 7.7]
18 years and above	85	Median	1.9	2.3	0.0
		Interquartile range	(0.0, 8.1)	(0.0, 5.8)	(-1.1, 1.5)
		Range	[0.0, 32.3]	[0.0, 35.5]	[-16.7, 12.3]
Total	108	Median	1.4	2.1	0.0 ($p = 0.70$)
		Interquartile range	(0.0, 6.7)	(0.0, 5.8)	(-1.1, 1.5)
		Range	[0.0, 32.3]	[0.0, 35.5]	[-16.7, 12.3]

PB0961 | Implication of Weight Excess in Hemophilic Arthropathy. The Results of a Colombian Reference Center

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Background: Hemophilia causes spontaneous bleeding in joints leading to a greater tendency to immobility and arthropathy. Decreased mobility implies lower physical activity and a sedentary lifestyle predisposing to an excess of weight (EOW). Weight excess is associated with loss of joint mobility, chronic pain and carries out a vicious cycle that generates susceptibility to arthropathy. Few data have been reported from middle-income countries.
Aims: To evaluate the implication of EOW in hemophilic arthropathy.
Methods: Subjects over five years old with a diagnosis of hemophilia A or B were included from a tertiary referral center in Bucaramanga, Colombia. Participants' height and weight were measured to calculate body mass index (BMI). For children (< 16 years old), EOW was defined by WHO nutritional charts, whereas for adults, EOW was defined as a BMI ≥ 25 kg/m^2. The association between independent variables and the presence of arthropathy was explored using the Chi-square test. A significance level of 5% was determined at two tails.
Results: Of 59 people with hemophilia (PWH), 51 (86.4%) had hemophilia A, and 8 (13.5%) had hemophilia B; 15 (25.3%) were classified as mild, 11 (18.6%) as moderate, and 33 (55.9%) as severe hemophilia; 41 (69.5%) had arthropathy, 13 (22%) had one joint compromise, and 28 (47.4%) had multiple joint compromises. The most frequently affected joint was knee (47.4%), followed by the ankle (38.9%) and elbow (33.9%). Mean BMI was 22.4 ± 4.84; 26 PWH (44.0%) had EOW. Those with EOW had a higher prevalence of one or multiple arthropathies compared with those with a normal weight (53.5% vs. 46.3% p = 0.025).
Conclusions: Prevalence of EOW is similar to the reported in high-income countries. Arthropathy was significantly higher in subjects with EOW; therefore, it is necessary to establish strategies for weight management, taking into account the specific characteristics of PWH.

PB0962 | Adverse Event Reporting in Haemophilia A Patients with and without Inhibitors Treated with Emicizumab: A Report from the Co-morbidity Working Party of the UK Haemophilia Centres Doctors' Organisation (UKHCDO)

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Background: Emicizumab has been prescribed in the UK for haemophilia A (HA) with inhibitors since February 2018 and for severe HA without inhibitors since July 2019. Long term safety has yet to be established.

Aims: A descriptive analysis of adverse events reported to the National Haemophilia Database (NHD) in UK patients treated with Emicizumab, February 2018 - January 2020.

Methods: UK haemophilia centres are required to report adverse events to the NHD. These are evaluated by the UKHCDO Co-morbidity Working Party. A descriptive review of events reported in Emicizumab treated patients is presented.

Results: There were 101 inhibitor and 71 non-inhibitor HA patients treated with Emicizumab for a median (IQR) of 13.1 (4.6-194); total 969 months and a median 2.0 (1.3-2.7), total 1080 months, respectively. Thrombotic microangiopathy and anti-drug antibodies were not reported. Three possible thrombotic events and two deaths were reported, described in tables 1 and table 2, respectively. Eight (4.5%) patients reported skin reactions: mild localised reactions in five (2.9%): systemic rash in two and increasingly severe, recurrent reactions leading to cessation of Emicizumab in one (0.6%). Small joint arthralgia was reported in two (1.2%) and GI symptoms in three (1.7%). Five (2.9%) reported headache occurring soon after dosing: mild and self-limiting in two; more severe requiring imaging in a further two and severe enough to require hospitalisation and drug cessation in one. Other reports included appendicitis (n = 1) and osteonecrosis (n = 1).

Conclusions: Continued follow-up is required to establish long-term safety, particularly in relation to thrombotic events. Headaches severe enough to require imaging are reported for the first time. Robust pharmacovigilance is required for all HA treatments to avoid reporting bias.

TABLE 1 Description of possible thrombotic events

Case	Age (years)	Event	Description	Contributory Risk Factors	Continued Emicizumab
1	78	NSTEMI*	Chest pain after first dose of Emicizumab associated with fever and hypotension. Troponin 227 ng/l, ECG normal. Angiography confirmed triple vessel disease but no acute thrombus.	Severe symptomatic IHD and port infection	Yes: without recurrent symptoms
2	53	Chronic SMA**thrombosis	Chronic SMA thrombosis identified on CT scan performed for long standing abdominal pain 15 months after starting Emicizumab. Collaterals were identified	Atherosclerosis with aortic calcification	Yes: without recurrent symptoms
3	32	Atypical cardiac event	Chest pain after third dose of Emicizumab associated with raised troponin > 5000 ng/l and non-specific ECG changes. Normal CT angiography.	-	Stopped pending further investigation

*Non ST elevation myocardial infarction, **superior mesenteric artery

TABLE 2 Causes of death

Case	Age	Cause of Death	Ante-mortem duration of Emicizumab treatment
1	51	Multi-organ failure complicating viral infection	6 months
2	27	Intra-abdominal bleeding whilst on Emicizumab prophylaxis, with significantly delayed presentation to hospital, hypovolaemic shock and multiorgan failure	6 months

PB0963 | One-Stage versus Chromogenic Factor IX Activity in Haemophilia B

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Background: Discrepancy between one-stage (OSA) and chromogenic factor activity assays (CSA) in haemophilia A is a well-known phenomenon caused by specific genetic variants. The laboratory and clinical relevance of Factor IX (FIX) assay discrepancy in persons with haemophilia B (PwHB) is less described.

Aims: To characterize one-stage and chromogenic FIX activity (FIX:C) in PwHB.

Methods: Samples from PwHB (N = 21; 16 families with moderate or mild phenotype) were included. OSA FIX:C was determined by using Dade ActinFSL (Siemens) on BCS-XP and Sysmex CS-2100i instruments (Siemens); CSA by Biophen Factor IX (Hyphen BioMed) and Rox Factor IX (Rossix) on BCS-XP. The *F9* gene variants were previously genotyped. The study was approved by the regional ethical committee.

Results: Median OSA FIX:C was 0.15 IU/mL (IQR, 0.06 - 0.24). Type I (quantitative) deficiency was present in four PwHB; 14 had type II (qualitative) deficiency, consistent with missense mutations; and unknown in three (confirmed by antigen levels in eleven). Bland-Altman plots revealed that FIX:C in OSA was systematically higher than CSA. This discrepancy was not found among 29 healthy controls. The median ratio of OSA/CSA (Hyphen) among PwHB was 2.5 (IQR, 1.9 - 4.0). Repeated measurement of stored aliquots yielded similar results and consistent assay discrepancy. Consistent with observations in PwHB, wild-type FIX levels below 0.10 IU/mL (diluted healthy control plasma) also showed a clear discrepancy between one-stage and chromogenic assays. Concentrating two PwHB-samples above 0.15 IU/mL resulted in persistent discrepancy. By using CSA as compared to OSA, 6/13 persons with mild and 4/4 with moderate hemophilia B would be reclassified to have a more severe diagnosis.

Conclusions: One-stage FIX:C yields systematically higher values among PwHB, in particular with FIX:C below 0.10 IU/mL. The choice of assay is important for classification of hemophilia B phenotypes. Discrepancy between OSA and CSA are determined by method and type of mutation in FIX.

PB0964 | Use of Emicizumab Prophylaxis in Adult and Pediatric Hemophilia A Patients with and without Inhibitors: Single Center Experience

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Background: Emicizumab received FDA approval for prophylaxis in hemophilia A patients with inhibitors in November 2017 and for those without inhibitors in October 2018. Hemostatic efficacy of emicizumab in real-world settings, is an area of ongoing interest and clinical relevance.

Aims: Describe a single Center's experience in adult and pediatric patient annualized bleeding rates (ABR) following transition from factor replacement or bypass therapy to emicizumab prophylaxis.

Methods: Retrospective record review of patients at the Gulf States Hemophilia and Thrombophilia Center of Houston, Texas on factor replacement or bypass therapy who initiated emicizumab following its licensure.

Results: Chart review included 69 patients (29 adults, 40 pediatric) with hemophilia A. Of the 29 adults, 12 (41.4%) experienced at least one breakthrough bleed event, 5 (27.8%) of which were trauma induced and 13 (72.2%) were considered spontaneous. Of the 18 reported breakthrough bleed events 8 (44.4%) were joint bleeds. An average ABR reduction of 85.4% (prior average ABR 9.65, post average ABR 1.41) was noted. Of the 40 pediatric patients, 10 (25.0%) experienced at least one breakthrough bleed event, 4 (20.0%) of which were trauma induced and 16 (80.0%) were considered spontaneous. Of the reported 20 breakthrough bleed events 12 (60.0%) were joint bleeds. An average ABR reduction of 81.8% (prior average ABR 3.13, post average ABR 0.57) was noted.

Conclusions: The adult population experienced a higher percentage of bleed events on emicizumab prophylaxis (41.4%) as compared to the pediatric population. However, the number of trauma induced versus spontaneous breakthrough bleed events is comparable between the adult and pediatric population. Despite the presence of breakthrough bleed events on emicizumab, average ABR for both the adult and pediatric population was reduced by > 80%. Additional observation period with this medication is merited.

PB0967 | Evaluation of Factors Associated with Prophylaxis Treatment Regimen among Men with Hemophilia B Participating in the Community Counts Registry from 2014-2018

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Background: Prophylaxis is recommended for severe men with Hemophilia B (MWHB) but it is unknown how widely it is adopted or what influences its use. The Community Counts Registry (Registry) provides a unique opportunity to evaluate current trends in prophylaxis usage among MWHB.

Aims: To examine factors associated with a prophylactic treatment regimen. We also considered whether treatment regimen varied according to the Hemophilia Treatment Center (HTC) where care was received.

Methods: We used marginal models to examine demographic and clinical factors associated with prophylactic compared to episodic treatment regimen among MWHB enrolled in the Registry between 2014 to 2018. Parameters were estimated with generalized estimating equations to adjust the standard errors, to account for correlated data within HTCs.

Results: Of 2,090 MWHB, 37.4% were using prophylaxis. The proportion of severe MWHB on prophylaxis varied significantly by HTC (Figure 1) and the intraclass correlation was 10.0%. In the multivariable analyses, the following were significantly associated with a prophylaxis regimen: history of a joint bleed (aOR 3.7), Hispanic ethnicity (aOR 1.7), and no insurance (aOR 0.4) (Table 1). We observed interaction effects between disease severity and chronic pain in the previous year and between enrollment age and history of central venous access device (CVAD). Severe MWHB with chronic pain in the previous year had higher odds (aOR 14.4) then non-severe MHWB with no chronic pain. MWHB ages 4 to12 years with a history of CVAD usage had higher odds (aOR 34.9) then ages 46 years or older with no history of CVAD usage.

Conclusions: A history of a joint bleed, health insurance, ethnicity, the interaction between enrollment age and history of CVAD, and between severity and chronic pain in the previous year, were significantly associated with use of a prophylactic regimen. Additionally, we provide strong evidence that treatment regimens for MWHB vary depending on the HTC.

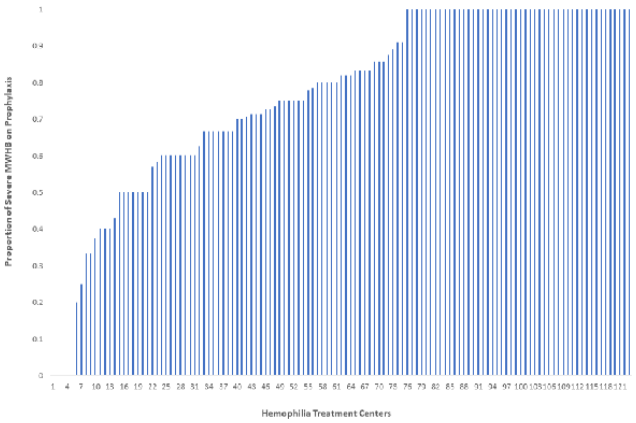

FIGURE 1 Proportion of Men with Severe Hemophilia B Using a Prophylaxis Treatment Regimen in the Registry by HTC

TABLE 1 Multivariable Factors Associated with a Prophylaxis Treatment Regimen using GEE Marginal Model

Characteristics	aOR (95% CI)	p-value	Characteristics	aOR (95% CI)	p-value
Ethnicity: Hispanic/Latino/Spanish Ethnicity	1.7 (1.2 - 2.5)	0.006	Disease Severity & Chronic Pain in past 12 months: Severe disease and Chronic Pain (yes)	14.4 (9.2 - 22.3)	0.0014
Not Hispanic/Latino/Spanish ethnicity	reference	reference	Non-Severe disease and absent Chronic Pain	reference	reference
History of Joint Bleed Event	3.7 (2.6 - 5.4)	<0.0001	Enrollment Age and History of CVAD usage: <3 years and CVAD history (yes)	22.0 (5.5 - 88.2)	0.0014
No History of Joint Bleed Event	reference	reference	4-12 years and CVAD history (yes)	34.9 (14.4 - 84.9)	0.0002
Health Insurance: Government	1.0 (0.7 - 1.3)	0.9606	13- 20 years and CVAD history (yes)	18.1 (7.6 - 43.4)	0.016
No health insurance	0.4 (0.2 - 0.9)	0.0262	21 - 30 years and CVAD history (yes)	6.9 (2.6 - 18.6)	0.3729
Other health insurance	1.3 (0.6 - 2.7)	0.4646	31 - 45 years and CVAD history (yes)	1.0 (0.4 - 3.0)	0.0806
Commercial health insurance	reference	reference	>=46 years and no CVAD history	reference	reference

PB0968 | Comorbidities in Patients with Severe Haemophilia: Birth Cohort Analysis of Taiwan Society of Thrombosis and Hemostasis Database

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Background: Hemophilia A (HA) and Hemophilia B (HB) caused by mutations in the genes encoding clotting factor VIII and factor IX are the commonest congenital disorders. The mainstay treatment in hemophilia is replacement of the missing clotting factor. But, one of the serious complications is transmission of virus which results in an increase of morbidity and mortality.

Aims: The study aimed to investigate comorbidities in patients with severe hemophilia by birth cohort analysis.

Methods: Uniform data collection form to register and annually monitor patients with severe haemophilia (PWH) in TSTH registry database from 2014 to Dec. 2018 were investigated. The critical change of hemophilia therapy in Taiwan was identified and used as landmarks to construct 3 birth cohorts, born prior to 1990, between 1990 and 1999 and after 2000. Data for the analysis included demographics, treatment regimens, and transfusion transmitted infections (TTI) encompassing HBV, HCV and HIV infection. Comorbidities including hypertension, diabetes mellitus, hyperlipidemia, cardiovascular disease, chronic kidney disease, cirrhosis, cancer, total joint replacement and death were investigated.

Results: A total of 245 severe PWH, 204 HA and 41 HB were enrolled. Increase of prophylactic rate and earlier diagnosis at age for severe hemophilia was noticed over time ($P < 0.001$). High body mass index was an emerging issue in elder PWH, compared to younger birth cohorts. The prevalence of HCV infection was significantly decreasing after introduction of recombinant clotting product, (odd ratios, 159.1, $P < 0.001$). PWH with HCV infection had a risk for hyperlipidemia, (odd ratios, 4.32, $P = 0.022$).

Conclusions: Age was an independent factor of HCV infection in PWH. Hyperlipidemia and cirrhosis were highly associated with HCV infection.

PB0969 | The Feasibility of Teaching Handheld Ultrasound (HHUS) for Home Use to Identify Bleeding in Hemophilia

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Background: Joint bleeds in hemophilia are best treated with immediate home administration of clotting factor. Most bleeds are reported subjectively (pain, tingling, swelling, and decreased mobility), however, subjective reporting is an unreliable measure of actual bleeding [Ceponis et al. Haemophilia 2013]. Ultrasound could be a more objective way to assess bleeding. Hemophilia providers can identify basic joint structures and effusions on handheld ultrasound (HHUS) [Zhou et al. Haemophilia 2018]. If patients could be taught to identify these basic structures and transmit images to their treatment center, more accurate diagnosis of bleeding vs. non-bleeding could be determined and more appropriate treatment initiated.

Aims: To assess whether patients with hemophilia can be trained to identify basic joint structures of the elbow, knee and ankle on HHUS and to transmit the live images to the HTC.

Methods: This study received ethical approval and informed consent was secured from all participants. Patients with hemophilia were recruited through the Washington Center for Bleeding Disorders and underwent a 1 day HHUS (Philips Lumify) training session conducted by the center's ultrasound-certified (RMSK) physical therapist. Subjects were instructed in ultrasound safety and equipment

TABLE 1 Training/Testing outline

Can patient perform the following actions?	Yes (left hand)	Yes (right hand)	No
1) Turn on/off the tablet 2) Turn on/off the Reacts app 3) Explain or understand basic principles of safety and handling of equipment			
Perform the following basic ultrasound probe techniques: 1)Translation 2)Heel-toe 3) Wag 4)Twist (also referred to as rotation, screw-driver) 5) Sono-compression			
Perform the following elbow US Probe placements (bilateral):			
Elbow 1)Anterior short axis 2) Anterior long axis 3)Posterior longitudinal axis			
Knee 1) Suprapatellar longitudinal axis 2) Medial patellofemoral recess 3) Lateral patellofemoral recess			
Ankle 1) Anterior longitudinal axis 2) Anterior short axis			

TABLE 2 Subject Characteristics

Age average (years)	38.1 (median 35.5, range 28-52)
Sex	all male
Hemophilia subtype and severity	All severe hemophilia A
Preexisting hemophilic arthropathy, N (%)	
Right elbow	5 (50%)
Left elbow	5 (50%)
Right knee	2 (20%)
Left knee	2 (20%)
Right ankle	8 (80%)
Left ankle	6 (60%)

handling, transducer techniques/positions, and live tele-ultrasound transmission utilizing the Reacts™ platform. Subjects then completed onsite testing utilizing this tele-ultrasound (Table1). Within 2 weeks, testing was repeated remotely in their home setting.

Results: Ten subjects, all men with severe hemophilia A with an average age of 38.1 years (median 35.5, range 28-52) participated (table 2). Two subjects underwent individual training and eight received group training. All 10 subjects passed all components of their immediate and delayed post-test.

Conclusions: Hemophilia patients, even with advanced arthropathy, can communicate with their treatment center to perform real-time home assessment for joint bleeding using HHUS. Further studies to validate HHUS during acute bleeding episodes are under way.

PB0970 | Comparison of Bleeding Pattern and Inhibitor Development among Hemophilia Patients: Prophylactic versus On-Demand Treatment

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Background: Availability of factor replacement has dramatically improved care among hemophilia patients worldwide. However, the severity and frequency of bleeding is variable and the optimal management is complex with many challenging management decisions. In Malaysia, though the number of patients on prophylaxis therapy continue to rise, there are still some patients who are on-demand therapy.

Aims: To compare bleeding patterns and inhibitor development among hemophilia patients who received prophylaxis versus on-demand therapy.

Methods: Data was obtained from National Registry on inhibitor screening program for hemophilia patients in hospitals run by Ministry of Health (MOH), Malaysia. All eligible hemophilia A and B patients who consented to participate in the registry were included. Data were collected prospectively from 1st February 2015 until 1st February 2018. Data analysed with descriptive statistics.

Results: The study samples consisted of 210 hemophilia patients from the 16 participating MOH Centers. Median age was two (IQR 0.7;7.0) years old. Majority were diagnosed at infancy (n = 99, 47.1%), followed by toddler and pre-school children with age between 2 to 5 years old (n = 42, 20%). Malays has the highest number of hemophilia (n = 104, 49.5%) and majority were diagnosed with Hemophilia A (n = 194, 92.4%). Sixty four percent (n = 134) of the study samples has moderate to severe disease however only 99 of cases (73.3%) were on prophylaxis. Overall median annual bleeding rates (ABR) was 2 (IQR 1.0;7.0). Mean ABR for moderate to severe hemophilia was lower among the prophylaxis arm (9.6%) with better Hemophilia Joint Health Score (HJHS), 12.6%. Six patients developed inhibitor during the study period and five were receiving on-demand therapy.

Conclusions: Prophylactic treatment was found to have better bleeding outcomes and joint health than on-demand therapy. Routine inhibitor screening is mandatory especially in patients receiving on-demand therapy.

PB0971 | The Impact of Switching Previously Treated Patients (PTPs) to Recombinant Factor VIII Fc Fusion Protein (rFVIIIFc) in Reducing the Annualized Bleeding Rate (ABR): A Single Center Experience

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Background: rFVIIIFc has proven to be safe and effective in patients with severe hemophilia A. We present the largest cohort of Spanish PTPs switched to rFVIIIFc.

Aims: To determine the annualized bleeding rate (ABR) and factor consumption associated with the use of rFVIIIFc compared to previous replacement FVIII preplacement therapy, both in prophylaxis and on-demand regimens.

Methods: A single-center, observational retrospective study conducted in Vall d'Hebron Hospital from Jan 1, 2017 to Dec 31, 2019. PTPs with severe hemophilia A switched from FVIII concentrates to rFVIIIFc were included. ABRs were compared using Poisson distribution test and the number of bleeding events using a negative binomial distribution model. Factor consumption was compared using Wilcoxon test

Results: The analysis included 51 patients. The mean age was 35.3 ± 15.4. The mean time of exposure was similar between the two arms: 18.7 months to previous FVIII product and 17.3 months to rFVIIIFc. 81% patients were on a prophylactic regimen both with previous FVIII product and rFVIIIFc.

There was a 55% reduction (95%CI 31-70%; $p < 0.001$) in the mean number of bleeding events and a reduction in mean ABR of 55% (95%CI 29-71%; $p < 0.001$) with rFVIIIFc compared to prior FVIII product.

Mean number of spontaneous bleeding events and spontaneous ABR presented a reduction of 58% (95%CI 26-76%; $p = 0.003$) and 56% (95%CI 18-77%; $p = 0.011$) respectively.

The monthly factor consumption was significantly lower with rFVIIIFc (12840- 21400UI; $p = 0.0305$).

The subgroup of patients on a prophylactic regimen with rFVIIIFc (n = 40) showed a 52% reduction (95%CI 30-75%; $p = 0.001$) in the number of bleeding events and a 51% reduction (95%CI 23-69%; $p = 0.002$) in mean ABR.

Conclusions: In this cohort the number of bleeding episodes and ABR with rFVIIIFc was significantly lower compared to prior FVIII products, regardless of the treatment regimen (prophylaxis/on-demand). Monthly factor consumption was also significantly reduced.

PB0972 | "Best of Both Worlds": Combination Treatment of Desmopressin and FVIII Concentrates in Hemophilia Patients Optimizes Therapy and Reduces Costs

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Background: Patients with non-severe hemophilia A may perioperatively be treated with desmopressin or factor VIII (FVIII) concentrate. Desmopressin leads to higher FVIII plasma levels, however, interpatient variability in FVIII rise is high. If FVIII increase is sufficient, minor procedures can be performed with desmopressin monotreatment. If insufficient, FVIII concentrate is used. Desmopressin combined with FVIII concentrate is not common practice. Because desmopressin is less expensive than concentrate, combined use may be cost-effective.

Aims: Assess accuracy, safety and cost-effectiveness of perioperative pharmacokinetic (PK)-guided combination treatment (desmopressin and FVIII concentrate) and FVIII concentrate savings in non-severe hemophilia patients.

Methods: Hemophilia A patients responsive to desmopressin undergoing a procedure were included. Desmopressin was administered intravenously (0.3 µg/kg). Based on previously determined FVIII response on desmopressin in the patient, additional FVIII concentrate was calculated using PK-guidance for a maximum of three days. FVIII plasma levels were determined before and after combination treatment (peak and trough levels). Hypothetical FVIII concentrate loading dose was calculated. A predicted FVIII level by the desmopressin-FVIII-PK-model was considered accurate if difference between measured and predicted FVIII level was < 0.2 IU/ml.

Results: Patient characteristics and savings of 18 procedures are described in table 1. Median age was 49.5 years (interquartile range 39.8 - 59.3). Figure 1 describes PK-model accuracy for FVIII peak level on day 0 and trough levels on day 1 and 2. At day 0, 10/18

TABLE 1 Patient characteristics

Characteristic	Number (%)
Hemophilia severity	
- Moderate	1 (5.6)
- Mild	17 (94.4)
Lowest measured FVIII (IU/ml)	0.13$ (0.675 - 0.25)
Loading dose FVIII savings (IU)	1375 (875 - 2000)
Bleeding risk procedure	
- Low	2* (11.1)
- Intermediate	6 (33.3)
- High	11* (55.6)
Performed procedure	
- Orthopedic	6* (33.3)
- Oral and maxillofacial	5* (27.8)
- Gastro-intestinal	2 (10.5)
- Urological	2 (10.5)
- Biopsy/excision	4 (21)
Days of combination treatment	
-<2	2 (11.1)
- 2	7 (38.9)
- 3	9 (50)
Bleeding complications	2 (11.1)

$ Reported in median and interquartile range (Q1 - Q3).
* One patient had a discectomy L5-S1 *and* a molar extraction

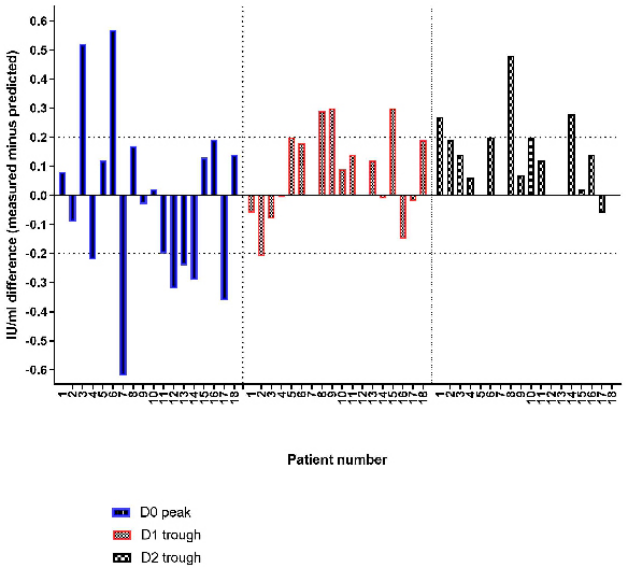

FIGURE 1 Comparison of measured FVIII and predicted FVIII peak and trough levels in patients with combination treatment

(55.5%) peak level predictions were accurate. At day 1, 11/15 (73.3%) trough level measurements were accurate and at day 2, 10/13 (76%). Modelling technique was continously adapted and improved based on study data, leading to better predictions.

Conclusions: Perioperative combination treatment of desmopressin and FVIII concentrate under PK guidance in non-severe hemophilia A patients provides an accurate dosing advice in the majority of patients. In addition, this resulted in considerable FVIII concentrate savings.

PB0973 | Monitoring of Hemlibra Prophylaxis in Children - Real World Data

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Background: Hemlibra, the bispecific monoclonal antibody bridging activated factor (F)IX and FX, is approved as prophylaxis for Hemophilia A (HA) patients with and without inhibitors. Real world data regarding its use in children are scarce.

Aims: To evaluate safety, efficacy and laboratory monitoring in a real world pediatric cohort receiving Hemlibra prophylaxis.

Methods: Plasma samples testing Hemlibra levels, aPTT and Thrombin generation (TG) parameters were obtained before therapy initiation, at weeks 2, 5 and every 3 months.

Results: Forty severe HA children, (median age: 5.5 years, range: 1 month- 18.5 y, including 11 infants) were enrolled, of whom 22 had no inhibitors. Patients were followed for a median of 42 weeks (range 6-100 weeks). Twenty patients (50%) experienced zero bleeds. Hemarthroses occurred mostly following trauma. Eight minor surgeries were safely performed in 7 children, yet another procedure (circumcision) was complicated by major bleeding. Bleeding rate was not different between infants (0-1 y) and older children. Positive correlation was found between the length of period treated with Hemlibra and any treated bleeds. Logistic regression indicated that presence of inhibitor significantly reduced bleeding risk .Neither thrombotic complications nor TMA were encountered.

Laboratory analyses disclosed statistically significant increase of Hemlibra plasma levels between week 2 and week 5. The extended aPTT values measured before treatment normalized at week 2 with additional significant shortening at week 5. Both ETP and peak height significantly increased from baseline after 5 weeks; however, TG did not reach the levels of normal controls. No differences were detected between inhibitor and non- inhibitor patients following loading period. Hemlibra levels, aPTT and TG parameters correlated positively. Notably lower TG was observed in very young infants.

Conclusions: Hemlibra prophylaxis was well tolerated in pediatric HA patients. TG interpretation in infants requires caution.

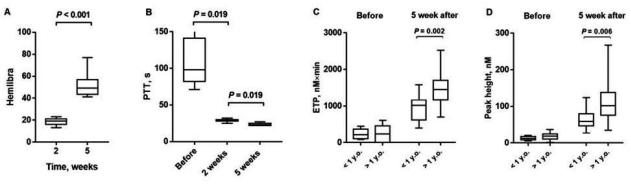

FIGURE 1

PB0974 | The Clinical Picture in Romanian Patients with Severe Forms of Hemophilia A: What Role did the Intron 22 Inversion and Age Play?

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Background: Hemophilia A (HA) is the most common congenital coagulopathy whose clinical picture is characterized by hemorrhagic events due to an inherited FVIII deficit. Severe HA forms (FVIII < 1%) are suspected due to the presence of important bleedings after minor injuries in early childhood and account for ≈ 50% of HA cases. A percentage of 45-50% of severe HA forms are positive for the intron 22 inversion (inv22).

Aims: To describe the clinical picture of Romanian patients with severe HA. To investigate the impact of age and inv22 presence on these findings.

Methods: Retrospective study of the medical records of patients with severe HA forms, diagnosed in the Hematology Department, Fundeni Clinical Institute, Bucharest, Romania. Inv22 was detected by inverse-shifting polymerase chain reaction. Written informed

TABLE 1 The Clinical Picture of Romanian Patients with Hemophilia A: Differences in Terms of Intron 22 Inversion Presence

Clinical Findings	inv22+	inv22-	P-value
Arthropathy	100.00%	90.48%	0.523
Hepatitis C	58.33%	38.10%	0.731
Gingivorrahagia	75.00%	28.57%	**0.014**
Muscular hematoma	50.00%	33.33%	0.465
Melena	25.00%	23.81%	0.100
Epistaxis	25.00%	19.05%	0.686
Anemia	25.00%	19.05%	0.686
Hematuria	33.33%	4.76%	**0.047**

TABLE 2 The Clinical Picture of Romanian Patients with Hemophilia A: Differences in Terms of Age

Clinical Findings	<35yo	≥35 yo	P-value
Arthropathy	100.00%	88.24%	0.484
Hepatitis C	18.75%	64.71%	**0.013**
Gingivorrahagia	50.00%	35.29%	0.491
Muscular hematoma	43.75%	29.41%	0.472
Melena	12.50%	29.41%	0.398
Epistaxis	12.50%	29.41%	0.398
Anemia	12.50%	29.41%	0.398
Hematuria	0.00%	23.53%	0.103
Intracranial hemorrhage	0.00%	5.88%	1.000

consent was sought from all patients. The Ethics Council approved the study (approval number 4203/31.01.2018). Regulations imposed by the national law and Helsinki Declaration (1975, revised in 5/2008) were respected.

Results: We included 33 patients with severe HA forms (mean age = 40.64 ± 14.92 years): 16 (48.48%) were aged < 35 years and 17 (51.52%) were aged ≥ 35 years. The clinical picture of the patients was dominated by: arthropathy (93.94%, N = 31), hepatitis C (45.45%, N = 15), gingivorrhagia (45.45%, N = 15), muscular hematoma (39.39%, N = 13), melena (24.24%, N = 8), epistaxis (21.21%, N = 7), anemia (21.21%, N = 7), hematuria (15.15%, N = 5), intracranial hemorrhage (3.03%, N = 1). Hepatitis C was more frequent in patients ≥ 35 years vs. < 35 years (64.71% vs. 18.75%, P = 0.0134) (Table 1).

Gingivorrhagias were more common in inv22-positive vs. inv22-negative cases (P = 0.0143), whereas hematuria was less common (P = 0.0471) (Table 2).

Conclusions: The clinical picture of Romanian patients with severe HA forms was dominated by bleeding events and hepatitis C. Hepatitis C was more frequent in patients aged ≥ 35 years, probably as a treatment-associated infection. Gingivorrhagias were more common and hematuria was less common in inv22-positive cases.

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PB0975 | Impact of a New Drug in the Real Child's Life

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Background: Inhibitors development in haemophilia patients is a severe complication. The Immune tolerance (ITI) is the only way to eradicate inhibitors. In February 2019, a novel recombinant, humanized, bispecific antibody (emicizumab) was licensed in Argentina for the prevention of bleeding in hemophilia A patients with inhibitors, but wasn't available until June/19.

Aims: Gain legal access to this drugs (it was not available in Argentina at that moment).

Methods: Clinical data from a 8 years old boy with severe haemophilia A, with inhibitor that failed two ITI.

Results: He was diagnosed at 8 month and after 18 ED he developed a high title factor VIII inhibitor. He underwent two ITI : first at 21 month old, required a central catheter (Port-a-cath). .After 14 months, the portal was removed due to infection. He required a second central catheter and after 43 months he stopped the ITI by failure of eradication of inhibitor (44UB). Second ITI was made at 6 years old and after 13 months, the ITI was stopped (12UB). He started prophylaxis with rFVIIa 90-120 ug/Kg, 3 times per week.

During bypass prophylaxis, the child had a mean of 2 episodes of bleeding by month with prolonged hospitalization. He had synovial hiperthrophy in both ankles. He missed several days of school, and had a lot of pain episodes, requiring treatment with morphine.

After judicial action, in March 2019 he started receiving emicizumab. After 11 months of treatment his health improved, he stopped having bleeding episodes and hospital admissions, resumed school and currently has the normal life of an 8 years'child .One tooth extraction was required, no factor supplementation and had not bleeding episodes. Also shows improvement in both ankles by ultrasonography.

Conclusions: It's the first successful case of treatment with emicizumab in Argentina, after a hard push the authorities.

PB0977 | Emicizumab and Surgery in Severe Haemophilia A with Inhibitor: Our First Experience

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Background: Surgical procedures in Haemophilia A with inhibitors is challenging with varrying bleeding patterns. Though emicizumab concurs almost normal haemostasis in most patients, there is little experience on it's efficacy in surgical procedures.

Aims: Report our experience in managing Haemophilia A with inhibitor on emicizumab.

Methods: Case report.

Results: This 17 year old was diagnosed with Severe Haemophilia A at 12 months and developed high titre inhibitor within the first 50 exposure days. Immune tolerance induction (ITI) was attempted at 10 years but failed. He was on-demand therapy with an annualised bleeding rate of 100 per year.

In March 2019, he fortunately received Emicizumab on compassionate use program.

He received loading dose of 150 mg weekly (2 mg/kg) for 4 weeks followed by 150 mg once every 2 weeks. This dose was chosen as only 150 mg vials were approved for compassionate use and the decision was to under dose him to prevent wastage.

Despite this reduced dose, he showed significant improvement in bleeding pattern. He stopped having weekly bleeds, was able to go to school daily and also managed some light sports.

In January 2020, during dental follow up it was noted that his lower 3rd molar has carries requiring extraction. He injected his routine emicizumab dose 3 days before planned extraction and took oral transxenamic acid 1 g TDS starting the day before procedure. Simple extraction was done and suture applied to secure haemostasis. No excessive bleeding was seen immediately post extration but 4 hours later there was a small haematoma. This resolved with transxenamic acid and ice gurgle. There was no further bleeding and no bypassing agents required. He continues with transxenamic acid and is due for suture removal in a couple of days.

Conclusions: Haemophilia A with inhibitors on emicizumab can possibly be managed without bypassing agents or blood test for simple surgical procedures.

PB0980 | Real-World Experience of rVIII-SingleChain Treatment in a French Single Center

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Background: rVIII-SingleChain (AFSTYLA®) is a B-domain-truncated recombinant FVIII product with higher von Willebrand factor binding affinity and covalent bonding between heavy and light chains to increase molecular stability. Clinical trials demonstrated its efficacy in adult and pediatric patients, leading to a reduction in treatment frequency compared with standard half-life (SHL) FVIII concentrates.

Aims: To evaluate in real life rVIII-SingleChain treatment versus previous regimen in patients from a French single center.

Methods: We retrospectively collected data following informed consent on patients with severe hemophilia A (HA) on prophylaxis with SHL FVIII followed by a switch to rVIII-SingleChain, based on individual needs and pharmacokinetic assessment according to routine clinical practice. We collected data on patient characteristics, PK parameters (half-life, trough level and time to 1%/5% thresholds), prophylaxis regimen, annualized bleeding rate (ABR) and patient feedback. We then compared data on prophylaxis with rVIII-SingleChain versus previous regimen. Study approval was obtained from the hospital *Ethics Committee*.

Results: Five patients with severe HA were treated with rVIII-SingleChain on prophylaxis for more than 12 months and were included for analysis. Bayesian PK analyses with the WAPPS-Hemo calculator revealed that for each patient half-live was increased (+58%) with rVIII-SingleChain compared with previous FVIII SHL (means: SHL = 10.96 h; rVIII-SingleChain = 17.3 h; $p < 0.0005$ paired t-test). This PK improvement was associated with a reduction in infusion frequency in 80% of patients (from 3 times to 2 times per week) and in factor consumption for 60% of patients. All patients had a lower ABR with rVIII-SingleChain and expressed their satisfaction, including increased sports practice for some. Patients reported neither adverse events nor anti-FVIII antibody response.

Conclusions: In accordance with former clinical studies and recent real life use in Germany, this study consolidates rVIII-SingleChain as an extended half-life rFVIII that improves PK parameters, prophylaxis regimen and patient quality of life.

PB0981 | Impact of Switching to Prophylaxis with Recombinant Factor IX Fc Fusion Protein Concentrate on Patient Reported Outcomes in People with Haemophilia B Using the PROBE Questionnaire

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Background: In 2017 all patients with severe Haemophilia B in Ireland switched to prophylaxis with recombinant factor IX Fc fusion protein (rFIXFc) concentrate from treatment with standard half-life (SHL) recombinant FIX. The Patient Reported Outcomes, Burdens and Experiences (PROBE) questionnaire was developed to assess patient reported outcomes (PRO). It has four major sections; demographics, general health problems, haemophilia related health problems and health related quality of life.

Aims: To evaluate impact of switching to prophylaxis with rFIXFc from treatment with SHL FIX on PRO at two years after switchover.

Methods: Following ethical approval and informed consent, patients completed PROBE questionnaires pre switchover and at 24 months. Descriptive statistics were used for analysis.

Results: 23 paired responses from 23 patients, median age 49 (31-72) years, with severe Haemophilia B were analysed. Acute pain, chronic pain and use of pain medication were reported at high rates at both 0 months (48%, 83% & 83%) and 24 months (64%, 74% & 95%). There was a reduction in chronic pain (83% to 74%) after 24 months of treatment with rFIXFc with a corresponding reduction in difficulties with activities of daily living (ADL). At 0 months, 61% reported ≤ 3 difficulties with ADL. In comparison, at 24 months 68% reported ≤ 3 ADL difficulties, with greatest improvement seen with doing heavy domestic tasks (17%), bending down (13%), walking (13%) and going upstairs (13%). There was a reduction in reported number of bleeds; 50% ≤3 bleeds/annum in year prior to switchover, compared to 87% ≤3 bleeds/annum at 24 months, with 35% reporting no bleeds.

Conclusions: From this initial data analysis there is a PRO benefit following a switch to rFIXFc prophylaxis with improvement in ADL, reduced chronic pain and bleeding rates. To further understand the patient experience post rFIXFc switch we plan to continue data analysis and perform qualitative interviews.

PB0982 | The CHiC Study: The Canadian Hemophilia Carriers Study - Preliminary Results

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Background: It is increasingly recognized that carriers of hemophilia A (HA) and B (HB) can experience abnormal bleeding regardless of their factor VIII (FVIII) or IX (FIX) level. The CHiC Study is a large national study comprehensively examining abnormal bleeding symptoms in hemophilia carriers utilizing CBDR-R (the Canadian Bleeding Disorder Registry Research Module).

Aims: This study aims to better understand the pathophysiology of abnormal hemophilia carrier bleeding.

Methods: The Research Ethics Boards at Queen's University and each participating centre granted clearance for this study. Adult hemophilia carriers from six bleeding centers across Canada were enrolled with informed consent. Questionnaires (Self-Administered Bleeding Assessment Tool, SF36v2) and hemophilia joint health scores (HJHS) were completed, and peripheral blood was collected. Assays including FVIII/IX one-stage (FVIII:OS/FIX:OS), FVIII chromogenic (FVIII:Ch), FVIII:Ag, and X Chromosome inactivation ratio (XIR) were performed in a centralized laboratory.

Results: Ninety-seven carriers are currently enrolled, and 79 blood samples have been received. Descriptive data is shown for carriers of hemophilia A (table 1) and B (table 2). In HA carriers, a weak correlation exists between bleeding score (BS) and FVIII:OS ($r = -0.315$, $p = 0.010$), and FVIII:Ch ($r = -0.285$, $p = 0.020$). FVIII:Ag correlates with FVIII:OS ($r = 0.731$, $p < 0.0001$), FVIII:Ch (0.653, $p < 0.0001$), and BS (-0.285, $p = 0.022$). No correlation is present between XIR and FVIII:OS ($r = -0.012$, $p = 0.923$), FVIII:Ch ($r = -0.093$, $p = 0.467$), BS ($r = -0.078$, $p = 0.555$) or HJHS ($r = 0.012$, $p = 0.939$). In HB carriers, a significant correlation exists between FIX:OS and HJHS ($r = 0.732$, $p = 0.039$), but not BS ($r = -0.612$, $p = 0.107$). Overall, a moderate correlation exists between BS and the SF36v2 physical component summary ($r = -0.400$, $p = 0.002$), and the SF36v2 mental component summary ($r = -0.325$, $p = 0.016$).

Conclusions: Consistent with previous studies, there is a weak correlation between factor level and bleeding score in HA carriers. Enrolment for this study is ongoing with future plans for modelling to identify the relative contribution of additional determinants of bleeding.

TABLE 1 Descriptive statistics from Hemophilia A (HA) carriers in the CHIC study

HA Carriers	Values	Max-Min	HA % Abnormal	Reference Values (Healthy)
Age (n = 68)	42	18-75	n/a	n/a
Bleeding Score (n = 80)	7	0-28	65%	0-5
FVIII:OS (IU/mL) (n = 70)	0.52	0.20-1.72	49%	0.5-1.50
FVIII:Ch (IU/mL) (n = 70)	0.50	0.09-1.72	50%	0.5-1.50
FVIII:Ag (IU/mL) (n = 67)	0.51	0.15-2.07	45%	0.5-1.50
XIR (n = 64)	57:43	34:66-85:15	10%	25:75-75:25
HJHS (n = 52)	3	0-22	18%	0-6
SF36v2 PCS (n = 56)	53.16	29.37-67.69	2%	<30
SF36v2 MCS (n = 56)	44.65	19.62-61.29	5%	<30

TABLE 2 Descriptive statistics from Hemophilia B (HB) carriers in the CHIC study

HB Carriers	Values	Max-Min	HB % Abnormal	Reference Values (Healthy)
Age (n = 8)	40	23-63	n/a	n/a
Bleeding Score (n = 10)	7	2-22	60%	0-5
FIX:OS (IU/mL) (n = 8)	0.69	0.11-1.30	13%	0.5-1.50
XIR (n = 9)	64:36	13:87-78:22	22%	25:75-75:25
HJHS (n = 9)	2	0-16	22%	0-6
SF36v2 PCS (n = 10)	57.05	34.81-61.88	0%	<30
SF36v2 MCS (n = 10)	45.18	19.62-57.13	20%	<30

PB0983 | Perioperative Management with Recombinant Factor IX Fc Fusion (rFIXFc): Irish and UK Experience

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Background: Extended half-life clotting factor concentrates are increasingly used for treatment of people with Haemophilia B, including perioperatively. Real world surgical data compliments clinical trial data and can inform optimal use and development of consensus guidelines.

Aims: To evaluate perioperative experience with recombinant factor IX Fc fusion (rFIXFc) concentrate with regards to clinical outcomes and to develop perioperative recommendations.

Methods: After ethical approval and informed consent, perioperative data (procedure, factor usage, factor levels, complications) was collected from patients with Haemophilia B who underwent a procedure with rFIXFc haemostatic cover, from four Haemophilia Comprehensive Care Centres in the UK and one in Ireland.

Results: Eight patients with Haemophilia B, median age 58 (20-70) years underwent ten major procedures (Table 1), data is available from nine. Preoperatively, patients received a median dose of 87 IU/kg (41-114 IU/kg) resulting in a median pre-operative FIX:C of 1.06 IU/mL (0.81-1.34 IU/mL). The median post-operative FIX:C on the day of surgery (D0) was 0.74 IU/mL, measured in 6/9 surgeries. Two infusions

TABLE 1 Major Procedures (one procedure not included as data not currently available)

Patient	Type of Surgery	Preoperative rFIXFc dose (IU/kg)	FIX:C level D0 post op (IU/mL) (number of infusions D0)	Median FIX:C level D1-3 (IU/mL)	Median FIX:C level D4-7 (IU/mL)	Total number of infusions (total days)	Total rFIXFc consumption (IU/kg)	Complications
1	Total knee replacement	94	0.74 (2)	0.69	0.52	18 (23)	812	Nil
2	Total knee replacement	81	0.77 (1)	0.56	0.53	19 (38)	1031	Nil
3	Total hip replacement	104	0.87 (2)	1.00	not available	28 (57)	1521	Nil
4	Ankle fusion	114	0.74 (2)	0.83	not available	not available (21)	784	Nil
5	Transurethral resection of prostate	75	no data (1)	0.57	not available	5 (11)	263	Nil
6	Haemorrhoidectomy	48	0.64 (2)	not available	not available	not available (9)	279	Nil
7	Anterior Resection	87	0.65 (2)	0.61	0.66	17 (19)	760	Nil
7	Reversal of Ileostomy	97	no data (1)	0.46	0.51	9 (12)	505	Post-operative ileus Post-operative bleeding more than expected Wound infection
7	Drainage of abdominal wall abscess	41	no data (1)	0.52	0.60	14 (18)	648	Nil

TABLE 2 Minor Procedures (Seven further procedures not classified in the above groups also performed)

Procedure Type	Number	Average treatment on day of procedure (IU/kg)	Average pre-procedure FIX:C level (IU/mL)	Average number of infusions D0	Median no of days of treatment	Median total dose per procedure (IU/kg)	Complications
Endoscopy	25	80	1	1	2 (1-2)	144 (42-159)	Delayed bleeding x 2 procedures
Joint Injection	17	98	1	1	1 (1-2)	102 (75-140)	Nil
Dental	6	50	0.99	1	1 (1-3)	44 (22-183)	Delayed bleeding x 1 procedure

were given on D0 for 5/9 surgeries, and on D1 post op the median FIX:C was 0.86 IU/mL (0.64-1.12 IU/mL, n = 4). In comparison, patients who received one infusion on D0 had a lower median D1 FIX:C of 0.46 IU/mL (0.4-0.56 IU/mL). 18 patients, median age 54 years (range 21-71 years) underwent 55 minor procedures (Table 2). Three of 55 procedures were associated with delayed bleeding.

Conclusions: rFIXFc provides effective perioperative haemostasis across a wide spectrum of major and minor surgeries. In all the reporting centres, adequate haemostasis was achieved with one or two infusions on the day of surgery. There was variation in dosing frequency and duration in the post operative period for major surgeries across centres. Ongoing collaboration between Haemophilia Comprehensive Care Centres is needed to develop a consensus approach.

PB0984 | Successful Parotid Tumor Surgery in Hemophilia a Patient after Immune Tolerance Induction (ITI) with High-Purity Human FVIII/ von Willebrand Factor (vWF)

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Background: Preoperative management of Hemophilia A patient remains a challenge especially on a tumor surgery field. The risk of major bleeding events after surgery is unpredictable. Immune tolerance induction (ITI) is the only proven method for removing inhibitors in hemophilia A patient receiving FVIII replacement.

Aims: To evaluate the efficacy of ITI on preoperative management of hemophilia A.

Methods: A case of 28 yo male, moderate hemophilia A (FVIII 1% in 2005), inhibitors < 0.05 BU, low responder, suffered from right parotid tumor since 2006 size 4x6x8 cm. Prophylactic treatment with human FVIII at increased doses switched into ITI with High-Purity Human FVIII/ von Willebrand Factor (vWF) 1000 iu weekly since October 2018. One year after ITI, FVIII level increase to 63%, and parotid tumor surgery with APTT target < 1.5 times normal was performed. The surgery went very well with minor bleeding at the surgery site and the pathology result was a benign parotid tumor.

Results: Surgery in hemophilia A patient is still very challenging even in the era of ITI. After 1 year of ITI, FVIII level increase to 63%. ITI is the regular infusion of FVIII to induce FVIII antigen-specific tolerance. Most of the current high purity pd-FVIII products carry almost 0% risk of inhibitor formation. There is data supporting the protective effect of vWF, a carrier protein of FVIII which is present in a large amount in most pd-FVIII products on inhibitor formation by reducing the immunogenicity of FVIII through preventing its entry into professional antigen presenting cells.

Conclusions: Successful ITI treatment with high-purity human FVIII/ vWF truly give promising result on perioperative management of Hemophilia A by preventing major bleeding events after surgery.

PB0985 | The 2-Years Successful Experience of Immune Tolerance Induction (ITI) with High-Purity Human FVIII/ von Willebrand Factor (vWF) in Hemophilia A Patients

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Background: Immune tolerance induction (ITI) is the only proven method for removing inhibitors in hemophilia A patients receiving FVIII replacement. ITI able to increase FVIII level, and important in management of low-responder patients whom FVIII treatment is unfeasible in spite of increased doses.

Aims: To measure the efficacy of ITI treatment.

Methods: Medical records of three adults with severe, moderate and mild hemophilia A with inhibitors < 0.05 BU on ITI at our center were reviewed. The efficacy of ITI measured by demonstrating an increase in FVIII level after 6 months and 2 years of ITI and significant reduction in all bleeding events during period of ITI. The quality of life (QoL) assessed by the ability to participate in normal daily activities.

Results: First patient, 38 yo, severe hemophilia A (FVIII 0.8% in 2012); second patient, 32 yo, moderate hemophilia A (FVIII 1% in 2012); and third patient, 31 yo, mild hemophilia A (FVIII 11% in 2010). All three of them were low-responder, usually receiving prophylactic treatment with human FVIII with increased doses. The ITI treatment with high-purity human FVIII/ von willebrand factor (vWF) 1000 iu weekly was started in August 2017. The FVIII level increase to 1%, 2%, and 12% after 6 months and 2%, 4%, and 12% after 2 years of ITI in 1st, 2nd, and 3rd patient respectively. No bleeding events observed and improvement in QoL showed in the restoration of normal daily activities.

Conclusions: Our study indicates a successful ITI treatment with high-purity human FVIII/ von willebrand factor (vWF). No adverse effects were detected. Potential benefits of avoiding hospitalizations and the prevention of long term complications, makes ITI treatment a real option.

PB0986 | Surrogate FVIII Activity in Patient and Constructed Plasma Samples Containing Emicizumab: A National External Quality Assurance Scheme for Blood Coagulation (UK NEQAS BC) Exercise

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Background: Emicizumab is used for the treatment of patients with severe haemophilia A with/without inhibitors. It is a bispecific antibody that mimics the properties of human FVIIIa activity by linking FIX and FX to activate FX.

TABLE 1 Unmodified One-stage FVIII assay results

Table 1		S19:10 FVIII 1-stage result	S19:11 FVIII 1-stage result		S19:12 FVIII 1-stage result
APTT Reagent	n	Median FVIII (IU/dL)	Median IU/dL (FVIII)	n	Median IU/dL (FVIII)
Siemens Actin FS	3	480.0	493.7	5	0.60
IL/Werfen Hemosil SynthasIL	6	687.6	764.9	6	0.40
Stago STA CK Prest	3	390.5	400	2	0.10
IL HemosIL APTT-SP	1	545.6	664.4	1	0.35
Grifols DG-APTT Synth	1	554.0	590	1	0.90

TABLE 2 Chromogenic assays

Table 2			S19:10		S19:11		S19:12	
Chromogenic Assay	Kit manufacturer	n	Median FVIII (IU/dL)	CV (%)	Median FVIII (IU/dL)	CV (%)	Median FVIII (IU/dL)	CV (%)
Chromogenic: bovine FIXa/FX	Siemens Bovine chromogenic	11	1.0	95.3	1.0	95.6	0.6	73.4
	Coamatic Chromogenix	10	1.0	115.8	1.0	119.2	0.7	125.0
	IL/Werfen Hemosil Electrochrome	1	2.4	-	2.8	-	0.7	-
Chromogenic: mixed human/ bovine FIXa/FX	Technoclone	1	2.2	-	2.7	-	<1.0	-

Aims: To obtain results from plasma samples containing emicizumab in a multicentre exercise and identify any variability between different reagents and test methods.

Methods: Participants performed FVIII assays, according to their normal laboratory practice, on 3 plasma samples S19:10, S19:11 and S19:12. Sample S19:10 was obtained from a patient with severe Haemophilia A treated with emicizumab for 3 weeks. Sample S19:11 was constructed from in-vitro addition of emicizumab into FVIII deficient plasma to mimic a post treatment level. Sample S19:12 was a pool of FVIII deficient plasma with no emicizumab present. All plasma samples were buffered and lyophilised before distribution.

Results: Two centres used emicizumab specific calibrator material (r2 diagnostics) with the one-stage assay so results were in µg/ml rather than IU/dl surrogate FVIII activity. Results are shown below. No results were provided by participants for sample S19:12:· Siemens Actin FS = 60.9 (S19:10) and 73.1 (S19:11) *IL/Werfen Hemosil SynthasIL = 53.0 (S19:10) and 64.7 (S19:11)·

*Stago STA CK Prest = 54.1 (S19:10) and 62.1 (S19:11)*Results from same centre.

Chromogenic assaysThe FVIII results generated from the bovine chromogenic kits, Siemens and Coamatic, were nearly 2.5 fold lower than results from the IL/Werfen Hemosil Electrochrome kit.

Conclusions: This UK NEQAS BC exercise identified a large variety of assay methods and reagents used for monitoring emicizumab plasma samples. The widespread use of unmodified one stage FVIII assay s suggests the need for guidance on when to use which test method.

PB0987 | FVIII Assay and APTT Results on Obizur in-vitro Spiked Plasma Samples: Analysis from the First UK National External Quality Assurance Scheme for Blood Coagulation (UK NEQAS BC) Obizur Exercise

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Background: Obizur is a recombinant porcine FVIII, given as a first-line therapy to some patients with acquired haemophilia A.

Aims: To obtain test results on plasma samples containing obizur in a multicentre exercise and identify any variability between different test methods and/or reagents.

Methods: In this exercise 29 of 37 participants performed APTT screens and FVIII assays on 2 samples: S19:13 and S19:14. Sample, S19:13, was constructed by in-vitro addition of obizur to FVIII deficient plasma to mimic a post obizur treatment level and sample S19:14, was a pool of FVIII deficient plasma with no added drug. Both samples were buffered with HEPES and Glycine and lyophilised prior to distribution.

Results: APTT Screen results: Centres, when performing an APTT most commonly used the IL Hemosil APTT reagent (n = 16). For this reagent a range of APTT ratio results between 1.14-1.32, with a CV of 4.7% for sample S19:13 and 2.94-6.0 with a CV of 19.8% for sample S19:14 were obtained.27 centres performed one stage FVIII

TABLE 1 One Stage FVIII assay results

APTT Reagent	n	Sample S19:13 (in-vitro spiked post treatment obizur level) Median (IU/dL)	Min (IU/dL)	Max (IU/dL)	CV (%)	Sample S19:14 (Pool of FVIII deficient plasma) Median (IU/dL)	Min (IU/dL)	Max (IU/dL)	CV (%)
IL Werfen Hemosil SynthasIL	14*	44.0	39.0	62.5	17.8	0.2	0.1	5.0	201.4
Siemens Actin FS	7	52.0	39.1	60.5	-	1.0	0.1	2.0	-
Pathromtin SL	2	45.3	44.2	46.3	-	0.6	-	1.0	-
Grifols DG-APTT Synth	1	52.6	-	-	-	0.5	-	-	-
STA CEPHASCREEN 10	1	67.0	-	-	-	1	-	-	-
Triniclot APTT HS	1	42.0	-	-	-	1	-	-	-
CK Prest	1*	58.0	-	-	-	FR	-	-	-

TABLE 2 Chromogenic FVIII results

Chromogenic Kit	n	Sample S19:13 (In-vitro spiked post treatment obizur level) Median (IU/dL)	Min (IU/dL)	Max (IU/dL)	CV (%)	Sample S19:14 (Pool of FVIII deficient plasma) Median (IU/dL)	Min (IU/dL)	Max (IU/dL)	CV (%)
Coamatic FVIII	1	22.0	-	-	-	FR	-	-	-
Unknown bovine	2	31.0	24.0	37.9	-	1.7	0.3	3.0	-

assays as shown in table 1 and 3 performed chromogenic FVIII as shown in table 2.

*1 participant used 2 different APTT reagents to test sample S19:14.

FR = Failed response.

FR = Failed response.

Conclusions: This first UK NEQAS BC exercise identified a large variety of testing methods and reagents used for the FVIII assay and APTT screening of plasma samples containing obizur. There was variability with nearly a 2-fold difference amongst the one-stage FVIII assay results and markedly lower chromogenic assay results suggesting the need for guidance on standardisation of methods and reagents used and careful interpretation of patient results.

PB0988 | The Role of Clinical Psychologist in Mothers of Children with Hemophilia

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Background: An innovative therapeutic management of hemophilia has been implemented at many hospitals in Japan. However, psychosocial support for hemophilia patients and their families is limited because there are few hemophilia treatment centers which can provide multidisciplinary care and support in Japan. Our hospital is a non-hemophilia treatment center and only medical doctors have seen patients with hemophilia. In 2013, one clinical psychologist joined the adult hemophilia care service. Since then the clinical psychologist has contributed to hemophilia health care, and resolved diverse medical and psychosocial issues.

Aims: This study reports on the assessment of psychosocial issues in mothers of children with hemophilia.

Methods: We evaluated 25 mothers (median age of 42 (range: 27-55)) of children with hemophilia. A clinical psychologist had interviews with patients and/or families independently of doctor's medical examination, and measured mood and emotion state by POMS2 (Profile of Mood States Second Edition).

Results: The main issues were remorse for their children with hemophilia and their husband′s family, anxiety about patient's bleeding and genetic problems of offspring. Their POMS2 showed the average scores in both positive mood state and negative mode state, because healthy mood and unhealthy mood were fifty-fifty. We noticed that many mothers were confused. This reflected the relevant mood such as “anxiety”. In addition, we found that there was unhealthy mood in the mothers who have patients with serious problems. They need a clinical psychologist. One year later, Total Mood Disturbance is getting better.

Conclusions: The clinical psychologist had a large impact on hemophilia mental health care and the health-related quality of life in hemophilia patients and families was greatly improved. Addressing the psychological problems in hemophilia is of great value and a clinical psychologist plays an important role.

PB0989 | Physical Activity, Sport and Haemophilia: An Observational Study on Risk Assessment

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Background: Many studies demonstrated beneficial effects of physical activity (PA) in persons with haemophilia (PWH): improved bone/muscular development (providing joint protection) and quality of life, reduced risk of injuries. However, PA practice is still controversial in PWH, lacking shared risk-benefit ratio assessment and recommendations.
Aims: This study aimed to assess the risk of PA in a cohort of PWH followed at an Italian haemophilia centre.
Methods: A 5-yr retrospective review of clinical records (2014-2019) of all severity PWH aged 9-65 yrs was conducted, collecting PA type, duration, frequency and exposure over the study period, using 5 risk categories (rated 1-3, Anderson & Forsyth, National Hemophilia Foundation 2005), in parallel with patients' age, disease severity, comorbidities, replacement treatment and clinical events (bleeding, traumas, injuries, with relationships with PA) were collected.
Results: One hundred PWH (A/B: 84/16, severe: 23/6; mean age 33.9 yrs) were enrolled. Thirteen patients did not report any physical exertion, 87 practiced heterogenous PA and sports (gym 17%, swimming 16%, soccer 13%, bicycling 9%, running 6%) 67% rated as safe-moderate. Physically active or not PWH were comparable regarding disease severity (severe 29% vs 23%), age (mean 33 vs 37 yrs) and total event number (mean, 1.67 vs 1.85), with tendency to significantly lower numbers in physically active PWH (1.22 vs 1.85) after excluding PA-related events. Treatment regimens were comparable in physically active PWH reporting events or not. Calculating a risk score based on risk category and total exposure to PA, the threshold 6 identified higher risk PWH, 43% reporting ≥ 1 event vs.13% of those with lower score (OR 5.05, 95%IC 1.75-14.6, p = 0.002).
Conclusions: In this cohort the large majority of PWH practice PA without increased risk, indeed showing lower numbers of clinical events. Risk score and threshold, possibly useful for individual risk-benefit assessment were identified, to be validated in larger cohorts and extended follow-up.

PB0990 | Low-Dose Prophylaxis versus On-Demand Treatment in Pakistan with Severe Hemophilia A: Updated

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Background: Prophylactic application of clotting factor concentrates particularly in children with hemophilia has become standard in countries with resources. In Pakistan prophylaxis is not practiced and majority of these patients are treated with on-demand Factor VIII concentrates to stop bleeding.
Aims: To access low dose prophylaxis vs. on demand treatment in severe Hemophilia A (HA) patients.
Methods: Prospective observational study conducted from 2018 to 2019. Fifteen severe HA children were enrolled and sub-grouped into prophylaxis (250 units or 20 IU/kg twice weekly) and twenty in on-demand treatment. Clinical and joint evaluation parameters were compared between both groups.
Results: A total of 35 patients were recruited in the study with 15 in the prophylaxis group (PG) and 20 in on demand group (DG). The median age of PG was 5 years (range 2-9 years), median body weight was 14 kg, (range 9-40 kg). However, in on DG median age 4 years (range 1-10 years) and median weight was 19.5 kg (range 8-33 kg). Annual bleeding rate (ABR) was 30(4.16%) in the PG i.e. (hemarthrosis 16(53.3%), oral bleeding 7(23.3%), epistaxis 4(13.3%) and hematoma 3(10%).However, ABR were significantly higher i.e.64(8.8%); hemarthrosis 22(73.3%), hematoma 13(43.3), gums bleeding 10 (66.7%), epistaxis 8(26.6%), and bruises 6(20%) and bleeding after trauma 5(33.3) in the DG. Similarly, days of hospitalization were lower in the PG (range 0-3) than in DG (range1-5). No patient developed FVIII inhibitor in the PG but 3 (15%) patients in the on-DG. Estimated given dose of factor was about 81120 IU/kg/year in the on DG and approximately 52000 IU/kg/year in the PG.
Conclusions: Establishment of a specialized center makes low dose prophylaxis for severe HA feasible in a country with limited resources and results into the expected marked clinical benefits. Patients can afford intensified medical care as long as drug costs are covered.

PB0991 | How to Treat Hemophilia A and B Patients Prophylactically on Standard and Extended Half-Life Factor Concentrates under Pharmacokinetic (PK) Guidance? First Experiences of a Prospective Cohort Study (OPTI-CLOT TARGET)

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Background: Pharmacokinetic (PK)-guided treatment can be applied to individualize replacement therapy in hemophilia patients, as

inter-individual differences in PK parameters influence coagulation factor VIII (FVIII) and factor IX (FIX) activity levels. Worldwide introduction of extended half-life (EHL) concentrates has further underlined this. Moreover, PK-guided dosing has the potential to increase insight into PK-pharmacodynamics (PK-PD) of factor concentrates in bleeding disorders e.g. associations between factor levels, bleeding (risk) and patient activity patterns.

Aims: Investigate the reliability and feasibility of PK-guided prophylactic dosing of factor concentrates in hemophilia A and B patients.

Methods: On May 1st 2019, a non-randomized, prospective observational cohort study was initiated for hemophilia A and B patients of all ages on prophylaxis, both standard half-life (SHL) and EHL concentrates (Netherlands trial Register:7523). Medical Ethical Committee approval was obtained. After informed consent, individual PK profiling is performed and patients start (iterative) PK-guided treatment for 36 weeks. The predicted dosing regimen is validated by a minimum of four factor levels. Detailed information on doses, blood sampling, bleeding events, activity patterns, expectations before and experiences after initiation of PK-guided dosing are documented.

Results: The first 15 patients have been included. Important results and conclusions will be presented, addressing current knowledge gaps in replacement therapy using SHL and novel EHL factor concentrates. Clinical dilemma's and challenges will be discussed and dwelled upon and include unexpected PK parameters, limitations of population PK models as provided by clinical trials, factor targeting during sporting activities and factor assay discrepancies which influence PK-guided dosing and clinical decision making.

Conclusions: The prospective OPTI-CLOT TARGET study, which implements PK-guided prophylaxis with factor concentrates, will provide new insights on how to manage prophylactic replacement therapy more optimally. Moreover, it may lead to a better understanding of the relationships between factor levels, bleeding (risk) and patient activity patterns, in order to optimize personalized treatment.

PB0993 | Data Mining: An Innovative Approach to Optimise *Post-Hoc* Analyses of Large Trial Data Sets into Clinically Relevant Results

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Background: Patients enrolled into clinical trials are a well characterised cohort. Typical safety/efficacy endpoints are critical for product licensing, which requires guidance on dosing in routine clinical care. However, there is no requirement to review data to identify predictors of good/poor outcome that can influence routine clinical practice.

Aims: Investigate the feasibility of *post hoc* analyses of pathfinder 2 cohorts using a new hybrid clinical-machine learning (CML) technology to identify treatment and patient characteristics, which predict good and poor patient outcomes, the latter particularly pertinent as it allows for treatment intensification.

Methods: Patients who participated in pathfinder 2 were included. Treatment and patient characteristics that influence outcomes were identified. Clinical measures relevant for predicting patient outcomes (baseline demographics, laboratory/clinical measurements and pharmacokinetics) were assessed by supervised CML using proprietary software.

Results: Preliminary insights indicate that the data are sufficient to support supervised CML. Joint status and target joints were re-evaluated and the resolution was observed during the main trial phase. Distinct patient profiles emerged, particularly around their previous treatment regimen. Utilising CML, adult/adolescent subgroups were defined by segmentation of baseline annualised bleeding rate (ABR) for 150 patients who received 50 IU/kg N8-GP every 4 days and evaluated over 18 months. A distinct group was patients with a baseline ABR ≥ 20 who achieved a positive ABR outcome. Interestingly, patients with a baseline ABR < 20 showed more heterogenous ABR outcomes. The heterogenous outcomes need to be investigated through evaluation of impact of other covariates, including nature of the bleeds, trough levels, joint pain, and medical comorbidities potentially to enable the development of prediction scores.

Conclusions: This *post-hoc* analysis can help identify disease-modifying parameters that may influence treatment decisions. Preliminary results of the CML technology appear promising in identifying relevant criteria for predicting treatment outcomes beyond the standard analysis in a large clinical trial data set.

PB0994 | First Episode of Macroscopic Hematuria in Hemophilia: Causes and Treatment

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Background: International guidelines regarding diagnostic strategies for hematuria differ and consequently clinical practice differs as well. In case of macroscopic hematuria, the Dutch guideline advises imaging and/or cystoscopy after urinalysis. Various expert opinions exist on the need for additional diagnostics in case of hematuria in hemophilia patients. Also treatment in these patients is debated but the usual practice is a single administration of clotting factor

TABLE 1 Patient characteristics

Characteristic	Number (%)
Hematuria episode	29 (63.0)
- Single	17 (27.0)
- Recurrent/persistent	
Severity hemophilia	
- Severe	11 (23.9)
- Moderate	13 (28.3)
- Mild	22 (47.8)
*Age first episode hematuria**	
- Severe hemophilia (n = 10), years	23.5 (20-37.25)
- Moderate hemophilia (n = 13), years	37 (21.5-46.5)
- Mild hemophilia (n = 21), years	41 (26-65.5)
Cause found in additional diagnostics (n = 38)	
- Yes	11 (28.9)
- No	27 (71.1)

*Median and interquartile range (Q1 - Q3). 2 had no reported episode date.

TABLE 2 Hematuria causes and treatment in first episode

Patients who underwent workup (n = 36)*	Number		
Causes directly detected (n = 9)			
- Kidney stones	3		
- Testicular torsion	1		
- Mechanical manipulation catheter	2		
- Extracorporeal shockwave lithotripsy	1		
- Kidney contusion after fall	1		
- Prostate carcinoma	1		
Detected causes during follow-up	2 (urothelial carcinoma; urinary tract infection)		
Treatment administration first episode (n = 34)	*Yes, single*	*Yes, repeated*	*No*
- Severe hemophilia	4		1
- Moderate hemophilia	3	3	5
- Mild hemophilia	2	3 6	7

* 2 patients had workup after first episode (at recurrence/persistence)

concentrate. Because of these different opinions and guidelines, there is no universally accepted standard of care.

Aims: To study if diagnostic workup and treatment in a first macroscopic hematuria episode in hemophilia patients is necessary to prevent sequelae and recurrence.

Methods: Adult hemophilia patients (A and B) with macroscopic hematuria were included in a retrospective cohort. Characteristics, information on hematuria episodes, additional diagnostics and treatment options were collected. Persistent hematuria was defined as a new episode of hematuria within 3 months after first presentation; hematuria after 3 months of first presentation as a recurrent episode. Additional diagnostics were performed by either the hemophilia treatment center or by a urologist. Imaging (CT, ultrasound or X-ray), urinalysis and/or cystoscopy were performed.

Results: Patient characteristics of 46 of all 491 patients of our treatment center are shown in table 1. Lifetime prevalence of macroscopic hematuria was 9.4% (46/491) and was most prevalent in severe hemophilia (29.8%, 17/57). Hematuria cause, treatment and characteristics are described in table 2. In total, 82.6% (38/46) had undergone additional diagnostics during or after the first episode of hematuria, revealing a cause in 26% (12/46).

Conclusions: Macroscopic hematuria in hemophilia patients can be an important clue of underlying pathology. Additional diagnostics in the first episode is indicated to prevent possible sequelae. Single treatment was given in the minority of patients.

PB0996 | Clinical Validation of Population Pharmacokinetic Model for Plasma-Derived Factor VIII/VWF Comparing 3 Years Follow-up

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Background: Individual pharmacokinetic profiling (iPK) in hemophilia A (HA) has become a useful tool to individualize prophylaxis using population PK models (PopPK). Recently a specific PopPK for plasma-derived factor VIII/VWF (pdFVIII/VWF) has been developed with WAPPS-Hemo.

Aims: Validate in clinical practice the novel Bayesian model for pdFVIII/VWF.

Methods: Prospective study in HA severe/moderate patients in prophylaxis with pdFVIII/VWF (Fanhdi®) comparing three periods of 1 year: standard prophylaxis (1P, 2016), early PK adoption (2P, 2017), and PK-guided prophylaxis (3P, 2018). PK parameters analyzed with WAPPS-Hemo were: half-life ($t_{1/2}$); trough level at 24, 48 and 72 h (TL24, TL48, TL72); and time to reach FVIII levels of 1, 2, 5% (T1%, T2%, T5%). Clinical variables included were: age, dose/kg, consumption, annualized joint bleeding rate (AJBR) and number of joint spontaneous and provoked bleeds. Wilcoxon and Kruskal Wallis tests (SPSS®) were used to compare the PK parameters and clinical variables between periods.

Results: Thirty patients were analyzed, 28 had severe HA and 2 moderate HA. Mean age was 31.2 years (range = 12.4-50.8). Eleven patients adjusted prophylaxis according PK and weight gain (10 increased dose or frequency and 1 patient reduced dose).

After PK-guided prophylaxis (3P), patients with joint bleeds were related to lower age, $t_{1/2}$, TL48, TL72 and T5% compared to patients with zero AJBR (Table 1). No differences in AJBR were observed comparing 1P and 2P, whereas a trend to higher consumption was reported (Table 2). However, after PK-guided prophylaxis (P1 vs P3) lower spontaneous joint bleeds and a trend lower AJBR were reported, but no differences in consumption (Table 2).

Conclusions: PK-guided prophylaxis allows the individualization of treatment, as well as improvements in bleeding control, especially in younger patients with short $t_{1/2}$. Collaborative projects as WAPPS-Hemo allow the development of specific PopPK for clotting factors without published PopPK.

TABLE 1 Analysis of joint bleeding causes using a specific pdFVIII/VWF PopPK (PK-guided tailoring period; 3P 2018)

PK parameters & clinical variables	Zero joint bleeds. Median	Zero joint bleeds. IQR	Joint bleeds ≥ 1. Median	Joint bleeds ≥ 1. IQR	P-value
Age (years)	40.5	20-52.5	8	4-32	0.016
Weight (kg)	62.5	58.5-69	32	23-72	0.134
t1/2 (h)	15.4	12.8-19.4	10.6	9.0-14.8	0.008
TL24 (IU/dL)	16.3	13.8-24.3	11.6	9.1-20.2	0.061
TL48 (IU/dL)	6.0	4.0-9.9	3.3	2.2-5.3	0.011
TL72 (IU/dL)	2.3	1.5-5.5	1.5	0.7-1.9	0.014
T5% (h)	52.1	43.6-74.8	40.0	33.8-49.5	0.016
T2%* (h)	71.5	57.3-86.0	61.0	49.3-70.0	0.061
T1%* (h)	93.1	90.0-144.0	84.7	63.0-101.5	0.084

TABLE 2 Comparisons between consumption and joint bleeding rates using Kruskal-Wallis test periods of PK study (1P 2016 vs. 2P 2017; and 1P 2016 vs.

Clinical variables	1P. Median	1p. IQR	2P. Median	2P. IQR	3P. Median	3P. IQR	P-value 1P vs 2P	P-value 1P vs 3P
Dose (IU)/kg/week	60.6	49.3-94.60	62.7	47.5-94.0	76.7	55.6-99.4	0.49	0.12
Consumption/kg (UI/kg)	3039.47	2573.2-4741.0	3436.6	2702.7-4959.5	3219.9	2605.0-4991.0	0.09	0.14
AJBR	0.0	0.0-2.0	0.0	0.0-1.0	0.0	0.0-1.0	1.00	0.09
Spontaneous joint bleeds	0.0	0.0-1.0	0.0	0.0-1.0	0.0	0.0-0.0	0.76	0.01
Provoked joint bleeds	0.0	0.0-0.0	1.0	1.0-1.0	0.0	0.0-1.0	0.43	0.48

PB0997 | Third Time's the Charm? Tachyphylaxis of Desmopressin Response in Non-Severe Hemophilia A Patients and Comparison to Earlier Response: The DAVID Study

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Background: Patients with non-severe hemophilia A may perioperatively be treated with desmopressin or factor VIII (FVIII) concentrate. Desmopressin leads to FVIII release, resulting in higher FVIII levels. However, interpatient variability on FVIII rise is high. If FVIII increase is sufficient, minor procedures can be performed with desmopressin monotherapy. If insufficient, FVIII concentrate is used. Data is limited on tachyphylaxis, a decreased response after repeated desmopressin administration. Therefore, we studied tachyphylaxis of desmopressin in hemophilia A patients who underwent surgery.

Aims: To study desmopressin response in hemophilia A patients who received repeated doses perioperatively and to determine extent and determinants of tachyphylaxis.

Methods: Hemophilia A patients responsive to desmopressin undergoing a procedure were included. Desmopressin was administered intravenously (0.3 µg/kg). Laboratory measurements were taken before and +/- 15 minutes after infusion. FVIII response levels to desmopressin after a test was collected from patients' medical files. The FVIII increase was calculated as the absolute difference between FVIII levels before and after desmopressin infusion on the respective day. Response to repeated administration of desmopressin (tachyphylaxis) between consecutive days was calculated as a percentage by dividing the latter and earlier FVIII increases with each other (for example: D1 divided by D0, times 100%).

Results: Patient characteristics and treatment days of 17 procedures are described in table 1. Figure 1 shows the absolute increase of FVIII after desmopressin test and treatment. Median percentage of tachyphylaxis was: D1/D0 41.2% (IQR 27.5% - 50%), D2/D0 was 36.4% (IQR 23.7% - 45.7%) and D2/D1 87% (IQR 72.2% - 93%). FVIII increase after desmopressin test dose was similar to the observed response perioperatively.

Conclusions: Physicians should be aware that even the second administration of desmopressin can result in much lower FVIII peak levels than previously reported. Desmopressin test response predicted the response to perioperative desmopressin administration accurately.

TABLE 1 Patient characteristics

Characteristic	Number (%)
Hemophilia severity	
- Moderate	1 (5.9)
- Mild	16 (94.1)
Lowest FVIII level (IU/ml)	0.15* (0.075 - 0.22)
Consecutive days desmopressin	
- <2 days	2 (11.1)
- 2 days	7 (38.9)
- 3 days	9 (50)

* Reported in median and interquartile range (Q1 - Q3)

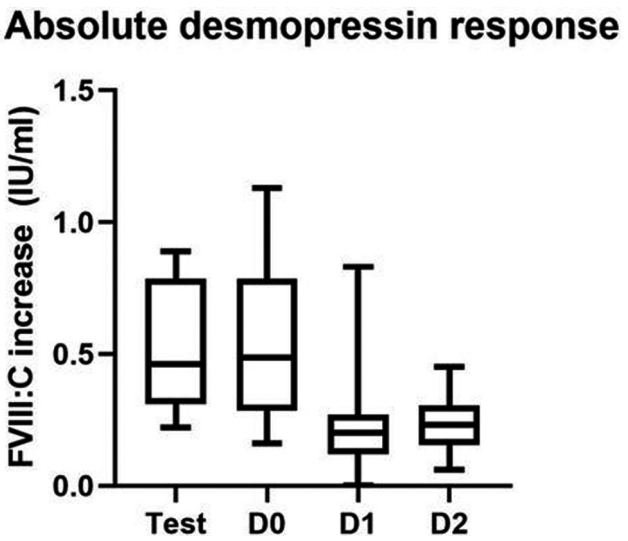

FIGURE 1 Desmopressin increases in earlier test and repeated doses

PB0998 | Incidence of Factor VIII and IX Inhibitors in Hemophilia A and B in China

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Background: The development of inhibitors of factor VIII(FVIII) and factor IX(FIX) is an important complication of hemophilia. China is a large country with a population of 1.37 billion, which has an estimated hemophilia population of at least 130,000. Because of the huge population bases, the population with this complication in China is also large and worth attention.

Aims: This abstract intended to show the distribution of hemophilia patients with inhibitors, retrospectively analyze the risk factors for inhibitor production, and describe the current situation of this population.

Methods: We used Chinese National Hemophilia Information Registration System to acquire information of patients with inhibitors. Then we conduct a questionnaire survey for FVIII and FIX inhibitor-positive patients by telephone.

Results: There have been 16083 hemophilia A patients and 2447 hemophilia B patients registered in the system since 2009. 766 patients with hemophilia A(4.76%) and 17 patients with hemophilia B(0.69%) developed inhibitors. These patients with inhibitors were distributed in 30 provinces in China, with more patients living in eastern coastal regions.(shown in Figure1.) Telephone follow-up is under way. From the available data, most people used to use a mix of plasma-derived and recombinant factors produced by various companies, that usually depended on which product was available at that time. Most people also heavily used FVIII or FIX before inhibitor production. They usually accepted prothrombin complex concentrate(PCC) and recombinant FVIIa(rFVIIa) for on-demand treatment after inhibitors production.

FIGURE 1 Incidence of factor VIII inhibitors in hemophilia A in China provinces

Conclusions: There were 783 hemophilia patients registered developing inhibitors in China, accounting for 4.23%(783/18530)of the registered patients with hemophilia since 2009. The incidence of inhibitors was higher in eastern coastal cities,that probably because the reimbursement rate was high and factor product was more available. The mixture of factors and high dose use might be two causes of inhibitor production.

PB0999 | Long-Term Immunogenicity, Efficacy and Safety of Simoctocog Alfa in Patients with Severe Haemophilia A who Had Completed the NuProtect PUP Study

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Background: The NuProtect (GENA-05) study assessed the immunogenicity, efficacy and safety of simoctocog alfa in 108 previously untreated patients (PUPs) with severe haemophilia A. The cumulative incidence of high-titre inhibitors was 17.6%.

Aims: The key objectives of the GENA-15 study were to investigate the long-term immunogenicity, efficacy and safety of simoctocog alfa in patients who had completed the NuProtect study.

Methods: GENA-15 was a prospective, multicentre, multinational, open-label, non-controlled phase 3b extension study. Participation was offered to all patients who completed the NuProtect study and had been treated for at least 100 exposure days (EDs). GENA-15 was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Results: Forty-eight patients were enrolled into GENA-15. All patients were included in the safety population. Efficacy data were available for 47 patients, all of whom received prophylaxis. Approximately 5 million international units (IU) of simoctocog alfa were administered to 48 patients with a mean of 179 EDs, a total of 8603 EDs, and a total of 8646 infusions. No patient developed FVIII inhibitors during GENA-15. The annualised bleeding rates (ABRs) during prophylaxis (negative binomial counting regression model) were 0.28 (95% confidence interval [CI]: 0.15, 0.53) [median 0, range 0-3.88] for spontaneous bleeding episodes (BEs) and 1.62 (95% CI: 1.09, 2.42) [median 1.0, range 0-8.68] for all BEs. Of 111 BEs treated with simoctocog alfa in 29 patients, efficacy of treatment was rated as “excellent” or “good” for 86 (77.5%). For two major surgeries (in two patients) the overall efficacy assessment was excellent. There were no adverse events assessed as related to study treatment by the Investigator.

Conclusions: No FVIII inhibitors developed during long-term simoctocog alfa prophylaxis in patients with severe haemophilia A who had completed the NuProtect PUP study. Simoctocog alfa prophylaxis was effective and well tolerated.

PB1000 | The Haemophilia A Patients whose FVIII: C by Chromogenic Substrate Assay were Higher than by One-Stage Substrate Assay Showed Risk of Development of Haemophiliac Arthropathy Silently

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Background: Reducing joint bleeding has been regarded as key to preventing development of hemophilic arthropathy. However, recent research has implied that such reductions do not completely protect against the onset of hemophilic arthropathy.
Aims: The aim was to confirm whether patients who had never experienced clinically evident joint bleeding (CEJB) did not develop hemophilic arthropathy, and also whether patients can develop hemophilic arthropathy without CEJB.
Methods: This study investigated 15 adult patients with hemophilia A who had never experienced CEJB to explore factors associated with hemophilic arthropathy without joint bleeding. Age, body mass index (BMI), factor VIII activity (FVIII:C) as measured by chromogenic substrate assay (CSA) and one-stage assay (OSA), and FVIII antigen (FVIII:Ag) were adopted as candidate factors. The causative mutation for hemophilia A was identified in all patients. Hemophilic arthropathy was estimated by Pettersson score (PS).
Results: While 10 patients showed a negative PS, the other five showed positive PS scores. These five patients all displayed higher FVIII:C by CSA than by OSA, and four kinds of mutations were identified in these five patients: p. Asp182Tyr, p. Thr314Ile, p. Arg1800His and p. Arg2169His. In terms of the mechanism of discrepancy between the two methods, p. Arg1800His was associated with enhanced binding affinity of FVIII for FX. However, the mechanisms underlying the other three mutations were not revealed.
Conclusions: Our study indicated that even hemophilia A patients who had never experienced CEJB risked developing hemophilic arthropathy. A distinct trend was seen for these patients to show higher FVIII:C by CSA than by OSA.

PB1001 | Immune Tolerance Induction (ITI) with a Single Factor VIII/von Willebrand Factor Concentrate in Haemophilia A Patients with Inhibitors - Update from the ObsITI Study

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Background: Immune tolerance induction (ITI) with FVIII is the only proven strategy for eradication of FVIII inhibitors. The ongoing, international, open-label, observational ITI study (ObsITI; NCT02207894) is evaluating ITI with a range of FVIII products.
Aims: This analysis reports interim data for prospective patients treated for ITI exclusively with a single plasma-derived, von Willebrand factor-stabilised, FVIII concentrate (pdFVIII/VWF, octanate®) between December 2005 (study initiation) and January 2019.
Methods: Complete success of ITI was defined as achievement of the following 3 criteria: inhibitor titre < 0.6 Bethesda units [BU]/mL; FVIII recovery ≥ 66%; FVIII half-life ≥ 6 h. Partial success required achievement of two criteria and partial response required achievement of one criterion. ITI success in this analysis was defined as complete or partial success.
Results: 100 patients completed ITI. Most patients had poor prognosis factors (91.0%) or were high titre inhibitor patients (86.0%). A negative inhibitor titre was achieved in 71 (71.0%) patients in a median time of 4.01 months (interquartile range [IQR] 1.61-8.97). ITI success was achieved by 70 (70.0%) patients [60 (60.0%) complete success and 10 (10.0%) partial success], 5 (5.0%) achieved a partial response, and ITI failed in 25 (25.0%) patients. Partial and complete success were achieved in a median time of 5.55 (IQR 3.19-12.48) and 11.25 (IQR 6.52-18.56) months, respectively. ITI success was achieved in 56 of 72 (77.8%) primary ITI patients (49 [68.1%] complete success and 7 [9.7%] partial success), and 42 of 52 (80.8%) primary ITI patients ≤ 12 years (35 [67.3%] complete success and 7 [13.5%] partial success). Only two serious adverse drug reactions (catheter infections of moderate intensity in the same patient) were considered possibly related to treatment.
Conclusions: ITI with octanate® led to a rapid and safe eradication of FVIII inhibitors and normalisation of FVIII pharmacokinetics in largely poor prognosis patients.

PB1002 | Dissecting the Paradox of "Severe" Hemophilia A (Clotting Factor VIII < 1%) with a Mild Clinical Phenotype in the Era of Prophylaxis

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Background: Hemophilia A (HA) severity is traditionally determined by clotting factor VIII (FVIII) levels, but up to 15% of individuals with severe hemophilia A (sHA, FVIII < 1%) have paradoxically mild clinical phenotypes. The Identification of these individuals is complicated by the lack of standardized methods for clinical phenotyping and the early initiation of prophylaxis that modifies phenotype. Identifying individuals with FVIII < 1% and a mild clinical phenotype may spare them from the burden of unnecessary treatment and reduce treatment cost, and studying this group of patients may lead to identification of novel genetic modifiers of bleeding and thrombosis.
Aims: To systematically review the literature of sHA for mild clinical phenotypes.

TABLE 1 Clinical phenotypes that may identify individuals with FVIII < 1% and mild bleeding in the era of prophylaxis. PPX-prophylaxis. PK-pharmacokinetics

Clinical phenotype metric	Measure of phenotype	Accounts for PPX intensity	Accounts for PPX PK and adherence	Effect of recall bias	Comments and reference
ISTH-BAT score	Composite score	-	-	+++	Not validated in PPX-treated sHA. May lack power to differentiate mild from non-mild clinical phenotypes due to saturation of the score even with mild bleeding symptoms. Borhany et al., Transfus Apher Sci. 2018;57(4):556.
Hemophilia severity score	Composite score	+	-	+++	Calculation of score is complex. Schulman et al., J Thromb Haemost. 2008 Jul;6(7):1113.
Age at first joint bleed before the initiation of PPX	Age (years)	N/A	N/A	+	Applicable only if PPX is initiated after the first bleeding event. Carcao et al. Blood. 2013;121(19):3946.
Joint severity score	Composite score	-	-	+	Affected by factors other than bleeding (e.g. inflammatory reaction). Aledort et al., J Intern Med. 1994 Oct;236(4):391.
Breakthrough bleeding event rate	Number of events per year	-	-	++	Bleeding events on PPX are very low in most individuals and the event rate may be affected by PPX dose or frequency adjustments in response to bleeding. Gringeri et al., J Thromb Haemost. 2011 Apr;9(4):700.
Breakthrough bleeding event rate with trough level-adjusted PPX	Number of events per year	+	+	++	Bleeding events on PPX are very low in most individuals and the event rate may be affected by PPX dose or frequency adjustments in response to bleeding or low trough levels. Petrini et al., Haemophilia. 2001;7(1):99.
Lowest intensity PPX regimen (dose and frequency) with no breakthrough bleeding events	Dosing regimen	+	-	+	Applicable only to dose-escalated PPX-treated individuals. Feldman et al., Lancet Haematol. 2018 Jun;5(6):e252.
FVIII consumption	FVIII consumption (IU/kg) per year	+	-	++	Van dijk et al., Haemophilia 2005;11:438.
Bleeding event rate after stopping prophylaxis	Number of events per year	N/A	N/A	+	Applicable only in patients who discontinue prophylaxis, such as adults. Fischer et al., Haemophilia. 2001;7:544.

Methods: We conducted a systematic literature review of PubMed for publications from 1980 through 2020 with the search terms hemophilia A and phenotype. The reference lists of studies with relevant data were also reviewed. We extracted data on clinical phenotypes that may distinguish mild bleeders from non-mild bleeders in the era of prophylaxis.

Results: Our initial search yielded 434 studies. Nine clinical phenotype metrics were identified. Most of these do not account for treatment intensity or adherence, or pharmacokinetics, and none account for hemostatic risk behavior or inhibitor status. Additional characteristics of each metric are listed in table 1. The phenotype metrics varied widely based on the depth and complexity of phenotypic assessment and variations in the populations to which they apply.

Conclusions: There are limited data to identify and study patients with sHA and a mild clinical phenotype. Among the clinical phenotype metrics that were identified, there is wide methodological variation, evidenced by divergent assessment tools and non-standardized concepts. The lack of standardized methods for clinical phenotyping of sHA is a major obstacle to improved understanding and treatment and thus improved standardization is essential for further evaluation of this important patient population.

PB1003 | Differences in Pharmacokinetic Evaluated by Clotting and Chromogenic Factor VIII Assays in Twelve Persons with Severe Hemophilia A on Prophylaxis with Recombinant Factor VIII Fc Fusion Protein (rFVIIIFc)

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Background: Nowadays pharmacokinetic (PK) approach is crucial in individualization of prophylaxis and became routinely recommended. It is well known the variability in FVIII measurements between different assays (one-stage or chromogenic assays) in samples from patients treated with FVIII products.

Aims: To evaluate how different FVIII assays affect the PK profile in hemophilia A (HA) persons undergoing prophylaxis with recombinant factor VIII Fc fusion protein (rFVIIIFc).

Methods: PKs were assessed in twelve severe HA patients that switched to prophylaxis with rFVIIIFc. At least three samples were obtained from each patient, before and after rFVIIIFc infusion; the same sample was tested for FVIII levels by one stage (Pathrontim, Siemens, Germany) and chromogenic (Electrachrome, IL, USA) assays. PK parameters were obtained with the WAPPS-Hemo platform (McMaster University, Ontario, Canada) after input of FVIII plasmatic levels. The balanced estimate terminal half-lives (HL) were compared between the two types of assays using a Wilcoxon test.

Results: The patients, all males, mean age 23.9 yr (range: 7-44), had mean vWF:Ag 0.99 IU/mL (range: 0.60-1.45 IU/mL). Infusion of rFVIIIFc varies between 16.7 IU/kg and 41.7 IU/kg (mean 24.7 IU/kg). The mean HL with chromogenic (16.9 hrs, range: 11-21) was 10.8% increased than mean HL with one-stage (14.9 hrs, range: 8.75-22.5) assays ($p = 0.05$). Individually HL with the different tests varies between 8.75 and -2.25 hours.

Conclusions: PK is being increasingly used to optimize prophylaxis regimes. However, in this small group of patients there is a marginal increase (11%) in terminal half-lives with chromogenic related to one-stage tests, but individually there may be striking differences (up to 1.8) in PK results between different FVIII testing methodologies. This raises questions about the correct PK profile (clotting or chromogenic assays) in patients on prophylaxis with rFVIIIFc and which FVIII assay to choose.

PB1004 | Haemophilia B Patients Switched to Recombinant Factor IX-Albumin Fusion Protein (rIX-FP): Ultrasound and Clinical Joint Assessment

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Background: Prophylaxis is the only strategy to prevent the onset/progression of haemophilic arthropathy. The introduction of extended half-life (EHL) concentrates improved personalization and convenience of prophylaxis by reducing infusion frequency, especially in haemophilia B. However, few real-life data are available, particularly on joint assessment. Ultrasound (US) is useful to early detect and monitor arthropathy.

Aims: This study aims to collect data about joint status in patients with haemophila B switching to a EHL concentrate, rIX-FP.

Methods: At annual check-up joints were evaluated by physical examination (Haemophilia Joint Health Score in children; Gilbert score in adults) and by ultrasound scan (Haemophilia Early Arthropathy Detection with UltraSound, HEAD-US). The last available scores before switch and on rIX-FP treatment were compared, together with patients' data (annualized bleeding rates [ABR], FIX trough levels, concentrate weekly dose) over comparable time periods on standard recombinant FIX (S-rFIX) and rIX-FP prophylaxis. Data mean ± 1 standard deviation are reported.

Results: Thirthy-six joints in 6 patients (5 severe, 1 moderate, age 31 ± 12 yrs) switched to rIX-FP (22 ± 10mo) were evaluated. Results about ABR, FIX through levels, concentrate weekly dose, HEAD-US and orthopedic joint scores are shown in Tab 1.

TABLE 1

	rIX-FP	S-rFIX	p
ABR	0.8 ± 1.6	0.1 ± 0.1	-
FIX trough level (%)	10.8 ± 1.9	3.9 ± 1.4	0.002
FIX concentrate weekly dose (IU/kg)	35.6 ± 12.1	89.8 ± 22.3	0.001
HEAD-US scores	0.94 ± 1.8	0.8 ± 1.6	-
Orthopedic scores	0.31 ± 0.7	0.25 ± 0.6	-

HEAD-US score was free of abnormalities at both assessments on 22/36 (61%) joints. Six joints (46% of those with HEAD-US ≥ 1) had improvement on rIX-FP prophylaxis, due to reduction of synovial hypertrophy. Twenty-height joints showed orthopedic score 0 at both assessments. No joint had score improvement, whereas score increased in 2. Nine joints (25%) with HEAD-US ≥ 1 were negative at physical examination, whereas 2 (6%) had HEAD-US = 0 but positive orthopedic scores.

Conclusions: Despite the limited patient sample, this study shows preserved joint status after switch to rIX-FP, associated with reduced rFIX consumption and sustained FIX trough levels. US scan reveals possible improvement of synovial hypertrophy and provides additional information about early abnormalities in joints negative at physical examination.

PB1005 | Non-Sever Hemophilia Is Not Benign? - Insights from the PROBE Study

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Background: There are limited data on the impact of haemophilia on health status and health-related quality of life (HRQL) in people affected by non-severe haemophilia.

Aims: To evaluate the health status of people living with mild or moderate haemophilia.

Methods: A cross-sectional, multinational study was conducted as part of the Patient Reported Outcomes, Burdens and Experiences (PROBE) project. Respondents without bleeding disorder (NoBD) and those with mild or moderate haemophilia were included. Respondents were asked to complete the PROBE questionnaire, which contains haemophilia-related questions, general health questions and HRQL. Results were compared between unaffected individuals and people with mild or moderate haemophilia.

Results: A total of 862 respondents, of whom 144 with moderate haemophilia, 143 with mild haemophilia and 575 with NoBD were included. Median age (first-third quartile) was 33 (23-46),42 (25-55) and 43 (35-54), respectively. In relation to bleeding in the previous 12 months, respondents with mild reported less bleeding frequency than those with moderate haemophilia, with similar patterns of bleeding frequency seen in both male and female cohorts. Reporting of acute pain is less in those with NoBD compared to the mild to moderate cohorts for both genders (male - 33%, 67%, 77%; female 38%,52%, 67%, respectively). Thirteen percent of those with NoBD reported an impact on activities of daily living compared with mild and moderate haemophilia who reported of 35% and 61%, respectively. The impact on quality of life due to mild haemophilia compared to those with NoBD was a reduction of 5.2%, 5.0% and 9.3% in VAS, EQ-5D-5L and PROBE Score respectively ($p \leq 0.001$).

Conclusions: People affected by mild or moderate hemophilia encountered a significant amount of haemophilia related sequalae. Future research is needed to identify the optimal management of moderate and mild hemophilia patients, with particular focus on early identification of patients with a severe clinical phenotype.

PB1008 | Prospective Comparative Analysis of Joint Structure and Function in Chinese Children with Severe Hemophilia A under Different Doses Factor VIII

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Background: Hemophilic arthropathy is the most serious complication of severe hemophilia A.

Aims: This is a interim research report for prospective comparative analysis of joint structure and function in Chinese children with severe hemophilia A under different doses FVIII.(China registration number:ChiCTR-ONC-17011186)

Methods: The study included 47 children(4-17 years old) with no inhibitor in severe hemophilia A.There were 9 cases in on-demand treatment group,18 in low-dose prophylaxis(FVIII10-15 IU/kg,2-3/week,and ≤ 30 IU/kg/week) group,and 20 in medium-dose prophylaxis(FVIII15-20 IU/kg,2-3/week,and 45-60 IU/kg/week) group.We investigated the changes of joints in children during the two-year period from bleeding phenotype(Annual bleeding rate,ABR/Annual target joint bleeding rate,ATJBR),HJHS(Hemophilia Joint Health Score),FISH(Functional Independence Score),ultrasound and MRI score.Median observation period was 22 months(658 days).

Results: There were no significant differences in median age,body weight,and BMI in each group($P > 0.05$).Children's ABR(12.34/year/patient) in medium-dose group was reduced by 238.6% and 49.4% compared with on-demand group(41.79/year/patient) and low-dose group(18.44/year/patient).The ATJBR from the most severe joint:the difference between on-demand group(7.07/year/patient),low-dose group(2.85/year/patient) and medium-dose group(0.94/year/patient) was statistically significant($P1 = 0.015$,$P2 = 0.003$,$P3 = 0.000$).The HJHS from the most severe target joint:on-demand group was degraded by 2.14 ± 2.41 points,compared with low-dose group(-0.35 ± 2.15) and medium-dose group(-0.69 ± 1.99) were statistically different($P1 = 0.020$,$P2 = 0.008$);but there was no statistical difference between low-dose and medium-dose groups($P = 0.650$).FISH score:there was a statistical difference between the on-demand and low-dose groups($P = 0.034$),while no difference between middle-dose and on-demand group,neither between low-dose and middle-dose group.The ultrasound score from the most severe target joint:85.7%(6/7) cases in on-demand group worsened,47.1%(8/17) in low-dose group and 36.8%(7/19) in middle-dose group, with no statistical difference between the groups.The MRI score from the most severe target joint:57.1%(4/7) progress in the on-demand group,which are statistically significant compared with 35.3%(6/17) in low-dose group and 23.5%(4/17) in middle-dose group($P1 = 0.026$,$P2 = 0.042$),however,no difference between low and middle dose groups($P = 0.201$).

Conclusions: Preliminary interim results show that medium-dose prophylaxis in children with severe hemophilia A in China is slightly

better than low-dose prophylaxis in protecting joint structure and function,and significantly better than on-demand therapy.

PB1009 | Pain Status in Patients with Hemophilia: Evaluation of Routine Pain Assessment in an Unselected Cohort of Patients with Hemophilia A and B

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Background: Pain is a common co-morbidity in patients with hemophilia (PWH). Most data on prevalence of pain is generated by patient surveys with potential selection bias or within clinical studies.
Aims: To analyze prevalence of pain in an unselected cohort of PWH visiting a single German hemophilia center for routine annual check-ups during the previous 5 years.
Methods: Assessment of pain intensity (numeric rating scale, NRS, 0 [no pain] to 10 [worst imaginable pain]) and location over the previous 3 months was carried out with an ad-hoc questionnaire. Pain interference was rated on a scale from 0 to 10 with higher values indicating more interference. Impact on mood was reported as none, moderate or severe. Demographic and clinical data were extracted from patient charts.
Results: 184 male adult PWH A or B visited the center, of whom 155 (84%) completed at least 1 questionnaire (93%, 81%, 65% of PWH with severe, moderate, mild hemophilia, respectively) with a median age at first assessment of 39 years (range, 18-77 years). 141/155 PWH (91%) reported episodes of pain with a median intensity (NRS) of 6 (range, 1-10), 5 (1-10), 3 (1-9) and median number of affected joints of 2, 2, 1 (range, 0-6) in PWH with severe, moderate, mild hemophilia, respectively. Only 9% of patients reported no pain and 15 patients (9.7%) had pain only in locations other than joints. Median pain interference in PWH with pain was rated 4 (range, 0-10); 51, 64, 22 PWH reported no, moderate and severe impact on mood, respectively.
Conclusions: This analysis of a routinely used pain assessment tool in an unselected population of PWH A and B reveals a high prevalence of pain episodes as well as relevant pain interference and impact on mood. Further analyses of pain over time and intervention effects will be performed.

PB1010 | The Impact of Physical Activity on the Pharmacokinetics of Factor VIII / IX in a Cohort of Pediatric Patients with Hemophilia A / B in Pharmacological Prophylaxis

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Background: Nowadays, prophylaxis is the gold-standard of treatment of patients with haemophilia, minimizing the consequences of joint bleeds and improving quality of life. Pharmacokinetics (a branch of pharmacology that studies distribution of a drug in the body) allows individualizing and optimizing the treatment of individual patients, so it is guaranteed a good hemostatic coverage and hemophiliacs should be encouraged to practice sports.
Aims: In a monocentric observational cross-over study, we studied the pharmacokinetics of FVIII / IX during physical activity. The aim of the study is to evaluate the changes in the pharmacokinetic curves during physical activity and compare the individual pharmacokinetic data with a population pharmacokinetic algorithm.
Methods: For each patient we evaluated the pharmacokinetic curves during mild activity (< 10000 steps / day) and moderate / intense (>10000 steps / day). Physical activity it has been monitored by a step counter. We evaluated the influence of physical activity on the standard pharmacokinetic curves and compared with individual pharmacokinetics (according to AICE guidelines) with a population pharmacokinetic algorithm (e.g. WAPPS).
Results: A total of 20 patients affected by hemophilia were enrolled. 15 patients (75%) were affected by hemophilia A while 5 patients (25%) where affected by hemophilia B. The pharmacokinetic curves performed during mild physical activity appeared significantly better in all times evaluated than those performed during moderate / intense activity.
Conclusions: The study of pharmacokinetic curves correlated to the physical activity allows to personalize the treatment and modulate therapy by increasing it in those who perform more physical activity and reducing it in those who perform less. The individualized therapy guarantees greater safety during sport but also savings when there is less movement.
Signed informed consent has been acquired from the patient's parents.
The study was approved by a recognized medical ethics committee.

PB1011 | Validation of an Algorithm to Identify Hemophilia A Patients in Administrative Database

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Background: Hemophilia A is an inherited bleeding disorder. Because of rarity of the condition, large populations are needed to identify

TABLE 1 Diagnostic accuracy of ICD code alone vs ICD code + coagulation FVIII < 0.4

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	c-statistic (from ROC analysis)
ICD code alone	95.2 (85.6- 98.7)	95.8 (94.8-96.6)	40.4 (32.4-48.8)	99.8 (99.5-99.9)	0.96
ICD code + low coagulation FVIII	93.5 (83.5-97.9)	99.3 (98.8-99.6)	80.5 (69.1-88.5)	99.8 (99.4 -99.9)	0.964

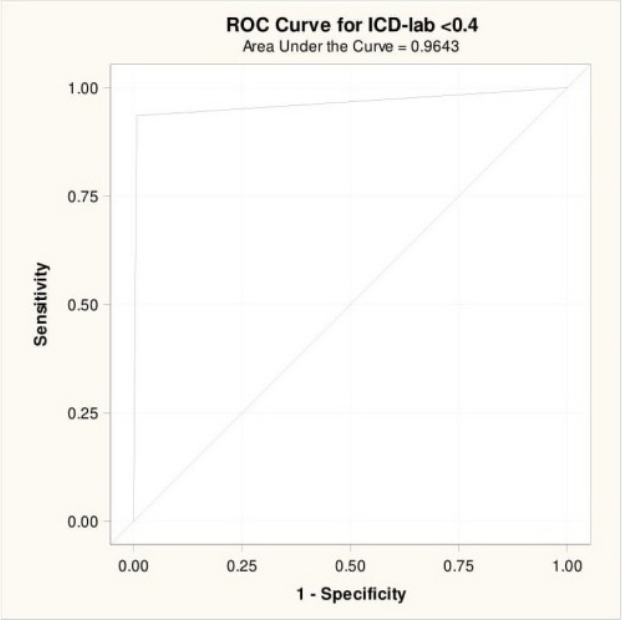

FIGURE 1 ROC curve of diagnostic algorithm using ICD code + coagulation FVIII < 0.4

adequate numbers of patients. Administrative databases offer large populations for study, although validation of the coding accuracy is essential.

Aims: The aim of this study was to evaluate the accuracy of using a combination of International Classification of Diseases (ICD) diagnostic codes and coagulation factor level for identifying hemophilia A within administrative databases.

Methods: Our baseline population was people of all ages who were tested for factor VIII (FVIII) activity in the province of Alberta, Canada between 2004-2019. To calculate the validity of our algorithm (ICD codes for hemophilia A and FVIII activity < 0.4), we aimed for a sample size which will allow us to detect high sensitivity and specificity. Medical charts of randomly selected patients were reviewed to confirm true cases of hemophilia A. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) were calculated.**Results**: Of 11053 patients who had FVIII testing, 2114 were randomly sampled for chart review. The mean age was 39 years and 71% were female. According to chart review, 62 (2.9%) patients have a confirmed diagnosis of hemophilia A. With our predefined algorithm, 72 (3.4%) patients met the combined ICD code and laboratory criteria. The sensitivity, specificity, PPV and NPV of our algorithm were 93.5%, 99.3%, 80.5% and 99.8% respectively (Table 1). Our diagnostic algorithm has excellent discriminant properties (c-statistic 0.964; Figure 1) and yielded similar results between pediatric and adult populations.

Conclusions: When combined with low (<0.4) coagulation factor level, ICD diagnostic codes obtained from administrative data provide a highly sensitive and specific method to ascertain cases of hemophilia A.

PB1012 | Association Between Aging and Health Status in Persons Leaving with Hemophilia and Controls Without a Bleeding Disorder - Insights from the PROBE Study

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Background: People with hemophilia (PWH) have a life expectancy disadvantage as compared to the general population but little is known about the impact of aging on health status and health-related quality of life (HRQL).

Aims: Assessing the characteristics of the association between aging, health status and HRQL in PWH and people with no bleeding disorders (PWNoBD).

Methods: A cross-sectional, multinational study was conducted as part of the Patient Reported Outcomes, Burdens and Experiences (PROBE) project. PWH and PWNoBD were asked to complete the PROBE questionnaire. Measures of health status and HRQL were the PROBE score, the EQ-5D-5L utility index, and the EuroQol visual analog scale (EQ-VAS) of global health. The association between

TABLE 1 Demographics of the study population

People with hemophilia (n = 1157)*				
	Age category			
	18 – 44	**45 – 64**	**65 – 74**	**≥ 75**
n (%)**	732 (63)	331 (29)	73 (6)	21 (2)
Income***				
Low Middle	65 (9)	10 (3)	0 (0)	0 (0)
Upper Middle	302 (41)	40 (12)	2 (3)	3 (14)
High	365 (50)	281 (85)	71 (97)	18 (86)
Male (%)	706 (96)	317 (96)	71 (97)	20 (95)
Hemophilia severity				
Mild (FL 0.05 – 0.40 IU/mL)	72 (10)	70 (21)	23 (32)	7 (33)
Moderate (FL 0.01-0.05 IU/mL)	136 (19)	50 (15)	7 (10)	8 (38)
Severe (FL < 0.01 IU/mL)	524 (72)	211 (64)	43 (59)	6 (29)
No. Of Comorbidities [Median (range)]	1 (0 -8)	1 (0 -9)	1 (0 – 7)	1 (0 – 4)
PROBE Score (n = 982) [median (Q1; Q3)]	0.76 (0.64; 0.85)	0.68 (0.57; 0.78)	0.71 (0.64; 0.80)	0.67 (0.52; 0.84)
EQ-5D (n = 1109) [median (Q1; Q3)]	0.82 (0.70; 0.92)	0.75 (0.62; 0.84)	0.76 (0.63; 0.88)	0.68 (0.52; 0.86)
VAS (n = 1228) [median (Q1; Q3)]	0.75 (0.60; 0.90)	0.70 (0.50; 0.80)	0.73 (0.60; 0.84)	0.62 (0.50; 0.80)
People with no bleeding disorders (n = 690)				
	Age category			
	19 – 44	**45 – 64**	**65 – 74**	**≥ 75**
n (%)**	335 (49)	272 (39)	71 (10)	12 (2)
Income***				
Low Middle	12 (4)	3 (1)	0 (0)	0 (0)
Upper Middle	95 (28)	53 (19)	15 (21)	4 (33)
High	228 (68)	216 (79)	56 (79)	8 (67)
Male (%)	144 (43)	116 (43)	36 (51)	4 (33)
No. Of Comorbidities [Median (range)]	0 (0 - 6)	0 (0 - 6)	0 (0 - 5)	1.5 (0 - 3)
PROBE Score (n = 580) [median (Q1; Q3)]	0.92 (0.83; 0.98)	0.90 (0.79; 0.96)	0.86 (0.79; 0.93)	0.75 (0.69; 0.89)
EQ-5D (n = 666) [median (Q1; Q3)]	0.92 (0.83; 0.98)	0.94 (0.87; 1.00)	0.94 (0.83; 1.00)	0.84 (0.50; 1.00)
VAS (n = 675) [median (Q1; Q3)]	0.85 (0.75; 0.90)	0.80 (0.75; 0.90)	0.85 (0.80; 0.90)	0.80 (0.65; 0.88)

TABLE 2 Results of the multivariate analysis using a generalized linear model

	PROBE Score n = 1562		*EQ-5D utility index n = 1775*		*EQ VAS n = 1803*	
Explanatory variable	**Coeff***	**95% CI**	**Coeff***	**95% CI**	**Coeff***	**95% CI**
Age	-0.0010	-0.0017; -0.0004	-0.0003	-0.0012; 0.0007	0.0000	-0.0009; 0.0009
*Interaction Age*PWH*	-0.0015	-0.0022; -0.0008	-0.0031	-0.0041; -0.0021	-0.0030	-0.0047; -0.0014
Male sex	0.0125	-0.0145; 0.0396	0.0129	-0.0145; 0.0404	0.0061	-0.0182; 0.0304
NoBD	Ref	-	Ref	-	Ref	-
Mild	-0.0282	-0.0749; 0.0184	0.0634	-0.0028; 0.1297	0.0851	-0.0001; 0.1702
Moderate	-0.1241	-0.1668; -0.0813	-0.0461	-0.1233; 0.0312	0.0014	-0.1011; 0.1039
Severe	-0.1152	-0.1582; -0.0722	-0.0780	-0.1524; -0.0036	-0.0143	-0.0942; 0.0657
Constant	0.9130	0.8855; 0.9405	0.9166	0.8720; 0.9613	0.8161	0.7686; 0.8636

*The regression coefficients can be interpreted as the adjusted mean difference in the score. For the PROBE score and VAS, that ranges from 0 to 1, the coefficient times 100 is equal to the percentage variation in the score, with a negative sign indicating a reduction. For EQ-5D utility value, that ranges from -0.594 to 1, the coefficient needs to be reported to a scale 0-100 before being interpreted as a percentage variation.
PROBE: Patient Reported Outcomes, Burdens and Experiences; EQ-5D: EuroQol 5-dimensions; EQ VAS: EuroQol visual analog scale; CI: confidence interval, PWH: person with hemophilia.

these measures and the age of participants was explored using a multivariable model adjusting for sex (all) and disease severity (PWH only) and stratifying by country.

Results: 1157 PWH and 690 PWNoBD completed the questionnaire in 33 countries from 2016 to 2019. Study population demographics are reported in Table 1. Eight percent of the PWH and 12% of the PWNoBD were aged ≥ 65 years. Multivariable analysis results are reported in Table 2. As expected, the EQ-5D utility index and EQ-VAS did not show a variation with aging in PWNoBD, while in PWH they were reduced respectively by 0.031 (95% confidence interval [CI] 0.021-0.041) and 0.030 (95% CI 0.014-0.047) every 10 years. Aging was associated with a mean reduction in the PROBE score of 0.010 (95% CI 0.004 to 0.017) every 10 years in PWNoBD. PWH showed an additional reduction in the PROBE score of 0.015 (95% CI 0.008 to 0.022) every 10 years.

Conclusions: Aging is associated with a steeper decrease in health status and HRQL in PWH than in PWNoBD. PROBE is more sensitive than EQ5D in measuring the association of aging for the specific domains measured in both PWH and PWNoBD.

*Unless otherwise specified **Row percentage (across age categories), all other percentages are within risk categories. ***This refers to the average per-capita income of the country according to the World Bank.

Q1: first quartile; Q2: third quartile; PROBE = patient reported outcomes burden and experience; VAS = visual analogue scale

PB1013 | Inhibitor Development in Hemophilia A - Refining the Risk Assessment

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Background: Several studies have investigated the intricate pathophysiological mechanisms leading to the development of FVIII inhibitors in hemophilia A patients. However, the etiology of their onset is very complex and is not yet fully understood. The inhibitors arise from a multicausal immune response involving both genetic (unmodifiable) and environmental (potentially modifiable) risk factors.

Aims: To address the need for a holistic approach to provide advanced risk management.

Methods: Report the current knowledge of genetic and environmental risk factors on inhibitor development.

Results: Two well-established unmodifiable risk factors are family history of inhibitors and the causative mutation's nature (type of genetic variant affecting the *F8)*.

Polymorphisms in immunomodulatory genes have also been found to play a role in differentiating individual response.

Although the mechanisms accounting for ethnicity differences remain somewhat unclear, it is well known that patients of African or Hispanic ancestry have an increased predisposition to develop inhibitors.

As for environmental factors, inhibitor development is surely affected by the source and type of the FVIII product and as well by treatment intensity.

Severe bleeds, surgery or concomitant infections have also been implicated in the context of concurrent immunological danger signals that up-regulate the immunologic reaction against exogenous FVIII.

Conclusions: Overall, inhibitor development is a phenomenon of multifactorial inheritance described by the liability/threshold model. Such an elaborate net of genetic and environmental interactions made it extremely difficult to create a clinical score system to quantify the individual risk.

Nowadays new large-scale hypothesis-generating approaches can provide the tools to study the personal specific array of multiple

risk factors concurrently and identify the unique risk profile of each patient. Moreover these innovative technologies may allow timely reassessing the risk when a transient factor comes along.
From these revolutionary resources will be rising the opportunity to evolve new preventive approaches and develop novel therapeutic strategies.

PB1014 | Subclinical deep venous thrombosis identified in 7,5% of people with hemophilia undergoing major orthopedic surgery in the absence of pharmacological thromboprophylaxis

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Background: Deep venous thrombosis (DVT) is a common postoperative complication in patients undergoing major orthopedic surgery of the lower limbs, such as total hip or knee replacement (THR, TKR). Routine pharmacological thromboprophylaxis with low-molecular-weight heparin (LMWH) or a direct oral anticoagulant agent is strongly recommended in patients undergoing these procedures. THR and TKR as well as ankle arthrodesis are frequently performed in people with hemophilia (PWH) suffering from chronic hemophilic arthropathy. Pharmacological thromboprophylaxis in this population remains controversial.
Aims: Identify the incidence of subclinical DVT in PWH undergoing major orthopedic surgery.
Methods: We report the results of a prospective study initiated in 2002 at the Cliniques universitaires Saint-Luc, Brussels, evaluating by systematic bilateral US-Doppler imaging the incidence of subclinical DVT in consecutive PWH referred for major orthopedic surgery and not receiving pharmacological thromboprophylaxis.
Results: The study included 45 different PWH (38 HA, 7 HB, 26 severe, 15 moderate and 4 mild forms) undergoing 66 orthopedic procedures of the lower limbs. Most procedures (89%) were performed with continuous infusion of clotting factor concentrates. Almost all patients received tranexamic acid on the day of surgery, wore compression stockings and started rehabilitation on day 1 post-op. No case of clinical DVT or PE was suspected. In total there were 5 cases (3 severe -1 moderate HA -1 moderate HB) of subclinical DVT which all were distal, not complicated by clinically patent PE. Two patients were treated with a short course (10-14 days) of LMWH and 3 had a spontaneous resolution. The overall incidence of DVT was 7.5% for all the procedures.
Conclusions: These data provide imaging-based evidence that the risk of DVT following major orthopedic surgery among PWH is low. Identified distal DVTs were distal and resolved spontaneously in most cases. Systematic pharmacological thromboprophylaxis in this specific population is probably for most patients not required.

PB1015 | Joint Damage in Non-severe Hemophilia A - Preliminary Results from an Explorative Study

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Background: Joint bleeds are the hallmark of severe hemophilia and may give rise to development of hemophilic arthropathy. In non-severe hemophilia, hemarthroses generally occur less often. However, it has been hypothesized that even subclinical joint bleeds may lead to joint deformation. To date, the extent of joint damage in non-severe hemophilia is unknown.
Aims: To describe the joint status in a cohort of non-severe hemophilia A patients.
Methods: The ongoing DYNAMO study evaluates joint status in non-severe hemophilia A patients (FVIII 2-35 IU/dL, age 24-55 years) at two time points two years apart. Elbows, knees and ankles are assessed by use of the Haemophilia Joint Health Score (HJHS) and by magnetic resonance imaging (MRI) on the same day. MRI's are reviewed by a musculoskeletal radiologist blinded to clinical data. The study was approved by ethics committees and written consent was obtained (ClinicalTrials.gov Identifier: NCT03623295).
Results: We included 27 non-severe hemophilia A patients with a median age of 43 years (IQR 30-51) and a median FVIII of 8 IU/dL (IQR 5-14). The median HJHS score was 3 out of 124 (IQR 2-6). The MRI IPSG score was calculated from 95 joints in 17 patients (7 joints were not evaluated). The median IPSG score was 5 out of 102 (IQR 2.5-10.0). Lifetime and 10-year bleeding history was retrospectively collected for 15 and 11 patients, respectively. Eight patients had a positive lifetime history of hemarthrosis. The median annual joint bleed rate was 0 (IQR 0.0-0.1). MR imaging demonstrated that ankles (26/33 joints) were more frequently affected than knees (18/34 joints) and elbows (5/28 joints). The most frequent findings over 33 ankle joints were hemosiderin (48%), effusion (42%), cartilage degradation (39%) and synovial hypertrophy (33%). Further analyses will be presented at ISTH 2020.
Conclusions: A substantial part of non-severe hemophilia patients in this cohort has minor joint damage.

PB1016 | Haemophilia Breakthrough in Developing Countries. Is it Possible?

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Background: Managing of haemophilia patients in developing countries is a challenge. Strong interaction of patients with the interdisciplinary team working all together as one is the initial step.

Aims: Develop customized clinical rounds with ultrasound evaluation support, facilitate access to an all-in-one evaluation by the interdisciplinary team and bring medical assessment closer to the recreational environment are our main goals.

Methods: Data review was obtained from a personalized assessment of patients during a non medical environment medical approach. Interdisciplinary team is made up of hematologist, dentistry, radiologist, orthopedist, therapist and psychologists. Type of haemophilia, age, treatment, oral health and articular status were collected, including active joints that were needed to be studied by ultrasound (US).

Results: 23 haemophiliac patients data was collected, 22 were Haemophilia A patients, 19 jun severe (3 of them with inhibitors and 17 receiving prophylaxis), and one severe Haemophilia B. Target joints were present in a 60%, with ankle affection in 13 patients, 8 in knee and 5 in elbow. Although almost all patients felt asymptomatic, only 34% demonstrated disease-free joint ultrasound. The rest presented synovial/cartilaginous/bone commitment including active bleeding in 2 patients that had to be infused. Odontological evaluation showed 56% of them had untreated caries.

Care strategies proposal has been based on 3 fundamentals:

1) Allow clinical round interdisciplinary evaluation to respond specific concerns and needs of each patient.

2) Establish personalized assessment from disciplines all together being carried out in one day

3) Optimize resources such as Ultrasound, an economic, harmless and highly diagnostic method, accessible to all levels of care.

Widely proven to be unconditional support in the Haemophiliac clinical evaluation.

Conclusions: Dynamic and fused coordination of a highly committed interdisciplinary team is the key to our achievements, adapting to patients needs and to our economic reality of developing south American country.

PB1017 | Measuring the Impact of Hemophilia on Families

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Background: Currently, there are no disease specific tools to measure the impact of hemophilia on families. While there are several hemophilia specific quality of life measures, none are valid for the entire pediatric age span, especially during the challenging time of diagnosis and management of young boys with hemophilia.

Aims: To develop and validate a tool to measure the impact on families of a diagnosis of hemophilia.

Methods: Phase I utilized focus groups with parents of boys with hemophilia and hemophilia health care providers (HCPs) across Canada to generate items for a hemophilia Family Impact Tool (H-FIT). Phase II implemented the H-FIT and the Pediatric Quality of Life Family Impact Module (PedsQL-FIM), to assess the validity of the new tool. All participating centres received ethics approval; all participants provided informed consent.

Results: Phase I participants were 23 parents and 20 HCPs in focus groups. Phase II participants were 72 parents who completed the H-FIT and the PedsQL-FIM.

The focus groups generated themes specific to hemophilia that were important to parents and HCPs, and were not covered by the generic PedsQL-FIM, such as: stress surrounding treatment administration, guilt resulting from the transmission of hemophilia, and confidence

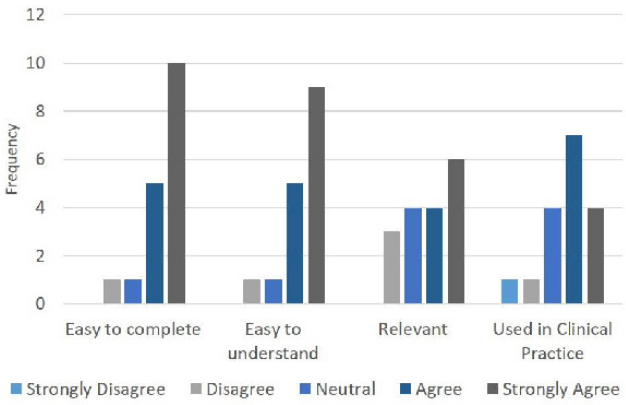

FIGURE 1 Parental agreement with ease of completion, ease of understanding, relevance to their families, and clinical usefulness of the H-FIT

educating others about hemophilia. This process generated 16 new items that were included in the H-FIT.

Data from Phase II demonstrated a strong correlation (r = 0.76, p < 0.001) between the H-FIT and PedsQL-FIM. The correlations for two age sub-groups were: r = 0.64 (p = 0.006) and 0.70 (p = 0.005) for parents of boys ≤ 4 (n = 17) and 5-7 (n = 14) years of age respectively. The majority of parents agreed that the H-FIT is relevant and should be used in clinical practice (Figure 1).

Conclusions: A novel, disease-specific tool has been developed to measure the impact of hemophilia on families, the H-FIT, which may be especially relevant for parents of boys ≤ 7 years.

PB1018 | ATHN 7: A Natural History Cohort Study of the Safety, Effectiveness, and Practice of Treatment for People with Hemophilia-Demographics and Preliminary Results

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Background: Introduction of non-factor molecules mimicking the effect of activated factor VIII or inhibiting endogenous anticoagulants mandates longitudinal understanding of their safety and effectiveness. Utilizing the national infrastructure of the American Thrombosis and Hemostasis Network (ATHN), ATHN 7: Hemophilia Natural History Study aims to collect such data in a cohort of participants with hemophilia.

Aims: The primary objective of ATHN 7: Hemophilia Natural History Study is to determine the safety of current FDA-approved hemophilia therapies. Safety will be measured by those events in the European Haemophilia Safety Surveillance (EUHASS) outcomes and other adverse events of special interest. Secondary objectives include determining the real-world effectiveness of therapies including patient-reported outcomes.

Methods: This longitudinal cohort study, currently open in 25 ATHN-affiliated sites in the United States, was approved by institutional review boards. All participants signed informed consent. Inclusion criteria included the presence of congenital hemophilia A or B. Participants were excluded if they had any other known bleeding disorder. See Figure 1 for data collection procedures.

Results: As of August 2019, 152 participants were enrolled. The mean age of participants was 19.1 years (range less than one to 80 years) with 144 having hemophilia A and 5 having hemophilia B. For three participants, a diagnosis was not provided. Ten (6.6%) participants had inhibitors at the time of enrollment. Fifty percent of participants were utilizing non-factor products, 45% recombinant factor, and 5% plasma-derived factor. To date, no safety events were reported.

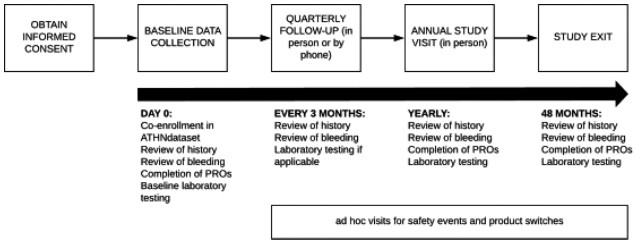

FIGURE 1 ATHN 7 Data Collection Procedures

Conclusions: ATHN 7: Hemophilia Natural History Study demonstrates the capability of ATHN's national infrastructure to prospectively collect longitudinal data to determine the safety and effectiveness of current therapies for those with hemophilia. The study is early in accrual, so final conclusions as to the safety and effectiveness of current hemophilia therapies cannot yet be drawn.

PB1019 | Management of Hemostatic Balance in Elective Joint Replacement Surgery in Patients with Haemophilia

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Background: Hemophilia is a genetic or acquired disease that leads to spontaneous and recurrent bleedings, which affect the joints and muscles, thus determining chronic damage to the cartilage which will lead to joint disease and hemophilic arthropathy. Even though hemophilic patients were initially thought to have a low incidence of atherothrombotic complications, it is now clear that atherothrombotic events occur.

Aims: This clinical study aims to underline the best manner of treating patients with elective joint replacement and hemophilia.

Methods: We analyzed five patients with hemophilia type A, aged between 35 and 62 years. Two of them had a severe form of hemophilia with factor VIII less than 1%, while the other three had a moderate form with factor VIII ranging between 1 and 5%. The five patients underwent total knee repair interventions and received substitution treatment with clotting factors but also prophylactic anticoagulant treatment.

Results: The postsurgical evolution of these patients was favorable, with similar hemostatic profile as the non-hemophilic patients. Moroctocog alfa is an efficient substitutive treatment that manages to normalize the hemostatic profile of patients.

Conclusions: Therefore, it is recommended to provide prophylactic antithrombotic therapy after the orthopedic interventions in hemophilic patients.

PB1020 | Treatment Patterns and Bleeding Outcomes in Persons with Severe Hemophilia A and B in a Real-World Setting

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Background: Current standard of care treatment for severe hemophilia A and B (SHA and SHB) is prophylactic intravenous replacement of coagulation factor VIII or IX (FVIII/FIX) to prevent spontaneous bleeding. Persons with hemophilia without prophylactic treatment receive therapy in case of bleeding, i.e. on-demand.
Aims: To assess treatment patterns, utilization of products, and bleeding outcomes in a real-world cohort of persons with SHA and SHB, defined as FVIII or FIX activity < 1%, in the era of standard half-life products.
Methods: Data was retrospectively collected from hemophilia-specific patient diaries used for home treatment, medical records and entries into the Austrian Hemophilia Registry from year 2012 to 2017, a time-period where extended-half life products were not routinely used.
Results: Fifty-three male persons with SHA (n = 47) and SHB (n = 6) were included; 26 with SHA and 5 with SHB were on prophylaxis, 8 and 1 switched therapy regimen and 13 and 0 received on-demand therapy. A detailed description of the study cohort and study results is shown in Table 1 and Table 2. Persons on prophylaxis used a mean factor FVIII or FIX dose of 71.7 and 40.1 IU/kg/week. Median (IQR) annualized bleeding rates (ABR) in SHA were 28.0 (23.4-31.3) in the on-demand and 4.9 (1.6-13.5) in the prophylaxis group, and 3.0 (2.0-6.8) in the prophylactic group of SHB. Three persons with SHA had zero bleeds during the observation period. On-demand therapy and hepatitis B and C were associated with higher ABR, but not age, weight and HIV positivity.
Conclusions: Bleeding rates and proportion of on-demand therapy in persons with hemophilia were high in our real-world cohort. Further improvement is needed, which might be facilitated with the advent of factor products with extended half-life or non-factor therapies.

TABLE 1 Description of the study cohort and demographics

	Severe Hemophilia A (n = 47)	Severe Hemophilia B (n = 6)
Median (IQR) age, years	38 (30-47)	38 (28.3-58.3)
Median (IQR) height, cm	178.5 (173.8-181.0)	176.5 (167.8-180.3)
Median (IQR) weight, kg	79 (69-90)	72.5 (68.8-84.8)
Median (IQR) body mass index (BMI)	24.7 (2.6-28.1)	23.8 (22.1-27.2)
Median (IQR) observation time, days	1096 (744-1461)	1374 (365-1552)
Median (IQR) number of infusions (i.e. exposure days) during observation per patient	325 (114-471)	294 (107-360)

TABLE 2 Treatment patterns and factor utilization in patients with severe hemophilia A and B

	Severe Hemophilia A (n = 47)	Severe Hemophilia B (n = 6)
Treatment regimen *Prophylaxis, n (%)*	26 (55.3)	5 (83.3)
On-Demand, n(%)	13 (27.7	0 (0)
"Switcher"-group, n (%)	8 (17.0)	1 (16.7)
Prescribed factor dosage in prophylaxis *Median (IQR) IU/kg/week*	*n = 31** *66.7 (53.3-87.0)*	*n = 6* *40.1 (30.9-57.3)*
Mean (±SD) IU/kg/week	71.7 (±23.4)	42.5 (±12.9)
Factor product type, n (%) *Recombinant*	35 (74.5)	2 (33.3)
Plasma-derived	12 (25.5	4 (66.7)
Median (IQR) factor consumption (IU/year)	190051 (89250-254892)	143655 (109662-223353)

*For prescribed factor dosage in the prophylaxis group, n is lower as this value could only be calculated for subjects who were on prophylaxis at some point in the observation period. This includes all subjects with SHA on prophylaxis and 5 subjects from the "Switcher"-group who were prescribed a defined prophylactic regimen. For 3 subjects of the „switcher-group" with SHA, prescribed weekly prophylactic infusions were unknown.

PB1021 | Comorbidities of Hemophilia & Factor XIII Deficiency Patients

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Background: Recent treatments, and widespread improvements in supportive care, are always modifying the demography of hemophilia. The life expectancy of hemophiliac patients is increasing.
On the other aspect, the prevalence of Comorbidities such as degenerative arthropathies, viral infections, cardiovascular diseases increases dramatically
Aims: To determine the prevalence of comorbidities among hemophilia A and hemophilia B and factorXIII deficiency patients in southeast of Iran

Methods: The 144 hemophilia and FactorXIII deficiency patients older than ten years old included in a cross-sectional study.
We collected the demographic information, and a thorough clinical examination carried out at baseline and d six-month intervals for two years from 2016 to 2018.
We assessed the joint health with Gilbert score, which is a physical examination score for the evaluation of hemophilic joints.
Results: The median age of patients was 24.7 ± 9.9. The median knee and elbow arthropathy, according to Gilbert score in hemophilia & FrXIIID patients was statistically significant (Pvalue < 0.0001).
Evaluation of viral profiles revealed 2 FrXIIID and 12 hemophilic patients infected with Hepatitis C Virus, which is statistically significant (Pvalue < 0.004).
The systolic and diastolic evaluation was without any significant correlation.
23(31.5%) FrXIIID patients and 11(15.5%) hemophilia patients had Intracranial hemorrhage, respectively.
3 (4.1%) FrXIIID patients, and 1 (1.4%) hemophilia patients diagnosed with Hepatocellular carcinoma, which is without significance.
(Pvalue < 0.320).
Conclusions: Hemophilic patients with comorbidities are tend to receive suboptimal carehence knowing the prevalence of specific comorbidities is essential.

PB1022 | The Clinical Phenotype of Patients with Non-severe Hemophilia A and B

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Background: Patients with non-severe hemophilia have a milder phenotype compared to patients with severe hemophilia. Nevertheless bleeding episodes elicited by trauma or surgery may have a strong impact on the lives of patients with non-severe hemophilia. Detailed knowledge on the frequency and severity of bleeding in non-severe hemophilia is scant.
Aims: To provide insight in the clinical phenotype of patients with non-severe hemophilia A and B.
Methods: The DYNAMO study is an observational cohort study including hemophilia A and B patients with a baseline clotting factor VIII/IX level of 2-35 IU/dL, aged 12-55 years. Global data on lifetime bleeding history and detailed information on all bleeding episodes from 2009 to present is collected from medical files. The study was approved by medical ethics committees and written consent was obtained (ClinicalTrials.gov Identifier: NCT03623295).
Results: In this preliminary analysis, 101 patients were included (72 HA, 29 HB). The median age was 34 years (IQR 26-48) and the median factor level was 11 IU/dL (IQR 5-17). Eighty patients (79%)

TABLE 1 Baseline characteristics, lifetime bleeding history and bleeding history treated with factor concentrate over the period 2009-present

	Hemophilia A (n=72)	Hemophilia B (n=29)	Total (n=101)
Median factor level in IU/dL	12 (IQR 5-17)	11 (IQR 4-16)	11 (IQR 5-17)
Lifetime bleeding history			
Treated bleed	57 (79%)	23 (79%)	80 (79%)
Treated joint bleed	34 (47%)	11 (38%)	45 (45%)
Intracranial hemorrhage	0	2 (7%)	2 (2%)
Bleeding history over last 10 years			
Number of bleeds	420	128	548
Number of joint bleeds	137	39	176
Number of muscle bleeds	143	46	189

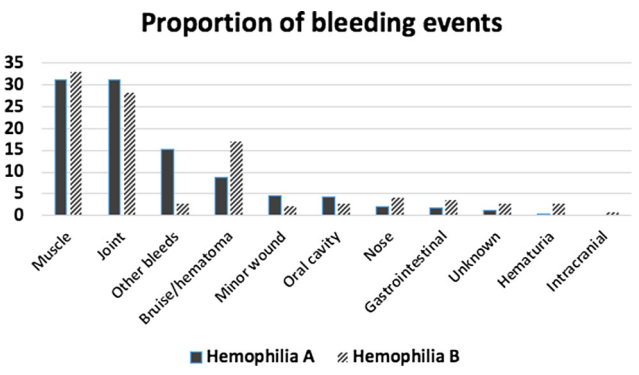

FIGURE 1 The proportion of treated bleeding events for the total hemophilia population (n = 101) over the period 2009-present

experienced at least one bleed that required treatment with factor concentrate (table 1). In the last 10 years, thirty-four patients experienced a total of 176 treated joint bleeds, of which 25 (14%) occurred spontaneously. The majority of all joint bleeds (134/176) and of spontaneous joint bleeds (19/25) were seen in 16 patients with moderate hemophilia. The proportion of joint bleeds out of the total number of treated bleeds was similar between hemophilia A and B (31% and 28% respectively, fig. 1).

Conclusions: Our results suggest that non-severe hemophilia patients may have serious bleeding episodes. On the other hand, 21% of patients have never been exposed to factor concentrate for the treatment of a bleed. These preliminary results highlight the need for careful monitoring of this patient population.

PB1023 | Efficacy of Platelet-rich Plasma in the Treatment of Hemophilic Arthropathy

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Background: The most clinical manifestation of haemophilia as a congenital bleeding disorder is the Intra-articular haemorrhage.
The regenerative methods such as intraarticular platelet-rich plasma may herald a new era for the treatment of young patients with Hemophilic Arthropathy.

Aims: This study aimed to evaluate the effectiveness of PRP treatment for the reduction of pain and improvement of physical function in hemophilic arthropathy patients.

Methods: Consecutive patients with hemophilic arthropathy included in a self-control open-label trial in Mofid Children's Hospital, Tehran Iran. All participants received three injections of PRP 2 weeks apart. The seriate self-reported and objective clinical outcomes evaluated before and three months after therapy.

Results: A total of 11patients with 14 joints treated at our centre with hemophilic arthropathy received three injections of PRP at 2-weeks intervals. All patients evaluated with a visual analogue scale (VAS), Hemophilia joint health score(HJHS), range of motion(ROM), and the existence of bleeding events before and after the treatment. The patients 9 (81.8%) boys, 2 girls(18.2%) completed the study. The Mean age was 14.7 years old (9-19 years old). Statistically, significant improvements noted in all of the evaluated measures in all of the patients.

Conclusions: Platelet-rich plasma therapy is a reasonable and minimally invasive procedure in which the chondroregenerative properties could be beneficial.

PB1024 | Quality of Life in Children with Congenital Bleeding Disorders: A Scoping Review of Current Research State and Knowledge Gaps

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Background: Quality of Life (QoL) in children with bleeding disorders can be affected. To measure QoL in developing children, input of children and parents is needed. This can be done by generic questionnaires and disease-specific questionnaires, that contain questions referring to typical disease-related issues, such as bleeding symptoms.

Aims: The aim of this scoping review was to analyze current knowledge on QoL in children with all forms of congenital bleeding disorders in order to identify knowledge gaps.

Methods: We performed a systematic literature search on April 29th 2019. Articles were screened on title/abstract by two independent reviewers using a preset inclusion tool. Eligible articles were read in full, leading to a final inclusion of 37 articles.

Results: Two-third of studies were performed in Europe and North-America, and published after 2010 (86,4%). Only 34,3% included children < 4 years of age. In 31 articles (83.8%) patients had hemophilia, but only 9 included a homogeneous group of the same disease and severity. Fourteen articles pooled results of patients with different types of bleeding disease or severity. Thirteen different generic and five different disease-specific questionnaires were used. Most studies only used self-reports, sometimes combined with proxy-reports. Ten articles used a reference group. Mean and SD were most often used as descriptive statistics.

Conclusions: QoL research in children with congenital bleeding disorders is an upcoming research field, but it is mainly performed in school-aged children with hemophilia, who are treated in well developed countries. Pitfalls are the pooling of various bleeding disorder types and severity, the lack of parent perspectives, the lack of non-hemophilia disease-specific questionnaires. Too many different generic QoL questionnaires are used, results not comparable between themselves. QoL was rarely measured to evaluate the effect of a therapy. Parameters as mean or SD are of less use in heterogeneous groups.

PB1025 | Immune Tolerance Induction (ITI) with a Plasma-derived Factor VIII for Patients with Hemophilia A and Inhibitors: A Retrospective Study

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Background: A serious complication in hemophilia A is the formation of inhibitors to clotting factors. The primary means for eradicating inhibitors is immune tolerance induction (ITI) therapy. Antihemophilic factor (human) is indicated for the treatment of hemophilia.

Aims: This multicenter retrospective data collection project evaluated patient- and treatment-related factors associated with outcomes following primary or rescue ITI with an antihemophilic factor (human) in patients with hemophilia A and high titer inhibitors.

Methods: Medical records of 13 inhibitor patients treated with antihemophilic factor (human) for primary or rescue ITI therapy between January 1, 2012, and July 31, 2017, was conducted in five US hemophilia treatment centers. To be eligible, patients were required to have a diagnosis of hemophilia A of any severity level, inhibitor to FVIII at the time of treatment initiation with antihemophilic factor (human), and ongoing treatment with antihemophilic factor (human) for primary or rescue ITI. Data were de-identified and analyzed descriptively. Outcome measures were defined, per the International Immune Tolerance consensus recommendations, as "complete success", "partial success", or "failure".

Results: All patients met the inclusion criteria. Ten of 13 patients (76.9%) had successful ITI; seven with complete success and three with partial success. Three patients failed ITI. As primary therapy, complete success was obtained with all but one of the six patients treated with antihemophilic factor (human). These six patients were all older than 7 at the initiation of ITI. Seven of the 13 patients had a combined previous 12 attempts with other products. Of these seven rescue patients, ITI with antihemophilic factor (human) was completely successful in two and partially successful in three.

Conclusions: While retrospective data has limitations, real-world evidence demonstrates that ITI with antihemophilic factor (human) concentrate can be successful or partially successful in diverse populations of moderately complex patients with hemophilia A and inhibitors.

PB1026 | Use of Desmopressin in the Treatment of Bleeding in Non-severe Haemophilia A

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Background: Desmopressin is a synthetic hormone used therapeutically in hemophilia A for the induction of an increase of endogenous factor VIII (FVIII). The response to desmopressin shows large inter-individual variation. When effective, desmopressin is an important alternative to the use of FVIII concentrates in the treatment of bleeding. Its benefits are its lower cost and lack of inhibitor risk when compared to FVIII concentrates. Presently we lack comparative information on the use of desmopressin and FVIII concentrates in the treatment of bleeds in non-severe hemophilia A.

Aims: To analyze the use of desmopressin in the treatment of bleeds in patients with non-severe hemophilia A.

Methods: The DYNAMO study is a multicenter cohort study that includes patients with hemophilia A with a baseline FVIII level of 2-35 IU/dL. Data on desmopressin and bleeding episodes over the past 10 years were collected. The study was approved by the medical

TABLE 1 Percentage of bleeds treated with desmopressin, FVIII or a combination of both per desmopressin response category

Desmopressin response	Inadequate (n = 8)	Adequate (n = 15)	Excellent (n = 41)	Total (n = 64)
Total number of bleeds	59	43	88	195
Treatment option				
FVIII	58 (98%)	16 (37%)	21 (23%)	95 (49%)
FVIII + desmopressin	-	11 (26%)	34 (37%)	45 (23%)
Desmopressin	-	16 (37%)	33 (35%)	49 (25%)

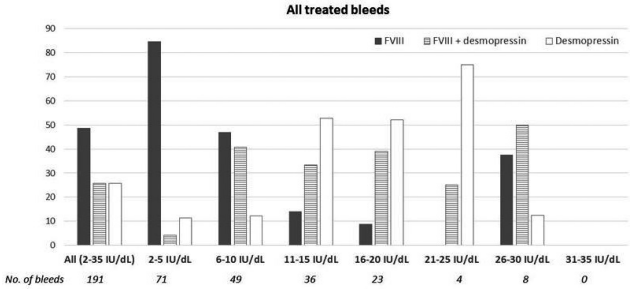

FIGURE 1 Percentage of bleeds treated with factor concentrate, desmopressin and a combination of both according to baseline FVIII activity

ethics committee and written consent was obtained (ClinicalTrials.gov Identifier:NCT03623295).

Results: In this preliminary analysis, we included 64 patients with non-severe hemophilia A in whom the desmopressin response was known. The median age was 31 years (IQR 23-47) and the median baseline FVIII level was 13 IU/dL (IQR 6-18). The desmopressin peak response was < 30 IU/dL (inadequate) in 8 (13%) patients, between 30-50 IU/dL (adequate) in 15 (23%) patients and > 50 IU/dL excellent in 41 (64%) patients. In total, 195 bleeds required treatment, with 95 (49%) treated with FVIII concentrates, 49 (25%) with desmopressin and 45 (23%) with a combination of both (table.1). Joint and muscle bleeds occurred most frequently (N = 110) and were mainly treated with FVIII concentrates (54%) or a combination of FVIII concentrates and desmopressin (30%)(figure 1).

Conclusions: Desmopressin was used either in combination or as solo treatment in 43% of all treated bleeds and should be considered as treatment option in patients with non-severe hemophilia.

PB1027 | The Parameters of Modified Clot Waveform Analysis Using the Plasmas of Hemophilia A Patients Treated with Emicizumab

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Background: Recently, emicizumab (Emi) has become used for hemophilia A patients. However, it has not been known how to evaluate patients' coagulation ability in the presence of emicizumab. Nogami et al reported that their coagulation ability was able to measure by using modified clot waveform analysis (mCWA) under a such situation.

Aims: Our objective is to clarify whether we can evaluate coagulation abilities in hemophilia A patients treated with Emi using mCWA.

Methods: We obtained plasmas drawn from seven hemophilia A patients (involved 2 with FVIII inhibitor) treated with Emi. We performed mCWA, in the same way as the method of Nogami et al, on the CS-5100 (Sysmex) instrument with a trigger reagent comprising an optimized mixture of tissue factor and ellagic acid. We divided a sample into two and stored below -80 degrees until measurement. Then, one was directly performed modified CWA, and the other was performed after incubating with anti-emicizumab antibody (AEA), which was provided by Chugai Cooperation. The parameters of mCWA were selected maximum velocity (adjusted-|min1|), coagulation maximum acceleration (adjusted-|min2|), coagulation maximum negative acceleration (adjusted-Max 2).

Results: The data of normal samples and AEA added samples measured with mCWA did not have the significant difference in all parameters. The detail was as follows. Median of adjusted-|min1| in normal samples was 4.31 (min3.70- max4.57) and that in adding samples was 4.34 (4.04- 4.43). Median of adjusted-|min2| in normal samples was 0.48 (0.40- 0.52) and that in adding samples was 0.51 (0.41-0.53). Median of adjusted-Max 2 was 0.17(0.13- 0.18) and 0.16(0.16-0.17), respectively.

Conclusions: This study is the first report about mCWA performed adding AEA to patients' specimens. There was no difference of the parameters in the samples with and without AEA. mCWA may be an indicator of patients' clotting activities in the presence of emicizumab.

PB1028 | Distribution of Hemophilia A and B within the INSIGHT Consortium

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Background: The distribution of hemophilia A and B is generally estimated to be 80/20 in the current literature. However, founder effects have been reported that lead to a higher prevalence of hemophilia B in certain regions. Information on the geographical variation in the distribution of hemophilia A and B is important to estimate the number of unidentified patients and regional burden of disease.
Aims: To give an overview of the geographical distribution of hemophilia A and B in Europe, Canada, and Australia among the centers of the INSIGHT consortium.
Methods: The 40 centers participating in the INSIGHT consortium were asked to provide anonymized data on the number of all hemophilia patients registered in their clinics in 2019. Requested information included the numbers of severe (< 1 IU/dL), moderate (2-5 IU/dL) and mild (6-40 IU/dL) hemophilia A, B and B Leyden patients. The numbers were pooled for the participating centers per country. Medical ethics committee approval was not required for this study.
Results: The majority of centers (83%) provided data on their hemophilia population. Data was reported on 8698 hemophilia A (83%) and 1707 hemophilia B patients (17%) (77 had a Leyden mutation). A median of 437 patients (IQR 259-630) were pooled per country. The mild and moderate form was present in 48% and 11% respectively of all hemophilia A patients. In hemophilia B, 45% and 22% of the patients had the mild and moderate form respectively. The highest proportion of hemophilia B was identified in participating centers in Ireland (28%), followed by Sweden (27%). The lowest proportion of hemophilia B was found in the participating centers in Spain (9%) (figure 1).

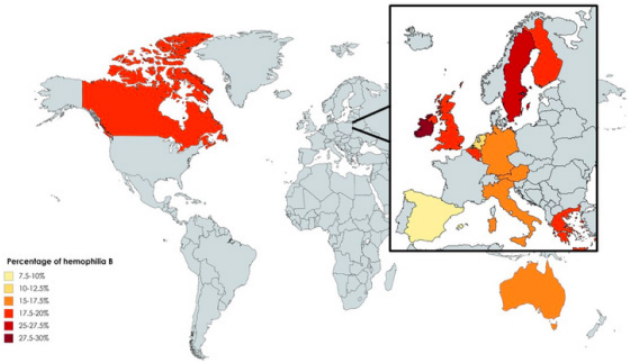

FIGURE 1 Percentage of hemophilia B population in 33 centers of the INSIGHT consortium

Conclusions: The distribution of hemophilia A and B varies between centers in different countries with overall a relative higher proportion of hemophilia B patients in participating centers in Ireland and orthern European countries.

PB1029 | Prophylaxis with Emicizumab in Children and Adults with Hemophilia A and Inhibitors: Real-World Data of 17 Patients from 4 Portuguese Hemophilia Treatment Centers

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Background: In Portugal, emicizumab, a bispecific monoclonal antibody that mimics FVIII, is only approved for the prevention of bleeding in persons with hemophilia A with FVIII inhibitors (PwHAi).

Aims: To evaluate the clinical efficacy and safety of emicizumab prophylaxis in PwHAi followed at 4 Portuguese Hemophilia Comprehensive Care Centers.

Methods: Emicizumab prophylaxis was evaluated in PwHAi previously receiving episodic/prophylactic bypassing agents (BPAs) treatment. Intraindividual comparison of bleeding rates (BR) and presence of target joints before and after starting emicizumab was done.

Results: Since January 2018, emicizumab prophylaxis was started in 17 patients, 10 children (4 months-12 years) and 7 adults (27-55 years) who were followed for a median of 71.4 weeks. Immune tolerance induction (ITI) was performed without success in 6 patients, and in the 5 younger children ITI was postponed mainly due to parents' refusal to CVL insertion. On emicizumab prophylaxis no patient experienced spontaneous bleeding and 12 patients had no treated bleeding events. The BR with emicizumab was 0.3 vs 9.1 with prior BPA, representing a 97% reduced risk of bleeding with emicizumab. Comparison of BR is more difficult in the 5 children in whom emicizumab was started at a very young age, but bleeding events in this group were unexpectedly rare. Although target joints were previously reported in 9 patients, after this follow-up period no target joint was identified in any patient. 7 patients were occasionally treated with recombinant activated FVII for trauma (hemarthrosis, epistaxis, 2 muscle hematomas and head trauma), bleeding after 2 minor surgeries and for prevention of bleeding before 1 minor procedure. No significant adverse events, namely thrombotic events, were observed.

Conclusions: Our experience suggests that emicizumab prophylaxis is effective and safe and leads to significantly less bleeding events and to resolution of target joints. We all are, however, aware how important is to maintain an active vigilance.

PB1030 | Thrombin Generation Assay to Tailored Treatment in Hemophilia a Patients

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TABLE 1 FVIII:C and TG parameters before and after therapy

	Lag time(min)	ETP(nM•min)	Peak(nM)	ttPeak(min)
HAS (n = 18)	6.0 ± 1.0	373 ± 142	28 ± 15	18 ± 3.0
Controls	6.0 ± 2.0	1350 ± 400	217 ± 67	9.0 ± 2.0
p HAS vs C	NS	<0.0001	<0.0001	<0.0001

Background: Patients with haemophilia A require different amounts of FVIII for reducing bleed rates. Treatments with FVIII replacement concentrates are available but they have variable effects on individual patients' global haemostasis .Thrombin generation assay (TGA) could be reliable for monitoring this therapy although with inter-individual variations.

Aims: To evaluate TGA profile in severe hemophilia A patients (HAS) treated with three different FVIII concentrates.

Methods: patients with FVIII:C < 1 IU/dL, without inhibitor, had not received FVIII for 72 h and were not bleeding at the time of the study, were enrolled after ethical approval and informed consent were obtained. FVIII:C (one-stage assay) and TG (calibrated automated thrombogram activated with tissue factor 1pM and phospholipids 4 μM) were assayed before and 15 min after plasma-derived FVIII concentrate (pdFVIII) or recombinant FVIII (rFVIII-X and rFVIII-A) infusions. TG parameters: lag time (LT), time to peak (ttP), thrombin peak (P), endogenous thrombin potential (ETP) were analysed.

Results: 6 patients were recruited for each group, mean age 43 ± 16 y (30-75 y). Before FVIII infusion HAS patients showed a significant decrease of TG compared with control group. No significant difference was found in LT between haemophilic patients and control. (Table 1). After infusion normal values of thrombin generation were achieved with all concentrates. The FVIII activity and TG parameters before and after therapy are summarized in Table 2.

Conclusions: FVIII replacement therapies, both rFVIII and pdFVIII showed different FVIII plasma levels. Nevertheless thrombin generation capacity reached normal values similar to healthy controls. TG assay may help for individual tailoring the replacement therapy in hemophilia patients.

TABLE 2 FVIII:C and TG parameters before and after therapy

	pdFVIII-B n = 6		FVIIIr-X n = 6		FVIIIr-A n = 6	
	Before	After	Before	After	Before	After
FVIII:C (IU/dL)	<1	31 ± 5.0	<1	41 ± 5.0	<1	55 ± 21
ETP(nM•min)	405 ± 196	1117 ± 279	293 ± 58	1211 ± 384	391 ± 20	1561 ± 334
Peak(nM)	31 ± 19	135 ± 16	20 ± 5.0	173 ± 38	30 ± 9.0	316 ± 99
Ttp (min)	18 ± 4.0	11 ± 1.0	17 ± 2.0	11 ± 1.0	17 ± 2.0	9.0 ± 2.0

PB1032 | Reference Ranges of HJHS Scores in Healthy Adult Males without Hemophilia

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Background: The Hemophilia Joint Health Score (HJHS) is an important tool to describe joint status in both pediatric and adult persons with hemophilia (PwH). Age-related, joint-health changes may result in elevated HJHS in healthy adult males, potentially confounding the interpretation of the HJHS in PwH.

Aims: We undertook to define age-related reference ranges for the HJHSv2.1 in healthy adult males.

Methods: Healthy males aged ≥ 18 years were eligible. Four age-defined strata were prespecified. Subjects with co-morbidities (i.e. rheumatoid arthritis, neurologic disorders) that may independently affect the HJHS were excluded. Subjects with mild sequelae from injury or other mild non-specific degenerative conditions were not excluded. Informed consent was obtained, and the study was approved by recognized ethics committees.

Results: 120 subjects were recruited: 40 aged 18-29; 35 aged 30-40; 22 aged 41-50 and 23 aged > 50 years. Median HJHS Total Scores (5th to 95th percentile range) (maximum score 124) were 2 (0-9) in those 18-29 years; 3 (0-12) in those 30-40 years; 3 (0-11) in those 41-50 years and 8 (2-19) in those > 50 years. *Figure 1* shows the boxplot of HJHS Total Score by age category. The heavy line for each box represents the median; the limits of the boxes show the 25th and 75th percentiles. HJHS scores increased as a function of age, Spearman's rho = 0.34 ($p < .001$). The ankles and knees most often accounted for increased scores. Swelling and crepitus were the most frequent abnormal items, while extension loss and loss of strength were rarely found.

Conclusions: The reference range of the HJHS Total Score in healthy adult males aged < 50 extends up to 12. This data is important for the accurate interpretation of joint outcomes using the HJHS in cohorts of adult PwH.

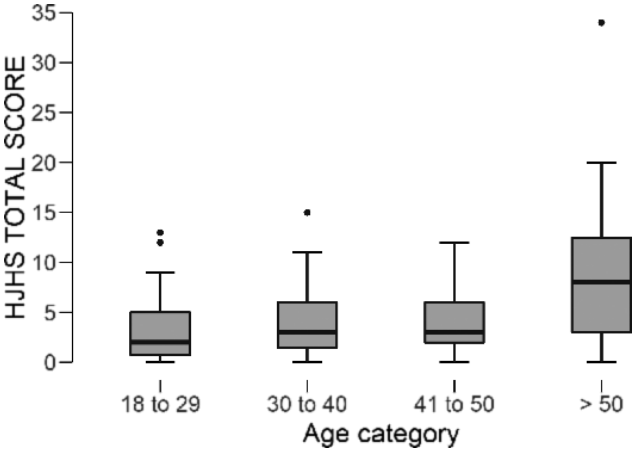

FIGURE 1 HJHS Total Score by Age Category

PB1033 | Value of Super Microvascular Imaging and Contrast-enhanced Ultrasound in Evaluating the Neovascularization of Synovial Hypertrophy in Hemophilia Joints

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Background: Joint bleeding occurred in hemophilia patient can stimulate synovial hyperplasia and neoangiogenesis. Our earlier study suggest the evaluation of neovascularization would help predict the possibility of recurrent joint bleeding. Therefore, exploring the general rule for neovascularization in the synovium after joint bleeding may contribute to the therapy for preventing recurrent joint bleeding.

Aims: To find a sensitive tool to evaluate the neovascularization and general rule of neovascularization in the synovium after joint bleeding.

Methods: 29 severe hemophilia A patients with 41 joints underwent gray-scale ultrasound, Color Doppler Flow Imaging (CDFI), SMI and CEUS examinations. Clinical data were collected from routine practice. Semi-quantitative analysis was based on the displaying proportion of blood flow signal in the synovium .Quantitative parameters were used to analyze the progression of neovascularization in synovium at different time points (1W, 2W, 3W, 3W-3 months,> 3 months) after joint bleeding, and the correlation between quantitative parameters and the frequency of joint bleeding in the past six months were also studied.

Results: Compared with semi-quantitative scores of SMI and CDFI, CEUS showed increased scores in 33 and 38 joints selectively. SMI vascular index, CEUS peak intensity (PT), and area under the curve (AUC) all peaked in the third week after joint bleeding. Frequency of joint bleeding in the past six months was positively correlated with SMI vascular index, and PT, ($r = 0.408$-0.597), $P < 0.05$, while negatively correlated with TP, ($r = -0.421$), $P < 0.05$.

Conclusions: SMI and CEUS seems to be more sensitive than CDFI in evaluating neovascularization of synovial hyperplasia. Besides, the peak value of synovial neovascularization in the third week after joint bleeding correlates positively with the frequency of joint bleeding during the past six months. Therefore, accurate evaluation of the

neovascularization and hyperplasia rule in the synovium may be beneficial in guiding targeted and individualized treatment.

PB1034 | A Multicenter Open-label Single Arm Trial to Evaluate Safety and Efficacy and Pharmacokinetics of Recombinant Human Coagulation Factor VIII (SCT800) in Previously Treated Patients with Severe Hemophilia A under Prophylaxis Therapy

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Background: SCT800 is a B-domain deleted recombinant factor VIII (rFVIII) product for hemophilia A.

Aims: This study is to assess efficacy, safety and pharmacokinetics of SCT800 for prophylaxis and treatment of bleeds in patients with severe hemophilia A.

Methods: This is a multi-center, phase 3, open-label trial(clinicaltrials.gov: NCT03815318), which was approved by the clinical committee of Hospital of Blood Disease,CAMS&PUMC. This study was sponsored by Sinocelltech Inc. All subjects must sign the Informed Consent Form before screening. In this clinical trial, males aged 12-65 years with FVIII < 1% and ≥ 150 exposure days (EDs) to any FVIII containing product and no history of inhibitors. Eligible patients received SCT800 25-50 IU/kg every other day or three times a week for approximately 24 weeks. Primary efficacy outcome was annualized bleeding rate (ABR).

Results: The analysis showed that a total of 73 patients were enrolled, and 71 patients (97.3%) completed the trial. The patients received prophylaxis with SCT 800 had a mean of 77.4 EDs during the trial. The overall median annualized bleeding rate was 2.080 (interquartile range:4.250) bleeds/patient/year. Based on patient reports and investigator's judgements, the success rate (defined as 'excellent' or 'good' hemostatic response) for treatment of bleeding episodes was 87%. 72% of bleeds resolved with one injection and 84% with up to two injections. Treatment-related adverse event (AE) incidence was 4.1% and there was one severe adverse event probably unrelated with study drug overall. No patient developed inhibitors. This is the preliminary analysis and the pharmacokinetic parameters are not obtained now.

Conclusions: These results in adult Previous Treated Patients indicate that SCT800 is effective and safe for the prevention and treatment of bleeds in adults with severe hemophilia A.

TABLE 1 Annualized bleeding rates

		Adolescents 12-18 years 32 patients	Adult ≥ 18 years 41 patients	All patients 73 patients
Total	N	20	19	39
	Median (IQR)	2.190(4.260)	0.000(4.250)	2.080(4.250)
	Estimated mean (95% CI)	3.087(2.0533-4.6418)	2.855(1.7184-4.7437)	2.957(2.1321-4.1004)
Spontaneous bleeds	N	13	10	23
	Median (IQR)	0.000(2.295)	0.000(2.110)	0.000(2.1659)
	Estimated mean (95% CI)	1.531(0.9475-2.4724)	1.152(0.6377-2.0817)	1.316(0.8964-1.9331)
Traumatic bleeds	N	13	12	15
	Median (IQR)	0.000(2.160)	0.000(2.050)	0.000(2.140)
	Estimated mean (95% CI)	1.180(0.6577-2.1179)	1.599(0.8231-3.1048)	1.415(0.8971-2.2315)

PB1035 | Pharmacokinetic and Clinical Improvements after PK-guided Switch from Standard Half-life to Extended Half-life Factor VIII Products

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Background: Extended half-life (EHL) factor VIII (FVIII) requires improvements in half-life ($t_{1/2}$) and area under the curve (AUC) of 1.3 and 1.25 times compared to standard half-life (SHL) products.

Aims: The aim of this study is compare pharmacokinetics (PK) after the switch from SHL to EHL in hemophilia A (HA) patients.

Methods: Multicenter comparative, cross-sectional, prospective study analyzing PK differences after switch from SHL to EHL (efmoroctocog-alfa [rFVIII-Fc] and rurioctocog-alfa pegol [PEG-rFVIII]). WAPPS-Hemo® was used to analyze PK parameters: $t_{1/2}$; AUC, peak level, trough level at 24, 48, 72 h and time to reach FVIII levels of 1, 2, 5%. Ratio of $t_{1/2}$and AUC, nº weekly doses, dose/kg/week, annual bleeding rate (ABR) and annual joint bleeding rate (AJBR) before/after the switch were compared. Wilcoxon and Kruskal-Wallis tests (SPSS®) were employed.

Results: Eighty-seven patients from 8 Spanish hospitals were analyzed (62 rFVIII-Fc; 25 PEG-rFVIII), 83 had severe HA and 4 moderate HA, with a median age of 30 years (range = 3-64).

All PK parameters were improved, ABR and AJBR were reduced, as well as dose/kg/week (16.9%, IQR:8.7-32.8%) and weekly infusion frequency (30%, IQR:0-33.3%) (Tables 1-2). In 14 younger patients the dose/kg/week was increased a median of 28.6% (IQR:11.7-40-7%). The median ratios of $t_{1/2}$ and AUC were 1.4 (IQR:1.3-1.6) and 1.7 (IQR:1.3-2.2) in the entire cohort. In patients with ≥ 12 years treated with EHL ratios of $t_{1/2}$ and AUC were 1.4 (IQR:1.3-1.7) and 1.8 (IQR:1.3-2.3), and in patients < 12 years treated with rFVIII-Fc were 1.3 (IQR:0.9-1.5) and 1.4 (IQR:1.1-2.1). No differences were observed in PK parameters in patients aged ≥ 12 years treated with rFVIII-Fc vs. PEG-rFVIII (Tables 1-2).

Conclusions: EHL FVIII have shown significant PK improvements, reductions in ABR and AJBR, avoiding one weekly infusion number and dose/kg/week. Outside the clinical trial setting, we have observed an increase in $t_{1/2}$ and AUC ratios accordingly to EHL definition.

TABLE 1 Comparisons between PK parameters after the switch between SHL to EHL FVIII (entire cohort) and between EHLs (patients aged ≥ 12 years)

PK parameters	SHL: median (IQR)	EHL: median (IQR)	P-value	rFVIII-Fc (n = 46): median (IQR)	PEG-rFVIII (n = 25): median (IQR)	P-value
t1/2 (h)	12.5 (9.5-15.8)	18.3 (13.3-23.5)	<0.001	20.3 (15.0-23.8)	19.8 (14.8-23.8)	0.736
AUC ((IU*h)/L)	9166 (7082-12349)	15805 (12184-21930)	<0.001	16456 (12682-21930)	17811 (13072-25327)	0.613
Peak level (IU/dL)	70.5 (56.8-81.0)	80.0 (68.0-97.5)	<0.001	76.0 (63.0-95.0)	94.0 (72.0-99.0)	0.099
Trough level at 24 h	13.1 (9.2-18.8)	24.6 (19.9-34.6)	<0.001	26.7 (20.7-34.6)	31.7 (22.7-39.9)	0.295
Trough level at 48 h	3.9 (2.0-7.1)	9.9 (5.9-17.4)	<0.001	10.8 (8.1-17.9)	14.9 (6.7-19.6)	0.576
Trough level at 72 h	1.5 (0.9-3.0)	4.1 (2.1-8.5)	<0.001	5.0 (2.9-8.5)	7.0 (2.3-9.4)	0.880
T5%	43.3 (33.0-58.8)	66.0 (51.5-91.0)	<0.001	71.9 (59.0-91.5)	83.0 (52.6-93.9)	0.970
T2%	63.4 (48.5-80.3)	93.0 (72.3-126.0)	<0.001	103.4 (82.0-127.4)	112.8 (74.9-129.8)	0.802
T1%	84.0 (67.9-109.8)	122.5 (92.6-160.3)	<0.001	136.5 (106.0-163.1)	143.8 (96.8-166.6)	0.634

TABLE 2 Comparisons between clinical variables after the switch between SHL to EHL FVIII (entire cohort) and between EHLs (patients aged ≥ 12 years)

Clinical variables	SHL: median (IQR)	EHL: median (IQR)	P-value	rFVIII-Fc (N = 46): median (IQR)	PEG-rFVIII (N = 25): median (IQR)	P-value
Age (years)	Not evaluated	Not evaluated	Not evaluated	33.5 (19.0-46.0)	37.0 (30.0-42.0)	0.665
Weigh (kg)	70 (55-84)	70 (57-87)	0.055	72 (64-90)	82 (72-94)	0.132
N° weekly doses	3.0 (2.0-3.0)	2.0 (1.8-2.3)	<0.001	2.0 (1.8-2.3)	2.0 (2.0-2.3)	0.831
Dose (IU/kg/week)	74.5 (58.0-105.2)	65.7 (44.8-96.5)	<0.001	60.5 (43.0-87.0)	51.7 (44.7-67.4)	0.191
ABR (N = 47)	2.0 (0.0-5.0)	0.0 (0.0-2.0)	0.001	1.0 (0.0-3.5)	0.0 (0.0-0.0)	0.148
AJBR (N = 26)	1.0 (0.0-4.0)	0.0 (0.0-1.0)	0.007	1.0 (0.0-1.0)	0.0 (0.0-0.0)	0.373

PB1036 | Molecular Mechanisms Underlying Haemophilia A and B Expression in 7 Females

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Background: Haemophilia A and B (HA and HB) are X-linked recessive bleeding disorders and female are usually benign carriers. Few cases of female haemophilia were reported so far.
Aims: To investigate the causes of hamophilia in 7 females.
Methods: Comprehensive analysis of *F8*, *F9* and the minimal promoter of *XIST* genotypes was performed in 5 unrelated HA and 2 HB females and related family members, XCI patterns (XIPs) have been conducted with methylation-based PCR assay using the androgen receptor *(AR)*. Karyotype and high-density single nucleotide polymorphisms (SNPs) genotyping array were also detected, linkage analysis was undertaken using microsatellite markers of X chromosome in the families with tendency of familial XCI skewing.
Results: 5 unique heterozygous *F8* mutations of Arg336*, Trp2081Gly, c.5998 + 1G>C, Ex2_6del (de novo, paternal allele), Ex2_26dup (de novo, maternal allele) in 5 female HA and 2 *F9* mutations of Gly409Glu and c.255 + 5G>A in 2 HB females. In the 7 females, no pathogenic variants were identified in the XIST promoter, and karyotype analysis revealed no obvious alternations. Macroarray showed an unreported large 431 kb duplication involving *F8* Ex2_26dup in the HA female, and a 2904 kb deletion at Xq28 in the HB female's paternal allele, and the *F9* c.255 + 5G>A mutation inherited from her mother. XIPs > 80:20 were detected in all the 7 females. The extremely XIPs > 95:5 were detected in 5 females of 3 generations in one HA family, and Linkage analysis revealed that inactivation X chromosome tracing to the same maternally allele.
Conclusions: The haemophilia expression in our cohort was caused by the X chromosome carrying the normal F8 or F9 being preferential inactivated, coordinated with the other X chromosome carrying the causative mutation. We postulate that the large deletion identified in the HB female resulted in extreme skewing of X-inactivation via a cell lethal mechanism.

PB1038 | Prophylactic Treatment with Low or Medium Dose Factor VIII for Children with Severe Hemophilia A in China: Evaluation of Joint Progression by Conventional Ultrasound and Hemophilia Joint Health Score

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Background: Prior research of prophylactic treatment for hemophilia joint diseases mainly focus on preventing joint bleeding, while lack of studies on the progression of hemophilia joint deterioration under current therapeutic regimen.
Aims: To evaluate the joint progression in hemophilia patients by conventional ultrasound (US) and Hemophilia Joint Health Score 2.1 evaluation (HJHS2.1)
Methods: 27 severe hemophilia A children were enrolled. Clinical data were collected by routine practice accompanied with US examination and HJHS2.1 assessment of both ankles, elbows and knees. According to the prophylactic dose and frequency, joints were divided into low-dose and medium-dose group, while normal joints and diseased joints were grouped based on the score of ultrasound assessment. The children were followed up for one year to evaluate joint progression.
Results: 157 joints from 27 patients showed that the variation in both US and HJHS2.1 evaluation via low-dose-treatment was more prevalent than that in medium-dose- treatment ($P > 0.05$). Compared with normal joints, the degree of deterioration in diseased joints was significantly higher both in US detection and HJHS assessment ($P < 0.05$). In our study, 74 joints were found without history of hemarthrosis. Among these joints, 18.9% of joints with variation in US scoring, whereas 14.9% of joints developed during HJHS2.1 assessment.
Conclusions: Despite the degree of joint deterioration in low-dose group has a tendency to develop faster compared with medium-dose group, the low-dose prophylactic scheme can still delay the progression of joint injury in a brief period to some extent, while the long-term effect of this regimen still needs to be ascertained. In addition, our study also suggests that worsening of the joint condition may

lead to exacerbation of joints. Hence, for the comprehensive evaluation of hemophilia joint diseases, it is of great importance to combine US examination with HJHS assessment.

PB1039 | FIGURE 1 Impact of Prophylactic Treatment Incorporation in Hemophilia A and B Hospitalization Rates of the State of São Paulo Patients - Brazil - from 2008 to 2018

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Background: In 2011 the Brazilian Ministry of Health implemented the long-term primary prophylactic for hemophilia A and B in the public Unified Healthcare System, SUS. Since the 1990s, government has been providing this high cost medication free of charge but only for treatment. However, publications are scarce regarding Brazilian hemophilia A and B patients' profile and hospitalization data. Impact of new therapies implementation is of utmost importance for the SUS resource allocation.
Aims: Retrospective analysis of the São Paulo State hospitalization database (SUS-SP) of patients with hemophilia A and B.
Methods: Hospitalization data was collected from the public healthcare database (SUS-SP from 2008 to 2018) providing diagnosis and number and days of hospitalization information. Hemophilia A and B patients were identified by International Classification of Diseases codes of D66 and D67. São Paulo State population was estimated by the Brazilian Institute of Geography and Statistics - IBGE, publications. Data was summarized descriptively. Categorical data is presented as frequencies and percentages. For continuous data, mean, standard deviation, median, minimum, and maximum are presented.
Results: In 2008, 448 hospitalizations occurred for the identified 2,000 hemophilic patients out of the total São Paulo 40 million inhabitants. The most frequent SUS-SP hospital admission reason reported was for the administration of coagulation factor with a 6.6 (mean) days length of hospital stay, (LOS median 3.5; min 1 - max 63 days). In 2018, SUS-SP had 2,600 hemophilia identified and 191 hospitalizations occurred (LOS mean 4.5 days, median 3.00; min zero - max 51 days) out of 43 million inhabitants in total, also mostly for the coagulation factor administration.
Conclusions: The long-term primary prophylaxis and treatment of SUS-SP hemophilia A and B underwent of in a 57,3% reduction in the hospitalization rate since 2011, reflecting improvements in SUS Brazil.

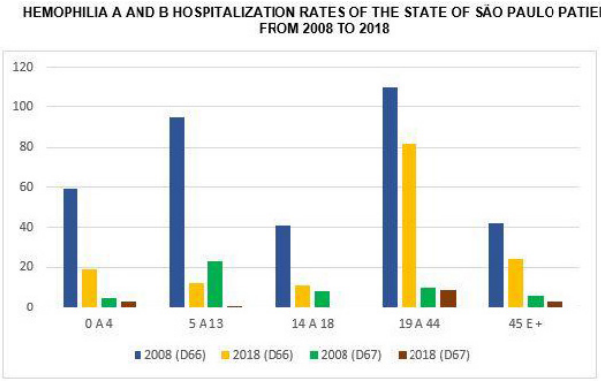

FIGURE 1 Hemophilia A and B Hospitalization Rates of the State of São Paulo Patients from 2008 to 2018

PB1040 | Is intracranial haemorrhage in haemophilia patients still a present risk in the third millennium? The final results of the EMO.REC Registry: a 10-year Italian study on intracranial haemorrhage in haemophilia patients

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Background: The most serious bleeding event in patients with haemophilia (PWHs) is certainly intracranial haemorrhage (ICH) which leads to disability and in some cases to death. Children ≤ 2 years and adults ≥ 50 years are the subjects most at risk for ICH.
Aims: To evaluate incidence, mortality, management and risk factors for ICH in an Italian population of PWHs
Methods: All ICHs in PWH were retrospectively and prospectively collected at 13 Italian Haemophilia Centres. All data of patients, ICHs management and outcomes were evaluated from 2009 to 2018.
Results: Overall 44 ICHs were found in 4990 haemophiliacs. ICH occurred in 10/15 children ≤ 2 yrs, none of them were on prophylaxis at the time of bleeding; one ICH occurred in a 4-year-old child during ITI. ICHs occurred in 29 adults, 44.8% with mild haemophilia. Overall patients with severe haemophilia were 61.4%; 100.0% (15/15) of children. Only 7/44 patients received a prophylactic regimen before ICH. Inhibitors were present in 13.6% of patients. 15/29 adult PWHs suffered from hypertension, 84.6% were mild subjects and 25.0% were moderate to severe ($p < 0.05$). ICH was spontaneous in the 69.8%. Surgery was required in 19/44 patients for cerebral hematoma evacuation. Patients treated with FVIII/FIX concentrates

or rFVIIa for at least three weeks were 76.7%. Subjects who died before starting or during treatment were 31.8%. Of the survivors 43.3% became permanently disabled. Only one-third of patients continued the prophylaxis after the acute treatment.

Conclusions: The final results obtained by our Registry confirm a high incidence of ICH in infants ≤ 2 years and in adults ≥ 50 years, especially in mild adult PWHs presenting concomitant hypertension. The majority of PWHs were treated on-demand during ICH, which indicates the important role of prophylaxis. Perhaps the use of new subcutaneous agents, as emicizumab, could be help to prevent the risk of ICH especially in children.

PB1041 | 3D Gait Analysis, Hemophilia Joint Health Score, Leg Muscle Laterality, and Biomarkers of Joint Damage: A Cross-sectional Comparative Assessment of Hemophilic Arthropathy

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Background: 3D gait analysis has been proposed as a reproducible and valid method to assess abnormal gait patterns and to monitor disease progression in patients with hemophilia (PWH).

Aims: This study aimed at comparing *Gait Deviation Index* (GDI) between adult PWH and healthy controls, and at opposing further established and exploratory outcome measures of hemophilic athropathy.

Methods: Male PWH aged between 18 and 49 years on prophylactic replacement therapy, and male healthy age-matched controls, passed through a cross-sectional assessment battery. Besides the 3D gait analysis (Vicon, Oxford, UK) derived GDI, secondary outcomes included kinematic, kinetic and spatio-temporal gait parameters, the *Hemophilia Joint Health Score* (HJHS), and in an exploratory approach, an assessment of electric impedance derived leg muscle laterality and a combined joint damage biomarker score. The latter represents a panel of four biomarkers, which were suggested to reflect general joint damage (sCOMP, sC1,2C, sCS846, uCTX-II), averaged after z-standardization and transformation to percentile.

Results: PWH (n = 18) walked slower, in shorter steps and accordingly with less functional range of motion in the hips and ankles, as opposed to healthy controls (n = 24). However, PWH did not differ significantly in GDI, the majority of gait parameters, and the biomarker panel score. Median levels of CTX-II were in PWH 72% higher, though. PWH had a higher mean HJHS (18.8 vs. 2.6, $p < .001$) and leg muscle laterality (4.3 vs. 1.5%, $p = .004$). A subgroup analysis revealed progressed gait pathology in PWH aged 26 to 49 years. Leg muscle laterality was strongly correlated with HJHS ($r = .76$, $p < .001$), whereas GDI was not ($r = -.39$, $p = .110$).

Conclusions: General gait pathology (GDI) was found in PWH aged 26-49 years, but not so in those aged 18-25 years. Leg muscle laterality emerged as a promising approach in detecting progression and physiological consequences of hemophilic joint arthropathy.

PB1043 | Pilot Study on Low-Dose Immune Tolerance Induction (ITI) Therapy in Severe Hemophilia A Children with High-Titer Inhibitors

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Background: Low dose of plasma-derived factor VIII/Von Willebrand factor (pdVWF/FVIII) complex concentrate might be effective as immune tolerance therapy (ITI) in inhibitor-positive severe Hemophilia A patients with high-titer inhibitors.

Aims: To assess the effectiveness of low-dose ITI regimen (time to achieve response, response rates, annual bleeding and joint bleeds during study period compared to recorded bleeds in the year before) and compare results with our publication in "Haemophilia", 2 decades ago.

Methods: A prospective study on previously treated children with severe hemophilia A having poor-risk status for ITI treatment success and high-titer inhibitors (inhibitor titer > 10 Bethesda units (BU) pre-ITI initiation; delay in ITI initiation [2-16 months]). Patients received ~50 IU/kg pdVWF/FVIII complex concentrate (Koate®), three times a week. Annual bleeding rate, annual joint bleeds and school attendance were compared retrospectively (12 months prior to study initiation)

Results: Fourteen patients with median age of 4.9 (2.4-11.2) years and median pre-ITI inhibitor titer 42.7 (11-168) BU were enrolled and followed-up during 6-22 months. Analysis at median 14 (9-22) months' follow-up showed a total response rate of 71.4%. This included success (achieving inhibitor < 0.6 BU) in 9 patients, (57.2%) in a median of 10.8 (5.2-15.8) months, and partial success (achieving inhibitor < 5 BU but > 0.6 BU) in 2 (14.2%). Compared to the pre-ITI period, median annual bleeds during ITI was 9 (75.0% reduction), and median annual joint bleeds was 4 (80% reduction). School attendance improved by 50%. Study limitations; single arm study and short follow-up (1-6 months after ITI discontinuation). However, ITI outcome in this study was better than reported 2 decades ago.

Conclusions: Low-dose ITI treatment strategy using pdVWF/FVIII complex concentrate (Koate®) achieved relatively satisfactory outcomes (71.4% response) in children with inhibitor-positive severe Hemophilia A and poor-risk status for ITI treatment success, improving school attendance.

PB1044 | Immune Tolerance Induction with Octanate® in Patients with Haemophilia A and Inhibitors: An Ongoing Case Series from a Malaysian Centre

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Background: Immune tolerance induction (ITI) is the primary treatment strategy in patients with haemophilia A who develop FVIII inhibitors and is the only clinically proven approach to eradicate inhibitors.

Aims: The aim of this analysis was to evaluate ITI outcomes in patients with inhibitors who were treated with a human plasma-derived factor VIII concentrate stabilised with von Willebrand factor (octanate®) according to routine clinical practice at a single haemophilia treatment centre in Malaysia.

Methods: Thirteen patients with severe haemophilia A and inhibitors were treated with octanate® for ITI. The ITI regimen was at the discretion of the treating physician. ITI success was defined as an undetectable inhibitor titre (< 0.6 Bethesda units [BU]/mL) on two occasions over a month apart.

Results: The median age at ITI start was 1.5 months (range 0.5-11). Eleven patients were high responders, 2 were low responders. Ten patients were primary ITI patients and 3 were rescue ITI patients who had a failed prior ITI attempt with another FVIII product. The starting ITI regimen was 100 IU/kg daily for 7 patients, 100 IU/kg 3x/week for 2 patients, and 50 IU/kg 3x/week for 5 patients. Four patients are still on ITI. Of the 9 patients to complete ITI, 8 (88.9%) achieved negative inhibitor titres in a median of 7.5 months (range 5-21). Five of the successful patients were on a 100 IU/kg daily regimen and 3 were on 50 IU/kg 3x/week. ITI failed in one patient, who was a high responder.

Conclusions: These data indicate a high success rate and rapid achievement of negative inhibitor titres with octanate® in patients with severe haemophilia A and inhibitors. Moderate dose regimens for ITI can successfully eradicate inhibitors and make ITI feasible in countries with limited resources.

PB1045 | MOdern Treatment of Inhibitor-PositiVe PATiEnts with Haemophilia A - MOTIVATE

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Background: Immune tolerance induction (ITI) is the only proven method to eradicate inhibitors in haemophilia A patients who develop a neutralising antibody to FVIII. While emicizumab or other bypassing agents are used to control bleeding in patients with inhibitors, they do not eliminate inhibitors. Although a single-centre study has been conducted, there are limited data on the use of ITI and emicizumab in combination and the outcomes of different treatment strategies.

Aims: The overall objectives of MOTIVATE are to characterise different approaches to the treatment and management of patients with haemophilia A and inhibitors and to evaluate the efficacy and safety of ITI and/or emicizumab.

Methods: MOTIVATE is comprised of two multicentre, investigator-initiated trials in the US and Europe. Data from the two trials will be pooled. Participants of any age with haemophilia A of any severity who have developed inhibitors to any FVIII product will be observed for a maximum of five years. Target enrolment is 120. The treatment approaches evaluated are standard ITI with FVIII (with Nuwiq®, octanate® or wilate®) (Group 1), ITI with emicizumab prophylaxis (Group 2), and prophylaxis with emicizumab or bypassing agents (Group 3). Participants are not randomised to any treatment group, and all treatments are at the discretion of the physician.

Results: The primary endpoints are the success of ITI in treatment groups that include ITI and the annualised bleeding rates across all groups. Optional sub-studies will explore factors that may influence ITI outcomes as well as the impact of different treatment approaches on diverse aspects of patient health, including joint and bone health and the risk of thrombosis.

Conclusions: MOTIVATE will collect real-world clinical experience on current approaches for the management of haemophilia A with inhibitors. The results will help facilitate discussions on the optimal treatment approach for people with haemophilia A and inhibitors.

PB1046 | The Health Status of Older Patients with Hemophilia in the Netherlands

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Background: Over the last 50 years, hemophilia management has improved tremendously, thereby increasing life expectancy.

Aims: Our aim was to describe the current health status of older (50 +) patients with severe hemophilia in the Netherlands.

Methods: In 2019, a cross-sectional study was performed among all patients with hemophilia in the Netherlands. (the HiN study) Information on joint impairment, age-related co-morbidities and health status (measured using the RAND-36, resulting in a score ranging from 0-100) were obtained by a self-reported questionnaire. Joint impairment was categorized as having no impairment, mild impairment (no limitations in daily life), moderate impairment (daily problems) or severe impairment (no movement in joint) in one of the 12 major joints. Information on HIV- and HCV status was obtained from medical records.

Results: In total, 116 patients with severe hemophilia older than 50 years were included. Overall median age was 61 (IQR: 56-67, range: 51-100).

Severe joint impairment was present in 81/115 patients (70.4%); 83 out of 113 individuals (73.5%) had undergone orthopedic surgery, the median number of surgical interventions was 2.5 (IQR: 1-4). The most common orthopedic interventions were joint-replacement surgery (56.0%) and joint fusion surgery (34.5%). The health status of older patients was worse than the general population. Patients performed worst on the domains of physical functioning (mean 43.4, SD: 26.7) and role limitations due to physical problems (mean: 52.7, SD: 43.6).

The most common age-related co-morbidities were hypertension (46.4%), hypercholesterolemia (17.3%), cancer (13.0%) and diabetes type 2 (10.9%). Twelve out of 101 patients (11.9%) were HIV-positive. Of 92 patients with information on HCV status, 87 (94.6%) were previously infected. Of these 87 patients, 2 patients (2.3%) were still HCV positive.

Conclusions: Besides hypertension, joint impairment is a major problem which severely impacts quality of life. Despite prophylactic treatment, most patients have undergone some form of orthopedic surgery.

PB1047 | Immune Tolerance Induction with Simoctocog Alfa in Patients with Haemophilia A: An Ongoing Multicentre Case Series from the UK

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Background: As the only clinically proven approach to eradicate inhibitors, immune tolerance induction (ITI) is the primary treatment strategy in patients with severe haemophilia A (HA) who develop inhibitors to FVIII.

Aims: The aim of this analysis was to evaluate ITI outcomes in patients with inhibitors who were treated with simoctocog alfa (Nuwiq®, a human cell line-derived recombinant FVIII) according to routine clinical practice in 3 UK centres.

Methods: Ten patients with severe HA and inhibitors were treated with simoctocog alfa for ITI. The ITI regimen was at the discretion of the treating physician. ITI outcome was assessed based on the following success criteria: undetectable inhibitor titre (< 0.6 BU/mL); FVIII recovery ≥ 66%; and FVIII half-life ≥ 6 hours. Tolerability, safety and the occurrence of bleeding episodes were assessed.

Results: Median age at ITI start was 18 months (range 9-193). Eight patients were high responders, 2 were low responders. The starting ITI regimen for 7 patients was 100 IU/kg daily, while one patient each received 100, 90 and 50 IU/kg every other day. Inhibitors were eradicated in 8 patients (80%). Two patients achieved complete success (all 3 criteria) after 6 and 14.5 months. Six patients achieved partial success (2 criteria), of whom 4 continue to receive ITI. During ITI, 6 patients (60%) experienced no spontaneous bleeds, 3 patients experienced 1 bleed, and 1 patient had 2 bleeds. Simoctocog alfa was well tolerated, with no adverse drug reactions reported.

Conclusions: In this ongoing cohort, 8 of 10 patients receiving ITI with simoctocog alfa have achieved an undetectable inhibitor titre and 6 patients have not experienced any spontaneous bleeds. These data indicate that ITI with simoctocog alfa is effective and well tolerated in patients with HA and inhibitors.

PB1048 | Correlations of Von Willebrand Factor Levels, Factor VIII (FVIII) Pharmacokinetics and Prophylaxis Dose in Severe Haemophilia A Patients Switching to rFVIII-Fc Studied by Individual and Population Tool-assisted PK Assessments

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Background: The assessment of individual pharmacokinetic (PK) of Factor VIII (FVIII) products allows personalisation of prophylaxis in haemophilia A patients to improve outcomes and reduce the i.v. injection burden. This is recommended particularly when switch of FVIII concentrate is planned. Population-PK tools may simplify performing individual PK assessments.

Aims: To study PK of efmoroctocog alfa (rFVIII-Fc) in candidates to switch and correlations with prophylaxis regimens and outcomes.

Methods: For PK assessments, FVIII activity (one-stage assay) was evaluated before and 0.25, 1, 3, 6, 24, 48, 72 and 96 hrs after injecting ~50 IU/Kg rFVIII-Fc. Baseline Willebrand Factor Antigen (VWF:Ag) was measured. PK parameters (terminal half-life HL; area under the curve, AUC; clearance, Cl; means ± 1 standard deviation) were calculated according to both non-compartimental analysis by the Phoenix WinNonlin (WN) software package and the population-PK tool Web-Accessible Population Pharmacokinetic Service-Hemophilia (WAPPS).

Results: Twenty patients were studied (severe/moderate, 18/3; 3 children/adolescents/adults 3/3/15). Different but highly correlated HL (18.8 ± 7.3 vs 16.9 ± 6.3 h; p = .012; Pearson's r = .906) and Cl (2.4 ± 1.1 vs 2.6 ± 1.2 ml/h/Kg; p = .001, r = 0.990) were found at WN and WAPPS assessments, with comparable AUC (2260 ± 1008 vs 2270 ± 1065 IU*h/dl, r = .80). All PK variables showed significant correlations with VWF:Ag at both assessments (HL: ≥.63, higher for WAPPS; p ≤ .002; AUC ≥ .74, p ≤ .001; Cl ≥ -.65, p = .001). Over mean 19 ± 11 mo. rFVIII-Fc prophylaxis, significant reductions of weekly dose (104 ± 25 vs. 95 ± 31 IU/Kg, first vs. last prescribed) were obtained by prolonging infusion intervals in 13/20 patients. Inverse correlations of last FVIII weekly dose with VWF:Ag were found (≥-.70, p = .001), as well with both AUC (≥-.74, p ≤ .001) and HL (≥-.81, p ≤ .001) assessments, coefficients increasing when compared to first prescribed dose.

Conclusions: Significant relationships are detected between individual rFVIII-Fc PK parameters and VWF:Ag levels, with implications on prophylaxis weekly dose and its reduction over treatment. Individual and WAPPS assessments provided different but highly correlated PK profiles.

PB1049 | Lack of Inhibitor Development after Switching to Rurioctocog Alfa Pegol in the American Thrombosis and Hemostasis Network (ATHN) 2: Factor Switching Study

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Background: Although surveillance in previously treated patients (PTPs) with hemophilia A switching factor products suggests no increased inhibitor development risk, real-world data on the incidence of inhibitor development following a switch to a new factor product is imperative. Rurioctocog alfa pegol (TAK-660, SHP660, BAX 855), an extended half-life factor VIII concentrate, received initial FDA approval in 2015.

Aims: The ATHN 2: Factor Switching Study provides the opportunity to longitudinally observe a cohort of PTPs switching to TAK-660 to determine the incidence of inhibitor development.

Methods: This longitudinal cohort study is being conducted in ATHN-affiliated sites in the United States (NCT02546622). The ATHN 2 protocol was approved by institutional review boards and participants signed informed consent. Inclusion criteria included moderate or severe hemophilia A on any FVIII concentrate prior to switching. Inhibitor titers were measured at baseline, after 10 and 50 exposure days (EDs). Participants with a history of an inhibitor were eligible.

Results: Fifty-two participants switching to TAK-660 were enrolled. Thirty-seven were in the prospective arm. The median enrollment age was 27 years of age (IQR 14-36). All participants were male. Thirty-two participants were Caucasian, 11 were African American, 3 were Asian, 3 were other races and 3 were unknown (race information). Forty-three participants had severe and 4 had moderate hemophilia A. Eight of the participants had a prior history of inhibitors, three transient and five successfully tolerized. The baseline inhibitor

titer for all participants was negative. No participant developed an inhibitor within one year of switch or 50 EDs.

Conclusions: No new inhibitors were noted amongst 52 hemophilia A participants over 50 EDs or 12 months, providing real-world evidence of the rarity of inhibitor development after product switch. Further data reporting participant satisfaction with TAK-660 and the results of other Patient Reported Outcomes (PROs) will be reported as data matures.

PB1050 | Treatment of Hemophilia A with rVIII-SingleChain in Italy: Physician and Patient Survey

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Background: rVIII-SingleChain is indicated for treatment and prophylaxis of bleeding in patients with hemophilia A (HA). The safety and efficacy of rVIII-SingleChain have previously been shown in the AFFINITY clinical trial program.

Aims: This survey evaluated clinical experience with rVIII-SingleChain from the perspective of both physicians and patients.

Methods: A web-based survey (July-September 2019) involving 14 Hemophilia Treatment Centers (HTCs) collected physician-reported data about HA patients receiving rVIII-SingleChain for ≥ 12 months. Additionally, a number of patients were interviewed.

Results: In total, 48 patients received rVIII-SingleChain for ≥ 12 months across the 14 HTCs; 38% were ≤ 18 years, 37% 19-40 years and 25% ≥41 years. Overall, 73% of patients had severe HA and 85% were receiving prophylaxis. Interviews were conducted with 26 patients; 38% were ≤ 18 years, 39% 19-40 years and 23% ≥41 years, 62% had severe HA and 85% were receiving prophylaxis. For patients who had received prophylaxis with a prior FVIII product, the median dosing frequency reduced from 3 to 2 infusions per week following the switch to rVIII-SingleChain. The annual median factor consumption was 4,500 IU/kg (range 2,215-7,200 IU/kg) with prior product and 4,320 IU/kg (range 2,215-6,646 IU/kg) with rVIII-SingleChain. Both physicians and patients reported a reduction in annualized spontaneous bleeding rate with rVIII-SingleChain compared with prior product (mean 2.15 vs. 0.89 and 2.46 vs. 0.63 events/year; median: 1.0 vs. 0.0 and 2.0 vs. 0.0, respectively). Mean satisfaction ratings (from 1; dissatisfied, to 5; very satisfied) for rVIII-SingleChain were quite high both for physicians (4.14, 86% satisfied/very satisfied) and for patients (4.18, 87% satisfied/very satisfied).

Conclusions: This survey suggested that switching to rVIII-SingleChain allowed patients to reduce their dosing frequency without increasing factor consumption or compromising protection. Both physicians and patients reported a positive experience with rVIII-SingleChain after at least 1 year of treatment.

PB1051 | Real-world Evidence on the Use of rIX-FP in Patients with Hemophilia B: Interim Results from Three Multinational Prospective, Non-interventional, Post-market Surveillance Studies

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Background: In clinical trials, rIX-FP, a long-acting fusion protein, led to low annualized bleeding rates with prophylaxis regimens up to every 21 days. Data on the effectiveness and safety of rIX-FP in routine clinical practice are required.

Aims: Prospective, non-interventional, multicenter studies are ongoing to gather data on effectiveness, safety and health-related quality of life outcomes in patients treated with rIX-FP during routine clinical practice in Europe.

Methods: Three non-interventional studies have been initiated in Italy, Belgium and Germany in September 2017, May 2017, and March 2018, respectively. All patients with hemophilia B are eligible for enrollment; however, the Italian study is not enrolling patients with mild hemophilia B. Patients are treated with rIX-FP up to every 14 days and routinely monitored every 3-12 months. Patients are followed for 2-3 years or until 100 exposure days. Patient data is collected, stored and anonymized in accordance with General Data Protection Regulations.

Results: In total, 114 patients have been enrolled in Italy (n = 60), Belgium (n = 16), and Germany (n = 38) up to December 31, 2019.

Patients range in age from 1-80 years, have between 0 and 646 exposure days to rIX-FP, and 64 patients (59%) have severe hemophilia B. At the data cut-off, 16 adverse events have been observed; one in the Italian cohort was considered related to rIX-FP and led to discontinuation of treatment. To date, no patients have developed inhibitors. Two patients in both Italy and Germany have discontinued rIX-FP treatment, one patient was lost to follow-up in Belgium. Efficacy and safety data collected will be reported after completion of the studies.

Conclusions: Initial data from these studies indicate that rIX-FP is well tolerated and leads to few adverse events in routine clinical use. Further data will be collected to assess the effectiveness and safety profile of rIX-FP in the real-world clinical setting.

PB1053 | A Simplified Health Education Program Improves Knowledge and Practices of Home Treatment in Hemophilia A Adolescents in a Developing Country

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Background: Home care represents a significant challenge in management of hemophilia in developing countries like Egypt. There are many barriers against optimum home treatment. Lack of knowledge and limited skills are of utmost importance.

Aims: To investigate whether or not a simplified educational health program can improve knowledge and practices of adolescents with hemophilia about home treatment.

Methods: A comparative cross sectional study was conducted on 50 adolescents with hemophilia A at hemophilia outpatient clinic of Zagazig university hospitals. Three tools were used in this study. The first was a structured interview questionnaire sheet. The second was a clinical checklist to evaluate self-infusion of the hemophiliac adolescents with Factor VIII. The third was the health education program. Patients were assessed at three time points (before health education program as a pretest, immediately after as a post test and 2 months after as a follow up test).

Results: The mean age of our patients was 14.74 years. 84% of the studied group didn't practice home treatment. Inability of self-injection, inability to calculate dose, lack of treatment products were the most common causes. There was a significant improvement in hemophiliac adolescents' knowledge and practices throughout three phases of the study regarding treatment and home care and this was particularly evident in the immediate post-test.

Conclusions: A simplified health education program can improve knowledge and practices of adolescents with hemophilia A regarding treatment and home care. Regular patient education through periodic refreshing courses is strongly recommended to maintain adequate knowledge and satisfactory practices.

PB1054 | Vitamin D Status and Risk of Osteoporosis in Hemophilia Patients

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Background: Generalized reduced bone mineral density and hemophilia are disorders of significant socioeconomic magnitude, both of which have a marked impact on the individuals' quality of life. The simultaneous occurrence of both illnesses and possible connections between the two has to our knowledge hardly been assessed in younger pediatric age.

Aims: We aimed to assess the level of Vit D in patients with Hemophilia and study the risk factors for osteoporosis in those patients.

Methods: We conduct a cross-sectional study on thirty hemophilia A patients. all patients subjected to assessment of; anthropometric measures; body mass index, Routine laboratory investigations; serum Ca, Ph, parathyroid hormone; Assay of serum Vitamin D level; Assay of serum Bone turnover markers (osteocalcin and B cros laps) and Dual energy X-ray absorptiometry (DEXA) scan on lumber spine and total body.

Results: The Mean age of patients was 12.17 ± 4.1 years. Mean Z score at L1-L4 was -1.56 ± 1.24. 40% of patients had sever deficiency of vitamin D (level < 10 ng/dl). BMD of L1-L4 was low in 90% and 66.7% of the studied patients with insuffient Vit D and severe deficiency of vitamin D respectively. The mean level of osteocalcin was significantly lower among patients with severe vitamin D deficiency than patients with insufficient vitamin D ($p < 0.05$) while the mean level of Beta cross laps was significantly higher among patients with severe vitamin D deficiency than patients with insufficient Vitamin D($p < 0.05$). There was highly significant difference between severity of hemophilia and each of mean vitamin D level and degree of vitamin D deficiency.

Conclusions: Vitamin D deficiency was prevalent in hemophilia patients with significant association with the disease severity, there was significant association of vitamin D deficiency with bone turnover markers (Osteocalcin and B-cros laps) that could impact bone health status in those patients.

PB1055 | ASCVD Risk Score among Haemophilia Patients: Time to Formally Evaluate Cardiovascular Risk in People with Haemophilia

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Background: Brazil has the fourth largest haemophilia population in the world. All the patients are followed in the haemophilia treatment

centres, which provide them interdisciplinary consultations and replacement factor for free. Since the last decade, Brazilian guidelines have been written in accordance to international standard-of-care. The mortality among Brazilian haemophilia people is decreasing and the relative incidence of cardiovascular (CVD) deaths is increasing.

Aims: The aim of the current analysis of the HemoCardio Study was to describe the CVD risk score according to ASCVD tool and its treatment recommendations.

Methods: Men with haemophilia 40 years or older were included. Patients who had previous CVD event or a low-density lipid cholesterol 190 mg/dL or higher were excluded. Interviews, medical file reviews, and blood tests were performed. The ASCVD tool (www.cvriskcalculator.com) was used to estimate CVD risk, considering age, gender, race, total cholesterol, high-density lipid cholesterol (HDL), systolic and diastolic blood pressures, treatment for systemic arterial hypertension, diabetes and smoking status.

Results: Among the 28 included patients, the median [interquartile range; IQR] age was 51.5 [IQR;46.0-58.8] years. The majority had haemophilia A, 42.9% were severe, 53.6% were on prophylaxis and 7.1% were inhibitor positive in the last year. The frequencies of obesity, current smokers, hypertension, diabetes, hypertriglyceridaemia, hypercholesterolaemia, and hypoHDLaemia were 21.4%, 14.3%, 64.3%, 18.5%, 15.4%, 50.0%, and 21.4%, respectively. Median ASCVD score was 6.0 [IQR;2.6-13.5], with 46.4% patients having a moderate-to-high risk. Statin use was suggested for 50.0% of the patients. Blood pressure was poorly controlled in half of the patients.

Conclusions: In conclusion, almost half of the men with haemophilia had a moderate-to-high ASCVD risk score with strong recommendations to improve control of dyslipidaemia and blood pressure.

PB1056 | Association between Platelet Glycoprotein Common SNVs and Bleeding Severity in Haemophilia Patients

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Background: Hemophilia bleeding severity is currently classified by the residual factor activity as mild (6-40%), moderate (1-5%) and severe (< 1%). This classification, however, is not an entirely reliable predictor of the bleeding phenotype of individual patients, but the reasons of this have not been clarified yet. Little information is available on the contribution of platelets to the severity of the bleeding phenotype of patients with haemophilia, but some observations suggest a possible compensating function of increased platelet reactivity in hemophilia.

Common single nucleotide variants (SNVs) of platelet receptors have been shown to account for interindividual differences in platelet reactivity among normal individuals.

Aims: To investigate if common SNVs influencing platelet reactivity have a different distribution in hemophiliacs with similar residual factor levels but with different bleeding phenotypes.

Methods: 57 patients with haemophilia A or B were enrolled and their bleeding score (BS) was calculated using the ISTH-BAT. Blood was collected in sodium citrate and DNA was extracted. SNVs of *ITGB3* (rs5918), *ITGA2B* (rs5911), *ITGA2* (rs1126643), *GP6* (rs1613662) and *SELP* (rs6136) were analyzed by PCR and Sanger sequencing.

Results: BS was significantly lower in patients carriers of the *ITGB3* rs5918 SNV (8 ± 0.7 vs 12 ± 1;$p < 0.05$), previously associated with increased platelet reactivity, and significantly higher in patients carriers of the *SELP* rs6136 SNV (19 ± 2 vs 9 ± 2;$p < 0.01$), previously associated with decreased platelet reactivity, despite similar residual factor levels (rs5918: 2.4 ± 3.9 vs 2.2 ± 1.7%, rs6136: 2.7 ± 2.4 vs 1.8 ± 1.9%; p=ns). Hemophilia patients with higher BS (9-34) showed a lower prevalence of the *ITGB3* rs5918 SNV (13.7%, $p < 0.01$) than patients with lower BS (0-8; 44%, p=ns).

Conclusions: These results suggest that common genetic variants known to modulate platelet reactivity can influence the bleeding phenotype of haemophilia patients. Thus, platelets may contribute to determine clinical disease severity in hemophilia.

PB1057 | Satisfaction and Preference of Hemophilia A Patients and Parents for the Treatments in Korea; Results from a Multicentered, Cross-sectional, Observational Study

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Background: Reports on patients' satisfaction and preferred characteristics for treatments would be worthwhile when choosing an optimal treatment reflecting the patient's perspective.

Aims: This was to describe the satisfaction and preference of hemophilia A patients/parents for the treatments in Korea.

Methods: This study was approved by National Institute for Bioethics Policy Review Board and conducted from Apr2018 to Sep2019 in 6 hospitals, representative for hemophilia treatments in Korea. Hemophilia A patients aged ≥ 16 years or parents of pediatric patients, who had used factor VIII concentrates for ≥ 1 month were enrolled, after obtaining informed consent. We used Treatment

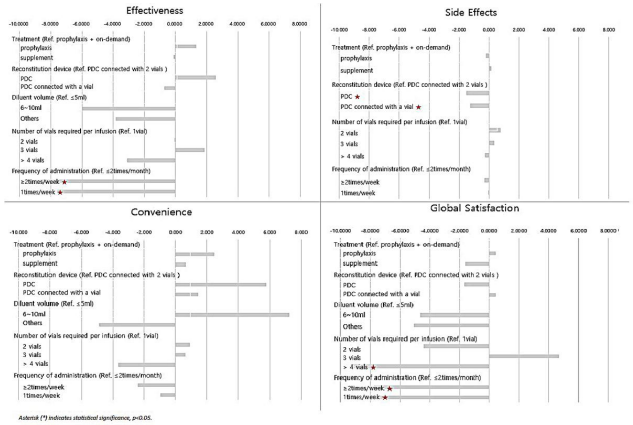

FIGURE 1 Risk factors for TSQM

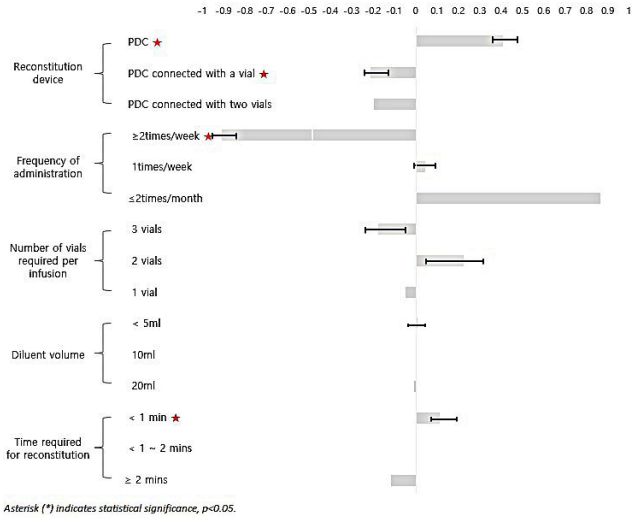

FIGURE 2 Choice-Based conjoint utilities and importance summary of medication attribution

Satisfaction Questionnaire For Medication(TSQM), consisted of 4 domains with each score(0-100) indicating the higher the better. For preference, we performed discrete-choice experiment with 10 series of 2 hypothetical treatment options created from *D-efficient* block design, which varied across 5 attributes(Fig 2). Multivariable linear regressions, count analysis, and conditional logistic regressions were performed. Positive Best-Worst(BW) score indicates predominantly "best" choice and higher part-worth utility indicates more preference.

Results: Of total 505 patients(aged mean31.5, treated for average102 months), 87.7% were severe, 53.5% treated as prophylaxis way, 80% used two connected vials while only 6.3% used prefilled dual chamber(PDC) syringe, and 52.3% experienced bleedings. Average TSQM were 64.6 in effectiveness, 97.9 in side effects, 57.1 in convenience, and 66.8 in global satisfaction. Type of device, number of vials, and administration frequency were associated with treatment satisfaction(Fig. 1). Concerning preference,<2times/month administration2(0.39BW) and PDC(0.22BW) were strongly preferred whereas ≥ 2times/month administration(-0.42BW) was mostly rejected. Lesser frequency and PDC showed higher part-worth utility(Fig. 2).

Conclusions: Hemophilia A patients in Korea were insufficiently satisfied with their treatments. Since patients reported the worst satisfaction in the convenience domain amongst 4 domains and gave a preference to simpler characteristics of treatment such as lesser frequency of administration and easier device type, more convenient treatment strategies are primarily required.

PB1058 | Case report: Inhibitor Improvement with Low Dose Immune Tolerance Induction (ITI) with rFVIIIFc and Emicizumab after Failed First Line Therapy with High Dose ITI

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Background: The eradication of inhibitors in patients with severe hemophilia A during immune tolerance induction (ITI) is a great challenge, especially if high dose ITI was not successful. Treatment success is evaluated by inhibitor titers, FVIII recovery, and half-life.

Aims: The change of IgG subtypes during ITI has not been evaluated in detail.

Methods: We report the course of a boy with severe hemophilia A, who was monitored for more than 4 years for coagulation, pharmacokinetic and immunological parameters (FVIII specific antibodies, isotypes, IgG subclasses, inhibitor titers) during ITI.

Results: The 5 years old boy developed high titer inhibitor (150 BU), increasing to 881 BU during ITI. After 10 months of ITI the inhibitor was 0.6 BU, increased after CVL infection to 7 BE and decreased very slowly without reaching < 2 BU. A change to rFVIIIFc resulted in only temporary improvement. After 3 years of ITI, treatment was switched to emicizumab and the ITI Atlanta protocol (rFVIIIFc 100 IE/kg 3x/week). After initial increase to 12.4 BU, inhibitor dropped down to < 0.6BU.

Initially, the FVIII specific antibodies belonged mainly to IgG1 together with IgG3 and IgG4 at high levels. IgG1, 3 and 4 increased after infection and vaccination. They did not decrease further but reached a plateau during ITI. After switch to emicizumab and a low dose ITI protocol all 3 subclasses IgG1, IgG3 and 4 increased again with a predominance of IgG4. The IgGs bound to the HC, A2, LC C1 and C2 domains of FVIII. Over time IgG1 and 3 decreased, while IgG4 reached a plateau. After 1 year only IgG4 was highly positive, IgG1 was barely detectable. They bound only to LC and C1.

Conclusions: Low-dose ITI can be effective in the presence of emicizumab even after failing first line high dose ITI. Antibody titers mirrored the clinical courses.

PB1059 | Impact of Extended Half-life Product versus Conventional Factor Product in Children with Hemophilia A - Findings from a Tertiary Care Centre

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Background: Until 2014, available FVIII products had Short Half-life (SHL) requiring frequent intravenous injections; FDA approved the first Extended Half-life (EHL) product known as Eloctate (rFVIII Fc). Pediatric hematology section at King Faisal Specialist Hospital and Research Centre, Riyadh, is a tertiary referral center for clotting factor deficiencies.

Aims: To report our experience with EHL products usage in pediatric (≤14 years) patients: Annual Bleeding Rate (ABR), Annual Factor Consumption (AFC) and compare bleed-free survival between EHL versus SHL products.

Methods: .Data collected on REDCap (Research Electronic Data Capture) software and analysis performed utilizing SPSS Version 20.0 software.

Results: Eloctate introduced to the institutional formulary on November 25th, 2017; from 107 HA cases in the pediatric service, 60 (58%) children (≤14 years) switched to Eloctate while 47 children remains on SHL products. Lesser infusion the main (67%) reason to switch followed by latest technology (18%) and 15% (9) switched to receive Eloctate Immune Tolerance Therapy (ITI) protocol. Prophylactic treatment observed in 67% (40) pre-switch, while 98% (59) are on prophylactic treatment.AFC was calculated for 46 patients who completed ≥ 12 months post-switch follow-up: 50% reduction in AFC, while bleed-free survival was calculated in both groups with 80% (48/60) children on EHL are bleed-free as compared to 59% (124/47) in SHL group; p-value = 0.074, longer follow-up warranted. Cost-effective analysis demonstrated reduction in bleeding -related hospital visits and admissions cost for EHL group, a detailed analysis is underway.

Conclusions: In our experience patients and families are aware and keen on adapting EHL products, findings suggest improved bleed-free survival and reduced AFC. Structured QoL survey would enable patient satisfaction levels between two groups. Preliminary financial assessments underlines cost-effectiveness of adapting EHL products but longer follow-up would enable a model (Markov) for countries in the region planning to integrate EHL in respective National hemophilia care programs.

PB1060 | Successful Use of Immunotherapy in the Management of Cancer in Patients with Hemophilia B

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Background: Persons with hemophilia (PWH) are at increased risk for cancer due to factors such as treatment-related HCV/HIV infection, in addition to prolonged life expectancy and consequences of aging. Use of immunotherapies in the management of cancer is increasing; the efficacy and safety of these agents in PWH represents a knowledge gap.

Aims: Report outcome of co-management of hemophilia B (HB) and metastatic malignancy, using immunotherapy-containing regimens.

Methods: Patient #1: Mild (10%) HB with metastatic hepatocellular carcinoma secondary to cirrhosis from HCV infection and baseline thrombocytopenia (~75K; PT 12.3s) was treated with Ytrium-90 liver directed therapy and palliative radiation therapy to bony metastases, followed by nivolumab. Response to therapy was excellent with complete resolution of measurable disease in liver and metastatic sites through year one. He subsequently progressed (abdominal lymph nodes); he received additional radiation therapy achieving a second remission while continuing nivolumab. Patients #2 & #3: Both cigarette smokers, developed extensive small cell lung cancer and were treated with standard therapy. Patient #2 (moderate; 3%) HB received 4 cycles of carboplatin/etoposide, 3 with atezolizumab; he progressed through treatment and transitioned to end of life care. Patient #3, (severe; < 1%) HB without an inhibitor on rFIX prophylaxis QOD, received 6 cycles of carboplatin/etoposide, 5 with atezolizumab with excellent treatment response; he remains on maintenance atezolizumab therapy at 7 months.

Results: No patient developed a serious adverse event related to treatment or FIX inhibitor, experienced a bleeding episode, or required rFIX regimen change with stable FIX levels at their baselines throughout treatment. No patient required hospitalization once treatment had begun.

Conclusions: PWH have increased complexity of care when presenting with malignancy but are able to receive standard cancer therapy that includes immunotherapy with close monitoring. All PWH should

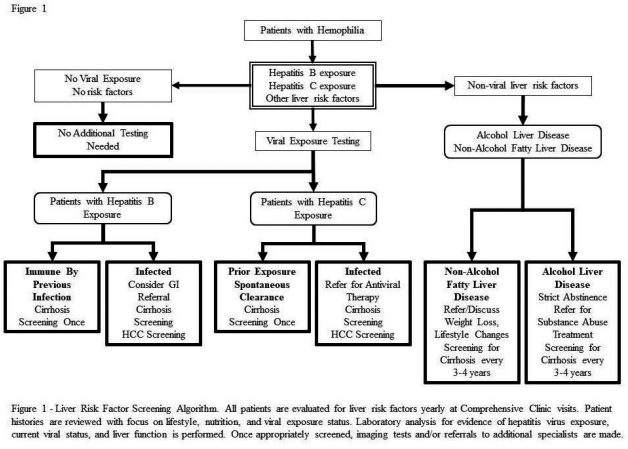

FIGURE 1 Liver Risk Factor Screening Algorithm

receive guideline based cancer screening as appropriate with particular focus on liver risk factor management.

PB1061 | rFVIIIFc at Lower Doses Can Achieve Clinically Meaningful Reduction of Annualized Bleed Rates in Children with Severe Haemophilia A

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Background: rFVIIIFc has shown efficacy in prophylaxis of PwHA. However, in developing countries lack of access to clotting factor concentrate (CFC) lead to patients not receiving prophylaxis. We present our experience in providing prophylaxis to children with haemophilia A using rFVIIIFc provided by the World federation of Hemophilia as part of the humanitarian aid program.

Aims: To assess the annualized bleed rate in children with severe haemophilia A who are on a low dose rFVIIIFc protocol.

Methods: Children with severe haemophilia A who have been either on episodic therapy or prophylaxis with conventional half -life FVIII who were given prophylaxis with rFVIIIFc were included in this analysis. The dose was 10-15 IU/kg once a week. Annualized bleed rate (AdBR) immediately prior to inclusion in this prophylaxis program was documented. All bleeds were then prospectively documented while on prophylaxis with special emphasis on joint or muscle bleeds. Inhibitors were monitored every 3-6 months depending on number of prior exposures.

Results: A total of 25 children received prophylaxis. Their median age was 8 (range: 1-14) years. The median exposures prior to starting rFVIIIFc was 18.5 (range: 2-225). AdBR prior to prophylaxis was 8 (range: 0-15). At a median dose of 15 IU/kg/week (range: 6-31), the median AdBR was 1 (range: 0-7) at a median follow-up of 100 (range: 26-123) weeks. Thirteen patients had 0-1 AdBR.Twelve patients had low titre inhibitor (≤5 BU) at enrolment. At a median exposure of 96 (range: 26-129), none developed high titre inhibitor during the study. Compliance was good with a median of 0 (range: 0-8) missed doses.

Conclusions: rFVIIIFc at lower doses of ~15 IU/kg/ week is effective in reduction of the median AdBR to ≤ 1/year with normal activities of daily living with the convenience of once a week dosing and lower costs.

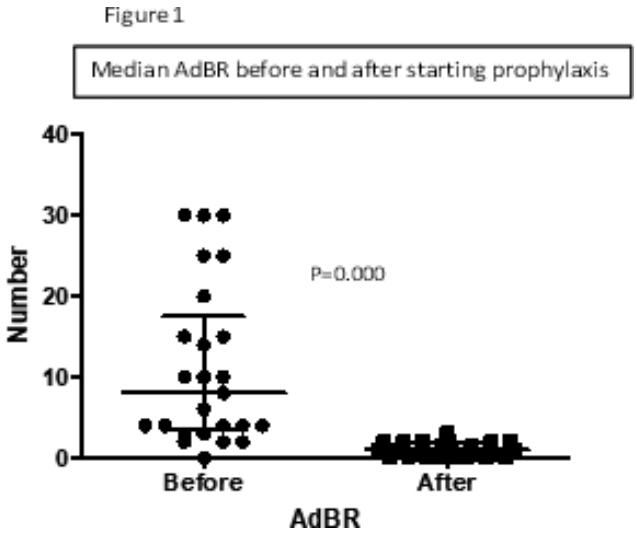

FIGURE 1 Median AdBR before and after starting prophylaxis

PB1062 | A Snapshot of a Prospective, Non-interventional Study to Evaluate Routine Practice Prophylactic Treatment Schedules - NIS-Previq

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Background: There is still a need to systematically analyze the prophylactic treatment of Haemophilia A (HA) in real life routine clinical practice since clinical trials are subjected to certain restrictions.

Aims: The prospective, observational study NIS-Previq is designed to collect information on prophylactic treatment of HA patients of all severities and ages treated with simoctocog alfa and 2 plasma derived von-Willebrand factor containing FVIII concentrates from one company.

Methods: Patients with HA of all ages receiving prophylaxis and general compliance to their therapy regimen are eligible to be enrolled after informed consent is given. All details of bleeding episodes and

TABLE 1 Number of patients by age group and selected demographic information at study entry (n patients)

Age Group(Years)	0-5	6-11	12-17	18-40	41-60	>60
Total No	10	8	6	11	5	1
Presence of at least 1 target joint	3	3	2	10	5	1
Previous Inhibitor activity	0	3	0	3	0	0
Non-severe Haemophilia A (>1%FVIII)	0	0	2	0	1	1

factor VIII administrations are recorded. Optional study elements comprise assessment of joint scores (HJHS), health related quality of life (SF-36) and a PK-assessment including dosing simulation for potential adaptation of therapy schedules with the full PK approach or the population-based PK using WAPPS-Hemo (www.wapps-hemo.org).

Results: As of January 2020, 7 German Haemophilia Centres enrolled 41 prophylactically treated patients, including 1 previously untreated patient and 1 with 15 previous exposure days. Total observation time was 834.2 months / 69.5 patient years with a mean of 20.35 months per patient (IQR: 7.2 - 27.5). In total, 31.6 Mio units FVIII were given, mainly for prophylactic purposes (88% of injections). The median ABR for spontaneous bleeds was 0 (IQR 0.9 - 3.3, for treated bleeds 2.0 (IQR 0.0 - 1.3). A traumatic cause was indicated for 64% of bleeds. The efficacy of treated bleeds was rated "excellent/good" in 95% of cases. 20 surgical procedures were performed in 12 patients.

No inhibitor formation or any other adverse drug reaction occurred during the observation period.

Conclusions: The snapshot on the study data so far confirm the good tolerability and efficacy of FVIII-based prophylaxis with 3 different FVIII concentrates.

PB1063 | Bleeding Outcomes, Factor Utilization and Cost Comparisons after Switching to an Extended Half-life Product for Prophylaxis in Haemophilia A in Austria

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Background: To prevent bleeding in severe hemophilia A (defined as factor VIII (FVIII) activity < 1%), regular replacement therapy with FVIII concentrates is required. Until recently only products with standard half-life (SHL) were available for prophylaxis. The therapeutic landscape has expanded with the availability of extended-half life products (EHL), which have been developed to reduce the burden of prophylaxis.

Aims: To compare prophylaxis regimen, clinical outcomes, factor utilization and costs in patients with severe haemophilia A, switched from prior prophylaxis with a SHL to efmoroctocog-alfa, a recombinant FVIII-Fc-fusion protein with extended half-life, the first approved EHL used in clinical practice.

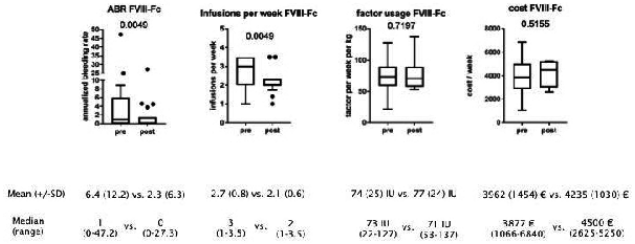

FIGURE 1 Comparison of ABR, number of infusions, factor utilization and costs with SHL prophylaxis (pre) compared to EHL/rFVIII-FC (post)

Methods: We performed a retrospective cohort study of persons with severe haemophilia A, who were switched from regular prophylaxis with SHL to prophylaxis with EHL (i.e. rFVIII-Fc) in Austria. All persons at participating sites who were switched until December 2018 and who had been treated with the EHL for at least 3 months were eligible. Data was collected from hemophilia-specific patient diaries and medical records. Furthermore, costs with EHL-prophylaxis were compared to prior prophylaxis regimen.

Results: Twenty male persons with severe haemophilia A (median (range) age and weight: 34 (9-75) years and 72.5 (27.5-109) kg) with evaluable data were included in this analysis. The two most common reasons for switching to rFVIII-Fc were high rates of bleeding with SHL and need of less frequent dosing/injections. Switch to rFVIII-Fc resulted in a significantly decreased annualized bleeding rate (ABR) and number of infusions. Factor utilization and costs were comparable to the prior prophylaxis with SHL, a slight increase in costs was not statistically significant. Detailed results are shown in Figure 1.

Conclusions: Switch to EHL (rFVIII-Fc) was associated with an improved clinical outcome, reflected by reduction of the ABR, and less frequent infusions, without a significantly higher factor usage or a significant cost increase.

PB1064 | Rapid Desensitization and Subsequent Immune Tolerance Induction in a Patient with Hypersensitivity and Inhibitor to Factor VIII

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Background: Therapy for hemophilia A, or factor VIII (FVIII) deficiency, generally consists of infusions of plasma-derived or recombinant FVIII (pdFVIII and rFVIII). Development of inhibitors renders FVIII infusions ineffective. A rare FVIII complication is development of hypersensitivity reactions, ranging from isolated urticaria to anaphylaxis.

Aims: Develop a management regimen for severe hypersensitivity to FVIII and a high-titer inhibitor.

Methods: This 4-year-old boy was diagnosed at birth with severe FVIII deficiency (< 1% activity). During treatment, he developed a

Day	Dose (U/kg)	Cumulative dose (U/kg)	Infusion time	Units per bag	Units (IU) infused	Volume (mL) infused	Premedication
1	0.01	0.01	15 min	1 IU	0.13	6.5	YES*
	0.02	0.03	15 min		0.26	13	
	0.04	0.07	15 min	10 IU	0.52	2.6	
	0.08	0.15	15 min		1	5	
	0.1	0.25	15 min		1.3	6.5	
	0.2	0.45	20 min		2.6	13	
	0.4	0.85	20 min	50 IU	5.2	5.2	
	0.8	1.65	20 min		10.4	10.4	
	1.5	3.15	20 min		19.5	19.5	
	3	6.15	30 min	250 IU	39	7.8	
	6	12.15	30 min		78	15.6	
	9	21.15	60 min		117	23.4	
	12	33.15	60 min	350 IU	155	22	
	15	48.15	60 min		195	27.9	
2	50	50	8h	3x217 IU over 160 min	650	250 mLx3	YES*
3	50	50	6h	2x325 IU over 3h	650	250 mLx2	YES*
4	50	50	4h	2x325 IU over 2h	650	250 mLx2	YES*
5	50	50	2h	650 IU	650	250 mL	YES*
6	50	50	1h	650 IU	650	250 mL	NO
7	50	50	30 min	650 IU	650	250 mL	NO
8	50	50	20 min	650 IU	650	250 mL	NO

FIGURE 1 Factor VIII Desensitization Protocol

high-titer inhibitor (140 BU). ITI was attempted with ALPHANATE, a pdFVIII, KOGENATE, a second generation rFVIII, and NUWIQ, a fourth generation rFVIII, but he suffered from increasingly severe hypersensitivity reactions to each product.

Results: The patient underwent 8 days of a desensitization protocol to NUWIQ, with a total daily dose of 50 IU/kg (Figure 1). Premedication (methylprednisolone/diphenhydramine) was given on days 1 through 5. ITI was initiated on day 9 with 50 IU/kg/day administered over 10 minutes; this interval was shortened to 5 minutes over the next week. He continued this dose daily until tolerance induction. His FVIII inhibitor titer was 7.2 BU at the start of ITI in 11/2018 and rose to 32 BU before declining to 0.0 BU in 3/2019. Desensitization to FVIII has been reported in at least 2 cases and consisted of step-wise increases of FVIII infusions over several weeks, with prolonged use of premedication and partial success. In contrast, this patient was desensitized over the course of 8 days with limited premedication, and started ITI on day 9. With an ITI regimen of 50 IU/Kg/day, this patient's inhibitor titer decreased to undetectable within 4 months.

Conclusions: The FVIII desensitization regimen described appears to offer a practical, cost-effective therapeutic approach to both the management of hypersensitivity as well as the induction of safe and effective immune tolerance.

PB1065 | Population Pharmacokinetic Simulation Analysis for Extended Dosing Regimens of Recombinant Coagulation Factor IX Albumin Fusion Protein (rIX-FP) in Adult and Pediatric Patients with Hemophilia B

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Background: rIX-FP, recombinant factor IX (FIX) fusion protein, was developed to extend the half-life of FIX in patients with hemophilia B. Population pharmacokinetics (PPK) has been evaluated using PK data from a phase 3b study that includes extended rIX-FP dosing regimens of 100 IU/kg every 21 days for adults and 75 IU/kg every 14 days for pediatric patients.

Aims: This analysis aims to characterize the PPK of rIX-FP, assess the impact of demographic and clinical factors, and simulate FIX activity-time profiles for different dosing regimens.

Methods: Patient blood samples were collected from 113 patients with hemophilia B (FIX ≤ 2%) aged 1 to 70 years to determine the FIX activity. A PPK model was developed in NONMEM 7 software, where goodness-of-fit (GOF) and prediction-corrected visual predictive check (pcVPC) were used for model evaluation. Exogenous FIX activity levels were simulated for multiple dosing scenarios.

Results: FIX activity was appropriately described using a 2-compartment PPK model. The final model precision was confirmed by the GOF and pcVPC. Median exogenous rIX-FP simulated trough was approximately 5% in patients ≥ 12 years receiving 100 IU/kg every 21 days, approximately 5% for pediatric patients aged 6 to < 12 years, and 3% for pediatric patients aged < 6 years. Both pediatric age groups received 75 IU/kg every 14 days.

Conclusions: FIX activity-time profiles for different dosing schedules can be predicted using the PPK model. The PPK simulation data suggests that most adult and pediatric patients with hemophilia B receiving rIX-FP on 21-day and 14-day extended interval dosing regimens reach sufficient FIX activity levels to transition from severe to a mild or moderate hemophilia classification.

PB1066 | Evaluation of Inhibitor Risk Prediction Tools Based on Genetic Risk Factors in Persons with Hemophilia A

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Background: Inhibitors are the most important treatment-related complication among persons with hemophilia. Currently, the only validated risk prediction tool relies on information about prior product exposure, limiting its utility to predict inhibitor risk in untreated or minimally-treated patients. A risk prediction tool based on patient-related risk factors that could be available before initiation of treatment could be more useful clinically.

Aims: This investigation evaluates six risk prediction tools based on genetic risk factors for inhibitors among persons with hemophilia A. The tools combine information on genotypes for variants in genes in the immune response pathway and the hemophilia genotype.

TABLE 1 Comparison of area under receiver operating curve (AUC) for inhibitor risk prediction tools

		All (AUC)	Mild/Moderate (AUC)	Severe (AUC)
Validated Inhibitor Prediction Tool (ter Avest et al. 2008)				0.74
Hemophilia Variant Categorization	Immune Response Variant Estimates			
Pathogenicity-based(1)	Meta-analysis(4)	0.58	0.67	0.59
Functionally-based(2)	Meta-analysis(4)	0.65	0.53	0.63
Evidence-based(3)	Meta-analysis(4)		0.72	0.63
Pathogenicity-based(1)	HIRS-based(5)	0.61	0.68	0.57
Functionally-based(2)	HIRS-based(5)	0.66	0.59	0.58
Evidence-based(3)	HIRS-based(5)		0.75	0.62

(1)Pathogenicity-based: hemophilia-causing variant weighted based on American College of Medical Genetics and Genomics pathogenicity criteria (2)Functionally-based: hemophilia-causing variant weighted based on predicted functional impact on factor VIII protein (3)Evidence-based: hemophilia-causing variant weighted based on previously-published estimates of effect (Mild/Moderate: Eckhardt et al. 2013; Severe: Gouw et al. 2012) (4)Meta-analysis: immune response gene variant genotypes weighted based on meta-analysis results (5) HIRS-based: immune response gene variant genotypes weighted based on estimates of effect derived from analysis in Hemophilia Inhibitor Research Study (HIRS) cohort

Methods: Variants in genes in the immune response pathway were weighted based on 1) estimates from a meta-analysis or 2) results of a prior investigation in this population and combined with 3 different hemophilia genotype categorization schemes, for a total of 6 tools evaluated. The ability of each tool to correctly predict inhibitor status was evaluated in 558 White, non-Hispanic participants in the Hemophilia Inhibitor Research Study (HIRS) with complete genetic information and informed consent. Tool performance was evaluated using the area under the receiver operating curve (AUC) after cross-validation.

Results: Among 450 inhibitor-negative and 108 inhibitor-positive HIRS participants, the prediction tool that combined information on immune-response genes previously-found to be associated with inhibitors in this population and categorized the hemophilia genotype based on previously-reported estimates of effect performed best (AUC = 0.75 and AUC = 0.62 among persons with mild/moderate and severe disease, respectively) (Table 1).

Conclusions: The best-performing tool did not perform as well as a previously-validated tool among persons with severe disease but performed well among persons with mild/moderate disease. Although cross-validation methods were used to reduce bias introduced by using the same data to simultaneously fit and evaluate the models, validation of these findings in other populations is warranted.

PB1067 | Lower Incidence of Cardiovascular Disease in Patients with Haemophilia

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Background: In theory haemophilia could lower CVD incidence by impaired thrombus formation. CVD mortality is reported to be lower in people with haemophilia (PwH) than the general population (GP). Reports on non-fatal CVD are lacking. Earlier we reported an unfavourable risk profile compared to the GP.

Aims: Compare the fatal and non-fatal incidence of CVD in PWH compared to the predicted incidence based on a risk profile validated for the GP.

Methods: A prospective, observational study in PwH aged 30 years or older at inclusion from The Netherlands and United Kingdom. The incidence of CVD was compared to the predicted 5 years incidence based on the QRISK2 - 2011 cardiovascular risk score. According to QRISK cardiovascular events are defined as: Myocardial infarction, Ischemic heart disease, stroke or transient ischaemic attacks. Analysis including and excluding haemorrhagic stroke was performed. The relative risk (RR, with 95% confidence interval), absolute risk reduction (ARR) and one-sided Fisher exact test were calculated.

Results: In total 560/595 patients with a valid QRISK score were followed for 5 years and analysed. 16 (lost to follow up) and 19 (non-CVD death) were excluded. 51.4% had severe haemophilia, the

mean age 47.1 years. The observed number of events 11 (1.9%), was significantly lower than the predicted number of 25 events (4.50%) (RR:0.44 (0.22 - 0.89); p=0.01; ARR 2.40%). When analysing the subgroups, excluding haemorrhagic stroke as an event, significant lower event rates were found in severe haemophilia: RR 0.27 (0.08 - 0.97); p = 0.03; ARR: 2.74%, non-severe: RR 0.35 (0.13 - 0.98); p = 0.03; ARR 3.21%.

Conclusions: A lower than predicted CVD event rate was found in PwH, supporting the hypothesis that haemophilia protects against CVD.

PB1068 | Utilizing Continuous Factor Infusion Replacement in a Hemophilia B Patient Undergoing Cardiac Surgery

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Background: The life expectancy of patients with hemophilia has improved over the decades, leading to increased prevalence of comorbidities requiring surgical interventions. This presents a complex therapeutic challenge of managing factor replacement at a very high cost financially.

Aims: We manage a 57-year-old male with mild hemophilia B admitted for a scheduled coronary artery bypass grafting (CABG) surgery utilizing continuous infusion factor replacement (CIFR) during his admission, and report on the cost savings associated with CFIR when compared to bolus dosing of recombinant factor IX (rFIX, BeneFIX®).

Methods: Pre-operatively, the patient's endogenous factor IX activity was 15%. We used the following formula to calculate the dosage needed to replete: Dosage needed= % (Desired FIX level-current FIX level) X weight (in kilograms) X 1.3. The goal was to maintain his FIX activity level at 80-100% on post-operative days (POD) 0-3, and at 60-80% on POD 4-14. We adjusted the doses daily based on the FIX activity level obtained each day working under the premise that 1 IU of rFIX increased FIX activity by 0.8%, and that the half-life of rFIX is 18-24 hours. The infusion rate was determined by the fact that FIX activity decreased by 3-4 IU//kg/hour. Each vial of rFIX (1000 IU) was mixed in 50 ml of normal saline for infusion and lasted 4-6 hours based on the target activity level. The patient was discharged on POD-9 on Aspirin and BeneFIX bolus dosing of 5000 IU (5 vials) every 12 hours administered via visiting home nurse for an additional 5 days (until POD-14).

TABLE 1 Number of Vials of rFIX Used in CIFR versus Bolus Dosing

	Number of vials used in CIFR	Number of vials used in Bolus dosing
POD 0-9	40 vial	116 vials
POD 10-14	50 vials	50 vials
Total	90	166

TABLE 2 Summary of Target Factor Activity Level and Amount of Drug Used

	Goal Factor level	Beneflx bolus dose (IU)	Factor Activity %	Beneflx Infusion rate (IU/hour)	Number of Vials (1000IU) Used with CIFR	Number of vials (1000IU) used for bolus dosing
POD-0	80-100%	7000 IU	101%	272 IU/hour		14 vials
POD-1	80-100%	3000 IU	69%	272 IU/hour		14 vials
POD 1 (after bolus)	80-100%		131%	272 IU/hour		
POD-2	80-100%		115%	272 IU/hour		14 vials
POD-3	80-100%	4000 IU	92%	272 IU/hour		14 vials
POD 4	60 80%		97%	200 IU/hour		10 vials
POD-5	60-80%		100%	200 IU/hour		10 vials
POD-6	60-80%		90%	200 IU/hour		10 vials
POD-7	60-80%		92%	200 IU/hour		10 vials
POD-8	60-80%			200 IU/hour		10 vials
POD-9 (Discharge)	60-80%			200 IU/hour		10 vials
					40 vials	116
POD 10 - 14	60-80%	5000IU q12h			50 vials	50 vials
					90 vials	166

Results: The patient required 65.5% less rFIX by utilizing the CIFR method while maintaining FIX activity levels at goal throughout his hospitalization.

Conclusions: CIFR achieved a steadier state of FIX activity, avoiding peaks and troughs while utilizing significantly less drug and saving over $800,000 in costs.

PB1069 | rFIXFc at Lower Doses Can Achieve Very Significant Reduction of Annualized Bleed Rates in Children with Severe Haemophilia B

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Background: rFIXFc has shown efficacy in prophylaxis of PwHB. However, in developing countries, lack of access to clotting factor concentrates (CFC) lead to patients not receiving prophylaxis. We present our experience in providing prophylaxis to children with haemophilia A using rFIXFc provided by the World Federation of Hemophilia through the humanitarian aid program.

Aims: To assess the annualized bleed rate in children with severe haemophilia B who are on prophylaxis with rFIXFc

Methods: Children with severe haemophilia B who have been either on episodic therapy or prophylaxis with conventional half- life FIX who were given prophylaxis with rFIXFc were included in this analysis. The dose was 15-20 IU/kg once in 2 weeks. Annualized bleed

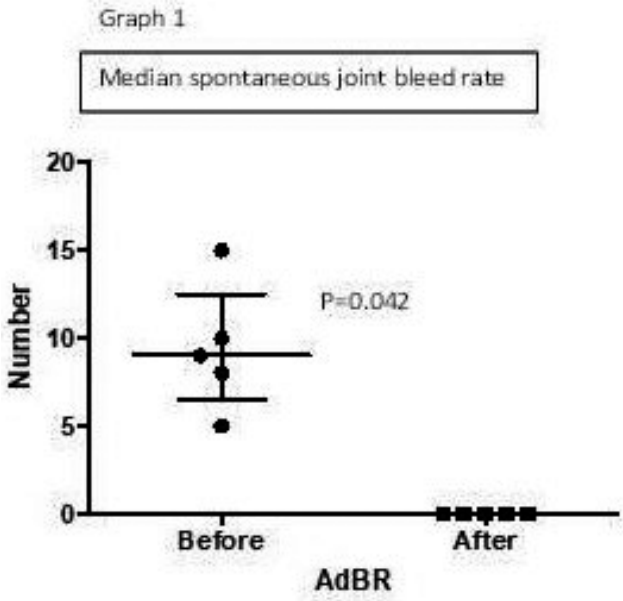

FIGURE 1 Median spontaneous joint bleed rate before and after starting prophylaxis

rate (AdBR) immediately prior to inclusion in this prophylaxis program was documented. All further bleeds were the prospectively documented while on prophylaxis with special emphasis on joint or muscle bleeds. Inhibitors were monitored every 3-6 months depending on prior exposures.

Results: A total of 5 children received prophylaxis. Their median age was 8 (range: 2-14) years. The median exposures prior to starting this prophylaxis program was 17 (range: 10-32). Median AdBR prior to prophylaxis was 9 (range: 5-15). At a median dose of 19 (range: 13-28) IU/kg once in 2 weeks, the median AdBR was 5 (range: 0-7) at a median follow-up of 110 weeks (range: 66-124). The median annualized spontaneous joint bleed rate was 0 (range: 0-0) during the same period. No patients had inhibitor at study entry or during prophylaxis at a median of 55 exposures (range: 32-63). Compliance was good with a median of 0 (range:0-3) missed doses.

Conclusions: rFIXFc at lower doses of ~20 IU/kg is effective in reducing spontaneous joint bleed rate to zero with normal activities of daily living at a convenient 2 weekly dosing with marked reduction in overall cost.

PB1070 | Hemostatic Management of Severe Hemophilia A (SHA) Patient with Dilated Myocardiopathy through Extracorporeal Membrane Oxygenation (ECMO), Ventricular Assist Device (VAD) and Heart Transplant (HT)

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Background: Dilated cardiomyopathy requires anticoagulation and support devices, ECMO and VAD, bridging to HT. Bleeding and thrombosis are common complications. Patients with SHA, make hemostatic management especially challenging.

Aims: To report the management of a pediatric SHA patient admitted with dilated cardiomyopathy: from initial anticoagulation, through V-A ECMO and VAD to HT.

Methods: Case report: 13 y.o. SHA patient without inhibitor, diagnosed with refractory dilated myocardiopathy. FVIII replacement and anticoagulation, from initial clinical treatment through implantation of ECMO and VAD, and final HT is reported.

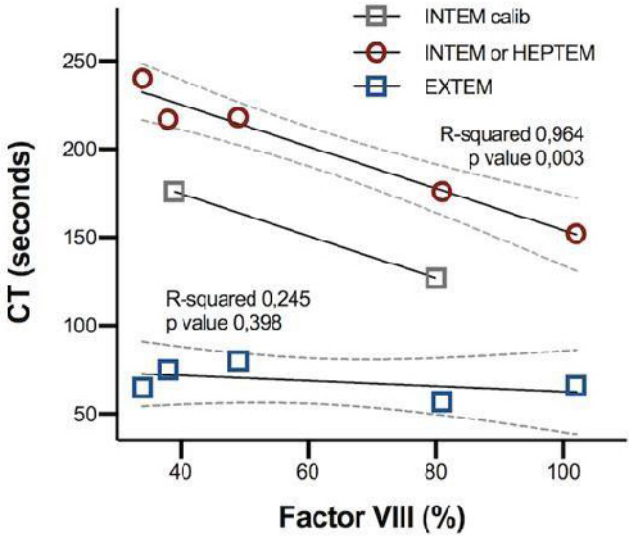

FIGURE 1 Correlation between ROTEM parameters and FVIII levels

TABLE 1 APTT, FVIII, AntiXa and ROTEM parameters at VAD and HT surgeries

Type of Cx and stage		APTT (sec)	FVIII (U/dL)	PT (% activity	AntiXa (IU/mL)	CT INTEM (sec)	CT HEPTEM (sec)	CT HEPTEM /CT INTEM RATIO	CT EXTEM (sec)
Reference or target range		24-40	50-150	70-120	0.3-0.7 (UFH)	120-220	120-220	Heparin Absence >0.8	45- 79
Test 2 weeks pre Surgery	Pre-Infusion of FVIII	38	39	97		176			56
Test 2 weeks pre Surgery	Post-Infusion of FVIII	37	80	97		127			66
VAD CCV surgery	Basal				0.42	320	217	0.68	75
VAD CCV surgery	CPB						253		90
VAD CCV surgery	Post Protamine	45	49	63		218	201	0.92	80
HT CCV surgery	Basal	36	81	89	0.29	176	169	0.96	56
HT CCV surgery	CPB						179		89
HT CCV surgery	Post Protamine	34	102	73		152	145	0.95	66

FVIII measurement: one stage clotting assay (3 dilutions). UFH and LMWH monitoring by chromogenic AntiXa activity. Thromboelastometry (ROTEM) for intraoperative monitoring.

Results: FVIII Concentrate was initially administered on continuous infusion (levels 30-50IU/dL) and UFH was started (antiXa 0.35-0.7U/mL). As he was stable, FVIII IV bolus/8 hours was given (aim 20-50IU/dL) and UFH replaced by LMWH (antiXa 0.5-0.1U/ml). After ECMO and VAD UFH was restarted. Prior to surgical procedures 2000U FVIII were administered, with additional 1000 U/2 hours during surgery to raise FVIII to 80-100UI/dL. Tranexamic acid was used during VAD and HT surgeries. Post CPB (cardiopulmonary bypass) blood loss was minimal.

APTT, FVIII, antiXa and ROTEM results in table 1. Correlation between ROTEM parameters and FVIII shown in figure 1. A good correlation between INTEM or HEPTEM CT and FVIII was seen, but all CT values were within normal range, implying a low sensitivity to detect 30 IU/dL FVIII.

The patient had a good clinical outcome, with a DVT (related to ECMO cannulation) as the only complication.

Conclusions: Assessing bleeding risk of every patient and setting adequate FVIII levels targets before anticoagulation and surgery, as well as evaluating bleeding and thrombotic risk related to support devices to manage anticoagulation by a multidisciplinary team, makes it possible to choose the best option for each patient.

PB1071 | No Peak of New-onset Inhibitors in the Aging Hemophilia Population: An International, Retrospective, Observational, Cohort Study of New Inhibitors in People with Hemophilia Aged 40 Years and Older (the NIAP Study)

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Background: A second peak of inhibitor development above age 60 in patients with severe hemophilia A has been suggested (Hay *et al*, *Blood* 2011), but not confirmed in other cohorts.

Aims: To further investigate, we identified and characterized patients with inhibitors diagnosed and treated within the frame of the ADVANCE Working Group, a European collaboration of hemophilia centers focused on the management of hemophilia at older ages.

Methods: Data were collected for 2,562 patients with hemophilia A aged ≥40 years from 16 centers over a retrospective period of 10 years.

Results: Fifty-eight patients were identified as having developed an inhibitor of any kind at age 40 and above; 19/1,222 (1.5%) severe of whom 5/276 (1.8%) were ≥60 years, 12/323 (3.7%) moderate, 25/1,017 (2.5%) mild and 2 not characterized. FVIII prophylaxis was in use at the time of inhibitor diagnosis/relapse in 74% of all severe HA patients, 33% moderate and 8% mild. Further data were collected on 43 patients via questionnaire on treatment, previous surgery and mutation. Overall, the use of prophylaxis was reported in 7/15 severe patients, 3/10 moderate and 1/18 mild patients. Among the 43 inhibitor patients, 32 were treated on-demand, of which the majority received rFVIII. All type of FVIII mutations were identified. The inhibitor was identified as a relapse of a previous inhibitor in 6 patients (3 severe). Most patients (26/43) had a history of surgery, trauma and/or serious infection in the 3 months preceding inhibitor identification; 23 of whom had a prior surgery (5/15 severe and 18/28 non-severe).

Conclusions: Although retrospectively collected, the evaluation of our relatively large cohort of closely monitored patients does not indicate a significantly higher incidence of new inhibitors than expected, and mainly associated with immune challenges. Most patients aged ≥40 years received FVIII prophylaxis, suggesting a potential value in regular exposure to maintain a tolerant state.

PB1072 | Real World Experience with Rurioctocog Alfa Pegol in Vall d'Hebron University Hospital

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Background: Prophylactic treatment with standard half-life coagulation factors (SHL) requires 3-4 weekly periodic infusions. Rurioctocog alfa pegol is an extended half-life (EHL) rFVIII (rFVIII-EHL) treatment that could be a more convenient and cost-effective therapeutic alternative than SHL, since it would reduce the number of weekly infusions (N.W.I).

Aims: The objective of the study was to describe the experience of the Vall d'Hebron University Hospital (Spain) with this rFVIII EHL, using pharmacokinetic (PK) parameters to individualize the new prophylactic regimen of our patients.

Methods: Ten severe hemophilia A patients were switched to prophylaxis with this rFVIII-EHL (mean age of 23.1 and SD 13.6, ranging from 12 to 50 years). A patient with mild hemophilia A in on demand treatment was excluded from the analysis. The assessment of the PK pharmacokinetic parameters was performed using the medical device myPKFiT®. In addition, A comparison between the previous treatment with FVIII SHL vs Rurioctocog alfa pegol was made for each patient .

Table 1 Regimens with FVIII-SHL vs EHL

Patient	Dose and prophylactic regimen (Nº weekly infusions, N.W.I.) with SHL	Dose and prophylactic regimen (Nº weekly infusions, N.W.I.) with rurioctocog alfa pegol
1	2000 UI M-Th (2)	2000 UI E/5 days (1)
2	1000 UI M-Th(2)	2000 UI E/72h (2)
3	1500 UI M y 2000 UI Th (2)	1500 UI M and 2000 UI Th (2)
4	3000 UI*	3000 UI E/7 days (1)
5	1500 UI M-W-F (3)	1500 UI T y 2000 UI F (2)
6	1000 UI M-Th y 1250 UI S (3)	2000 UI M-Th (2)
7	2000 UI M-Th (2)	3000 UI E/6 days (1)
8	750 UI T-Th-S (3)	2500 UI E/5 days (1)
9	3000 UI M-Th (2)	3000 UI E/5 days(1)
10	750 UI M-W-F (3)	1500 UI T and 2000 UI F (2)

* on demand treatment

Table 2 PK characteristics of patients treated with rurioctocog alfa pegol

Patient	Age (years)	Weight (Kg)	Half-life (h)	Vd (dl/kg)	Cl (dl/h/kg)
1	42	65	24	0.4	0.012
2	18	63	19.4	0.4	0.013
3	12	36	11.8	0.4	0.024
4	50	96	17.3	0.3	0.012
5	14	59	13.3	0.4	0.018
6	11	50.7	19.9	0.5	0.018
7	23	67	18.4	0.4	0.016
8	17	55	13	0.5	0.027
9	31	100	11.8	0.3	0.019
10	13	59.5	16.2	0.4	0.015

FIGURE 1

Results: Table 1 shows the dose comparison, prophylactic regimen and N.W.I of patients undergoing treatment with SHL (9 under prophylactic treatment with Advate® and 1 with Refacto®) vs the new (rFVIII- EHL). The N.W.I was reduced by 35% after the FVIII switching (23 infusions / week (n = 10) with the SHL vs. 15 infusions / week (n = 10) with the EHL, as it shown in Table 1). The PK characteristics of the patients after the upgrade are shown in Table 2.

Conclusions: Our experience shows that rurioctocog alfa pegol is a therapeutic alternative that allows, according to the PK patient parameters, to reduce the frequency of administration by 35% compared to SHL treatment. This reduction in the number of weekly infusions means a significantly lower disease burden for the patient and less coagulation factor consumption.

PB1073 | Switch to Extended Half-life Recombinant Single Chain Factor VIII in Haemophilia A Patients. Experience in One Centre

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Background: The use of extended half-life (EHL) recombinant factor VIII (rFVIII) products started around five years ago, with wide clinical

experience nowadays. Reducing monthly doses without increasing bleeding events or lowering FVIII trough levels, have been achieved in several studies.

Aims: We evaluate the switch to recombinant single chain FVIII in patients followed-up at a Haemophilia Treatment Center.

Methods: Nine haemophilia A patients switched from conventional rFVIII to EHL single chain rFVIII are described here: six patients with severe haemophilia (mean FVIII:C 0.5 IU/dL); one moderate (FVIII:C 1,3 IU/dL) and two mild haemophilia (mean FVIII:C 9.5 IU/dL). Median age was 31 years (2-55). Three patients were on prophylaxis and the other six on demand treatment. Only the youngest patient was previously untreated (PUP). All patients had a mean annualized bleeding rate of 0.5 (0-2).

Mean follow-up period was 18 months (9-24). Efficacy and potential adverse events were evaluated through clinical endpoints.

Results: Using single chain rFVIII allowed decreasing by 11% the amount of IU administered (from 55000 to 49000 IU/month). The youngest patient (the PUP) started scaled dose prophylaxis; the other two patients on prophylaxis regimen kept the number of injections and dose, in order to obtain a longer period above 1 IU/dL and thus adapting the prophylaxis to their lifestyle and haemorrhage phenotype.

Neither breakthrough bleeds nor inhibitor development were observed in any patient.

Two major surgery (included one orthopedic surgery) and three dental procedures were carried on without complications during follow-up period.

All patients interviewed agreed that this change had significantly increased their quality of life.

Conclusions: Although these are preliminary results, due to the limited number of patients and follow-up period, the switch to single chain rFVIII has been effective and safe in all age groups patients (including one PUP) with all severities haemophilia A.

PB1074 | Feasibility of Implementation of Point-of-Care Musculoskeletal Ultrasound in Joint Status Assessment in Children with Hemophilia

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Background: Hemophilic arthropathy results from repeated hemarthrosis in patients with hemophilia. Objective evaluation of joint status using point of care musculoskeletal ultrasound (POC-MSKUS) may provide longitudinal joint assessment and complement acute bleeding episode evaluation. Ultrasound is cost-effective, accessible, non-radiating, and sensitive to soft tissue changes in early arthropathy. Wide-scale implementation of POC-MSKUS in hemophilia treatment centers is hindered by concerns about feasibility of implementation in the comprehensive care model.

TABLE 1 Measures of feasibility

Feasibility Measures	Value
Recruitment rate	25/25 (100%)
Patients completed 6 joint evaluation by POC-MSKUS	25/25 (100%)
6 joint POC-MSKUS evaluation completed in <75 min	25/25 (100%)
Mean time for POC-MSKUS evaluation, (95% CI)	47 min (41.5, 52.5)
Number of trained providers completing each POC-MSKUS evaluation	2
Average patient satisfaction score (range 1-5), (95% CI)	4.8 (4.68, 4.92)

TABLE 2 Inter-rater reliability of HEAD-US scores expressed as percent correlation and Intraclass Correlation Coefficient (ICC)

HEAD-US Score	% Correlation	ICC
Elbow (n=50)	94 %	0.96
Knee (n=50)	90 %	0.39
Ankle (n=50)	86 %	0.96
Soft Tissue (n=150)	95 %	0.74
Cartilage (n=150)	94 %	0.92
Bone (n=150)	97 %	0.53
Joint additive score (n=25)	72 %	0.96

Aims: To evaluate the feasibility of implementation of POC-MSKUS in the pediatric comprehensive hemophilia care model.

Methods: Pediatric patients with hemophilia A or B underwent bilateral elbows, knees and ankles POC-MSKUS evaluation by a pediatric hematologist or physical therapist with 1-5 years of scanning experience and prior formal training. Joints were scored using the Hemophilia Early Arthropathy Detection with Ultrasound (HEAD-US) system, and read by a second blinded hematologist or physical therapist. Intraclass Correlation Coefficient (ICC) was used to assess inter-rater reliability of scores. Feasibility was assessed by recruitment rate, proportion of patients completing POC-MSKUS in the allotted time frame, and 5-point Likert scale patient satisfaction survey.

Results: 25 patients underwent POC-MSKUS evaluation, age 4-18 (mean 9.8 years), 84% were severe and 88% on prophylaxis. Recruitment rate was 100%, all 6 joint POC-MSKUS evaluations were completed in under 75 minutes (mean 47, CI 95% 41.5-52.5). Participants reported highly favorable satisfaction scores on a 5-point Likert scale (mean 4.8, CI 95% 4.68-4.92) (Table 1). Preliminary results for inter-rater reliability were fair for knees (ICC 0.39) and excellent for elbows (ICC 0.96), ankles (ICC 0.96), and total joint scores (ICC 0.96) (Table 2).

Conclusions: We demonstrate that POC-MSKUS can be feasibly implemented in the comprehensive hemophilia care model with good inter-rater reliability in ultrasound assessment of hemophilic arthropathy between different providers. Formal training, teamwork and maintenance of competence are key to successful implementation.

PB1075 | A Descriptive Study of Physical Activity and Fitness Level Profiles of Nigerians with Haemophila

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Background: .Physical activity is beneficial to persons with haemophilia (PWH) because it's been shown to improve joint, bone and muscle health. Even though awareness is being created and PWH are encouraged to indulge in physical activities, no study has described the level of physical activities of our PWH.
Aims: Description of physical activity and fitness levels of PWH in Nigeria.
Methods: A cross-sectional survey of PWH where a pre-tested, pre-validated, interviewer-administered modified PAQ_CAP1_1 (NHANES 2015) questionnaire was used. Measurable outcomes include level and intensity of physical activity and participation in physical fitness.
Results: Twenty-seven PWH participated, majority were ≤20years (77.7%), have haemophilia A (92.6%) with moderate severity (59.3%). The annualized bleeding rate was ≥6 in 44.4% of them, 85.2% had target joints while 88.9% had developed arthropathy. More than half (59.2%) of them performed little or no physical activity. Of those that perform significant activity, only 22.2% were continuously active for a minimum of 10minutes a day with mean number of days in a week of 4.8(1.5)days. Most of the respondents (74.1%) did not take up to 10minutes walk a day in a typical week. Only 33.3% of the subjects engaged in vigorous sports fitness, mostly a day in a week (44.4%) of about 20minutes (55.6%). About 57.2% of the participants performed moderate intensity sports 5days a week. The highest physical activities engaged by the subjects were walking (30.8%) and football (23.1%). Sixty-three percent of the subjects engaged in physical activities to strengthen their muscles and 40.7% spent 10-30 minutes to be physically active each day.
Conclusions: A good number of PWH in Nigeria still do not engage in significant physical activity and fitness. Walking was still the major activity performed by them. More efforts should be made toward educating PWH and their care givers on the need to engage in more physical activities.

PB1076 | Factor VIII trough Levels in Patients with Hemophilia A on Secondary Prophylaxis in Relation to Clinical and Radiological Outcomes

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Background: Pharmacokinetics guided dosing may be based on at least actual FVIII trough and peak levels instead of current FVIII estimates based on body weight and in-vivo recovery based dosing.
Aims: To assess the trough and peak level of factor VIII in patients with hemophilia A on low dose prophylaxis and its impact on the clinical and radiological joint status.
Methods: A follow up study was performed in Ain-Shams University, Pediatrics Hospital, Hemophilia Clinic. It included 25 children and adolescents with hemophilia A on prophylactic factor VIII Recombinant 3rd generation with a dose of 45 IU/Kg/week in 2 doses was used. Trough level of factor VIII was done before prophylactic dose and peak level post-infusion through chromogenic assay using STAGO-Immuno-Def VIII reagent. Clinical joint score and Functional Independence Score of Hemophilia (FISH) were done. Radiological joint scores were done using conventional x-ray, ultrasound and MRI. Cut-off level of trough levels was studied at 1% and 1%-5% of factor VIII.
Results: 4 patients (21.1%) had trough level < 1%, 13 patients (68.4%) had trough level between 1%-5%, 2 patients (10.5%) had trough level > 5%. No significant difference between trough level of factor VIII and clinical joint scores and FISH scores of patients. Median score of the worst joint of patients with trough level >5% was 5 in comparison to 8 and 7 in patients with trough level 1%-5% and < 1% respectively. Mean FISH of patients with trough level >5% was 9.50 ± 2.12 in comparison to 12.92 ± 5.41 and 11.00 ± 3.56 for patients with troughs 1%-5% and < 1% that was respectively.
Conclusions: Low trough levels alone did not warrant intensification of the prophylaxis regimen; the dose and dosing frequency may need be adjusted according to individual activity and bleeding pattern.

PB1077 | Successful Inhibitor Eradication for Hemophilia A Children with Poor-risk High-titer Inhibitor Using Low-dose Immune Tolerance Induction Strategy

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Background: Low-dose immune tolerance induction (ITI) using plasma-derived factor VIII/von Willebrand factor (pdFVIII/VWF) +/- immunosuppression showed satisfactory success rate and economically practical in China in our pilot study.

Aims: To confirm the efficacy of this strategy in hemophilia A inhibitor children with poor-ITI risk.

Methods: This was a single center, prospective study in 53 hemophilia A inhibitor children from September 2016 to October, 2018. All had pre-ITI inhibitors ≥10 BU +/- other poor-ITI risk and received ITI at ~50 FVIII IU/kg (domestic pdFVIII/VWF) every-other-day alone, or combined with immunosuppression (rituximab and prednisone).

Results: Forty-six subjects [median age 3.5 (IQR, 2.5-6.4) years] with pre-ITI inhibitor titer 29.5 (IQR, 15.0- 64.8) BU were included in final analysis with a total response rate 82.6% (38/46) [success (negative inhibitor) 71.7% (33/46) in median 8.6 (IQR, 4.4-10.4) months, partial success 10.9% (5/46)]. In subjects only used ITI alone, success was achieved in 94.1% (16/17), and 58.6% (17/29) in subjects added immunosuppression. The mean monthly bleeding rate during ITI was 0.49, a 59.3% declination from 1.18 in the pre-ITI period. Younger age (< 4.5 years) was predictor for ITI success. Relapse occurred in 8 of 33 successes but 7 of these returned to negative. No severe adverse reaction was reported.

Conclusions: In poor ITI-risk subjects, this low-dose ITI (pdFVIII/VWF) +/- immunosuppression strategy showed a success rate of ~70% which is similar to that in other high/intermediate-dose regimens. Younger age was related to better response. We are performing longer follow-up to further confirm the results.

PB1078 | Total Knee Arthroplasty in Patients with Hemophilia A and B: Impact on Quality of Life

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Background: Hemophilic arthropathy is a prevalent disease in patients with Hemophilia A and B that causes negative consequences on joint function, ability to perform daily activities, chronic pain and impairment on quality of life. Treatment of advanced cases consists of total arthroplasty with joint replacement, even in young patients, to restore their daily activities.

Aims: The aim of this study is to evaluate clinical results and impact onquality of life in hemophilic patients having advanced knee arthropaty and compare pre and post surgery data.

Methods: Seven patients were invited to participate, all Hemophilic A(6) and B(1), with indication of total knee arthroplasty (mean 37y, median 35y, range:30-47y). After patients signed the informed consent, a general questionnaire was applied, Haem-A-Qol, FISH and HJHS scores, previous the surgery. Six months later, it was applied again for re-evaluation. Bleeding rateswere also analyzed, pre and post surgery factor concentrate consumption.

TABLE 1 Evaluation of AJBR, HAEM-A -QOL, FISH and HJHS before surgery

NUMBER OF PATIENTS	AGE	AJBR	HAEMOQOL(%)			FISH	HJHS
			PHYSICAL HEALTH	BURDEN OF HEMOPHILIA	TOTAL		TOTAL
1	30	0	35	0	15.7	20	18
2	47	0	50	50	29.75	18	31
3	34	3	85	62.5	40.83	20	21
4	47	11	90	81.25	39.22	18	21
5	35	10	55	43.75	41.12	21	25
MEAN	38.60	4.8	63	47.5	33.32	19.4	23
MEDIAN	35	3	55	50	39.22	20	21

TABLE 2 Evaluation of AJBR, HAEM-A -QOL, FISH and HJHS after surgery

NUMBER OF PATIENTS	AGE	AJBR	HAEMOQOL			FISH	HJHS
		AJBR	PHYSICAL HEALTH	BURDEN OF HEMOPHILIA	TOTAL		TOTAL
1	30	1	35	0	9.2	23	17
2	47	4	25	25	29.43	21	13
3	34	0	30	6.25	34.16	23	11
4	47	1.09	65	75	33.5	23	14
5	35	9	5	0	13.81	29	11
MEAN	38.60	1.21	32	13	29	23.8	13
MEDIAN	35	1	30	12.5	29.43	23	13

Results: Five of seven patients were analyzed, because two of them didn't reach 6 months after surgery. The median rate of AJBR was 4.8 (range 0 to 11) and 1.21 (range 0 to 4); HJHS was 23 (range 18 to 31) and 13 (range 11 to 17); FISH score was 19.4(range 18 to 21) and 23.8 (range 21 to 29), Haem-A -QOL was 33.32%(range 15,7 to 41,12) and 29% (range 9.2 to 34.16) pre and post surgery.

Conclusions: Despite the moderate increase in HAEM-A QOL, due to other arthropathies that these patients have, when domain 1 and 2 related to physical health and the burden of hemophilia were analized, we found significant results. Regarding HJSH and FISH scores, all patients presented improvement, with positive impact in their daily activities.

PB1079 | Inhibitor Formation and Immune Tolerance Induction with Fc-fusion-Protein Recombinant Factor IX in Severe Hemophilia B

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Background: Availability of new generation of extended half-life products is revolutionizing the care of hemophilia patients, however, their use in immune tolerance induction (ITI) in hemophilia-B is not well-established.

Aims: We report an Indigenous Canadians boy who was diagnosed with severe hemophilia B at the age of 20 months following prolonged oral cavity bleeding. Genetic studies revealed a large deletion in the *FIX* gene; his mother was a *de-novo* carrier.

Methods: Patient was subsequently started on weekly prophylaxis with Fc-fusion-protein rFIX, Alprolix with resolution of bleeding. To minimize the number of required infusions, Alprolix was chosen. Despite this, due to difficult venous access, after 7 exposure-days a Port-a-Cath was inserted. However, during the procedure he developed an anaphylactic reaction and was subsequently diagnosed to have an inhibitor (0.62 BU/ml). After a two-week "holiday" window in which no further infusions of Alprolix were given patient was started on ITI according to the Beutel protocol. Given

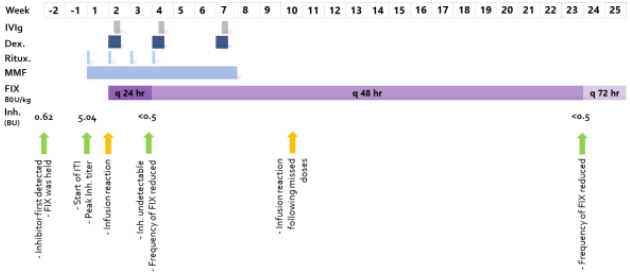

FIGURE 1 Course of ITI

some reports of successful ITI with Fc-fusion-protein rFVIII in hemophilia-A, Alprolix was chosen. Prior to starting ITI, patient's peak inhibitor titer was 5.04 BU/ml. The boy was initially commenced on daily Alprolix (80 IU/kg). Over the next 24 weeks he was gradually weaned to every-3-days. Since the start of ITI, patient has had two infusion reactions, both episodes occurring after a >4 day gap between Alprolix infusions.

Results: Patient has achieved Bethesda negativity, a good recovery (47%) and an estimated half-life of ~36 hrs, 24 weeks following the start of ITI. Patient has not needed any bypassing agent, nor had any proteinuria since starting ITI.

Conclusions: To our knowledge this is the first reported case of the use of extended half-life product as "first-line" ITI in a hemophilia-B with inhibitor, and suggests that Alprolix may be a promising option to improve the outcome of ITI in this challenging group of patients.

PB1080 | Clinical Improvement of Haemophilic Arthropathy in a Female Pediatric Patient with Severe Hemophilia A Following Treatment with Emicizumab

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Background: Emicizumab, a subcutaneously administrated bispecific monoclonal antibody that bridges activated factor IX and factor X, was recently approved for the prophylactic treatment of patients with hemophilia A following demonstration of a reduced rate of bleeding events and improvement of health-related quality of life.

Aims: Describe the use of Emicizumab in a pediatric female with severe hemophilia A.

Methods: Here we report Emicizumab therapy in a 6-year-old female with severe haemophilia A, factor VIII activity < 1%, compound heterozygous (intron 22 inversion and missense variant c.6742T>C). The patient was initiated on intravenous prophylactic infusions (second generation standard half-life product) when 2 years-old after multiple soft tissue bleeds and epistaxis leading to iron deficiency anemia. Patient was eventually transitioned to prophylactic therapy with Emicizumab weekly due to persistence of multiple episodes of epistaxis, multiple emergency department (ED) visits for acute bleeding events, an annualized bleeding rate (ABR) higher than 10, poor adherence, and development of heamophilia target joint (3 or more joint bleeds in a period of 6 months) in her ankles (bilateral) and right knee.

Results: The patient received Emicizumab for approximately 2 years with abolition of joint bleeds, control of epistaxis, reduction of ABR to 2, and no ED visits. There was multimodal assessment using POCUS imaging and MRI and documented clinical improvement.

Conclusions: We believe this to be the first case describing a female with severe haemophilia A using Emicizumab, and the first pediatric patient with resolution of haemophilic arthropathy after 2 years on Emicizumab therapy. There is currently a lack of data supporting the safety of Emicizumab in female patients of reproductive age; but our experience suggests that Emicizumab may be considered in female patients before puberty, and particularly those with haemophilic arthropathy. Our experience highlights the need for further studies of Emicizumab.

PB1081 | Emicizumab Prophylaxis in a Racially Diverse Pediatric Population with Hemophilia A with and without Inhibitors

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Background: Emicizumab is a recombinant, humanized, bispecific monoclonal antibody that bridges factor (F)IXa and FX to restore the function of missing activity FVIII to promote hemostasis in patients with hemophilia A (PHA). Clinical trials have shown the efficacy and safety of emicizumab in PHA with and without inhibitors, however, real-world data on the hemostatic efficacy, especially in a racially diverse, pediatric population is lacking.

Aims: To report real-world data of efficacy and safety in a diverse pediatric population with hemophilia A on emicizumab prophylaxis by retrospective chart review.

Methods: We reviewed medical records of PHA receiving emicizumab prophylaxis at The Bleeding Disorders and Thrombosis Program at the University of Texas Southwestern, Dallas and collected demographic and clinical information.

Results: We identified 30 patients with severe hemophilia A on emicizumab prophylaxis with a median age of 11.2 (range: 1-17) years without inhibitor (54%), and 47% with inhibitor. Fifty percent were Caucasians; the rest included Hispanics (26.7%), Blacks (13.3%) and Asian (10%). All patients received 4 weekly induction doses of emicizumab (3 mg/kg) followed by weekly maintenance dosing (1.5 mg/kg) in 22 patients and biweekly dosing (3 mg/kg) in 8 patients. Eight (26.7%) patients, whom 50% were Caucasians experienced bleeding while on emicizumab prophylaxis. Five (16.7%), whom four (80%) had history of inhibitor, experienced a breakthrough bleed while on emicizumab prophylaxis. Three (60%) were joint bleeds, one was an iliopsoas bleed, and one was a soft tissue bleed. All were on weekly maintenance dose except one on biweekly dosing. Four underwent

surgical procedure, all with a history of inhibitor. Three (60%) developed post-operative bleeding. There were no reported side effects.
Conclusions: We report the largest real-world data of a racially diverse pediatric population of PHA with and without inhibitors receiving emizicumab prophylaxis. Breakthrough and post-operative bleeding occur in PHA on emicizumab prophylaxis similarly in different racial backgrounds.

PB1082 | Mode of Delivery in Haemophilia: Vaginal Delivery and Cesarean Section Carry Similar Risk for Inhibitor Development in Severe Haemophilia A

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Background: The role of the gut microbiota on the immune system in haemophilia A is a potential risk factor for the development of anti-Factor VIII inhibitory antibodies. The infant gut microbiota has never been directly investigated in the haemophilia population. However, the mode of delivery is proposed to be a major influence on the composition of the early gut microbiota.
Aims: The aim of the study was to compare the incidence of inhibitors in children born by vaginal delivery and cesarean section.
Methods: Data was analysed using SPSS.
Results: 956 children with severe haemophilia A that had reached 50 exposure days of FVIII treatment before their second birthday were included in this PedNet multicentre study. A family history of HA was identified in 45.1% (n=431) and prenatal diagnosis made in 6.3% (n=59). There were 337 cases of inhibitors in total. 60.4% (n=577) had a high-risk genetic mutation, 9.5% (n=91) had a family history of inhibitors and 14.5% (n=139) had intensive first treatment. Cesarean section was performed in 33.4% (n=320). No difference in risk of inhibitor development was observed in infants born by cesarean section compared to vaginal delivery overall (36.0% vs 31.3%, OR 1.15, CI 0.95-1.38). Additionally, cesarean section delivery was added into a logistic regression model using the established risk factors of (a) family history of inhibitors, (b) high risk genetic mutation and (c) intensive first treatment, and no improvement in model performance was observed. Further separate analyses of 8 subgroups defined by a combination of the presence or absence of the three previously mentioned established risk factors also showed no difference in the rate of inhibitor development (table 1) although the numbers of inhibitor positive patients in these subgroups was small.
Conclusions: In summary, no difference in the risk of inhibitor development was found between infants born by vaginal delivery or cesarean section.

TABLE 1

Table 1. Subgroup analysis based on established risk factors.

Subgroup defined by established risk factors			n=	Inhibitor developed (n=)	C-section (n=)	C-section AND inhibitor developed	Odds Ratio (95% Confidence Interval)
Family history of inhibitors	High risk genetic mutation	Intensive first treatment					
✓	✓	✓	11	100% (11)	45% (5)	n=5	NA
✓	✓	✗	47	49% (23)	49% (23)	n=11	0.96 (0.53-1.77)
✓	✗	✓	4	50% (2)	0% (0)	n=0	NA
✓	✗	✗	29	31% (9)	41% (12)	n=5	1.59 (0.68-3.66)
✓	✗	✗	29	31% (9)	41% (12)	n=5	1.59 (0.68-3.66)
✗	✓	✓	74	58% (43)	27% (20)	n=14	1.68 (0.73-3.89)
✗	✓	✗	445	35% (157)	37% (163)	n=57	0.99 (0.76-1.28)
✗	✗	✓	50	14% (28)	30% (15)	n=3	0.64 (0.21-1.94)
✗	✗	✗	296	19% (55)	79% (70)	n=19	1.39 (0.91-2.12)

PB1083 | Inhibitor Epidemiology among People Living with Haemophilia A in South West Nigeria

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Background: One of the most significant complications of haemophilia treatment is the development of an inhibitor to exogenous factor viii administered in Haemophilia. As a result of the immune response, genetic predisposition and environmental risk factors. This usually contributes to the treatment challenge and decrease the quality of life
Aims: The aim of this study is to determine the prevalence, pattern and possible risk factors for development of Inhibitors in haemophilia A patients attending clinics in south west Nigeria
Methods: Information such as age, sex, clinical history, annual bleed rate, infusion history including types of factor replacement therapies they have been exposed to were retrieved from the World Bleeding Disorder Registry. Samples were taken and APTT based Inhibitor screening was carried out.
Results: A total of 40 haemophilia A patients were enrolled in this study, all are Male patients with age range between 2 years and 30 years of age. 75% were severe Haemophilacs, 22.5% moderate and 2.5% were mild. Fourty percent of Haemophilia A had positive inhibitor screening. 75% of those that tested positive to inhibitors had time dependent inhibitors while 25% have a pattern that appears as time independent inhibitors(likely high titre factor viii inhibitor). All the patient with inhibitors are of severe phenotype using clinical and laboratory parameters.
Conclusions: Factor vii inhibitors causes major challenge in Haemophilia management. routine screening and genetic testing can aid early identification and proper anagement plan

PB1084 | Is Radiosynovectomy a Tool in Advanced Hemophilic Arthropathy?

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Background: The treatment of hemophilic arthropathy is challenging. In the initial cases, where synovitis is the main component, radiosynovectomy (RS) is a safe and effective option. For advanced cases, with joint destruction, arthroplasties and arthrodesis are usually indicated. However, there is no well-defined treatment for cases that fall between these two extremes.
Aims: Report a series of cases in hemophilic patients undergoing Ytrium (^{90} Y) radiosynovectomy for the treatment of cases that are intermediate between these two conditions.
Methods: Fifteen RS (^{90} Y) were performed in 7 severe hemophilia A patients, being 9 in ankles, 5 in knees and 1 in elbow. The median age was 32 years-old (average=34.7). Six (86%) patients had at least 2 joints treated. All patients were assessed using the *Functional Independence Score in Hemophilia* (FISH), *Hemophilia Joint Health Score* (HJHS), Gilbert Score and the functional tests *timed up and go* (TUG) and the sit and stand test 5 times (TSL5). The patients were evaluated before and 1, 3 and 6 months after the procedure, with the final evaluation at 12 months. The median HJHS of the affected joints was 10 (average=10.3)
Results: All patients showed an improvement in synovitis, as assessed by edema on the Gilbert and HJHS scales. Only 3 patients showed a functional improvement, maintained for 12 months in the evaluation through TUG and TSL5. The other 4 patients showed an improvement, but it was lost, with results similar to the initial evaluations after 6 months. The FISH functional scale showed no significant changes.
Conclusions: Although RS shows improvement in patients′ synovitis, it has not been translated into a clinically relevant improvement for most patients. However, other forms of treatment should be investigated for these cases, since 83% of patients who did not have a sustained functional improvement do not wish to be operated.

PB1085 | Surgery in People with Hemophilia and Inhibitors: 10 Years of Experience in a Portuguese Comprehensive Care Centre

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Background: The development of inhibitors is considered the most challenging complication of hemophilia treatment. Bypassing agents, recombinant FVIIa (rFVIIa) and activated prothrombin complex concentrate (aPCC), have shown to be fair effective in patients with inhibitors undergoing surgery, although not so effective as lacking factor.
Aims: Report of 10 years experience of a portuguese hemophilia comprehensive care centre in patients with severe hemophilia with inhibitors (high responders) undergoing surgery.
Methods: Severe hemophilic patients (plasma factor levels < 1 IU/dL) with inhibitors who underwent surgery between 2010 and 2019 were included in a retrospective study. Data related to patient profile, procedure performed and treatment administered was collected from hospital electronic applications (Sclinic, SISLAB and SIBAS).
Results: 26 surgeries were performed in 7 patients: 7 (26.9%) were orthopedic and 19 (73.1%) refered to other procedures.All patients had severe hemophilia: 6 (85,7%) hemophilia A and 1 (14,3%) hemophilia B. The median age was 23,7 years (2-50).
The median inhibitor titer before procedure was 78,5 Bethesda Units (0,6-282). In 25 (96,1%) cases, patients received bypassing agent (rFVIIa was given in 16 (61,5%) surgeries and aPPC in 9 (34,6%)). One patient (3,9%) received FVIII concentrate (CFVIII).
All pediatric patients received rFVIIa, and adults aPCC and/or CFVIII. The median dose administered in each case was 27,6 mg/day of rFVIIa, 5.737 IU/day of aPPC and 4.797 IU/day of CFVIII.
The median length of hospitalization was 5,5 days (1-27). The hemostasis control was achieved in all cases and no major adverse events were recorded, haemorrhagic or thrombotic.
Conclusions: In our centre patients with hemophilia and inhibitors undergoing surgery have been effectively and safely managed with bypassing agents, rFVIIa either/or aPPC.
Patients with high titer inhibitors but low titer before procedure, were treated with CFVIII, overcoming the inhibitor and before the anamnestic response, achieving a better hemostatic outcome and saving costs.

PB1086 | Immune Tolerance Induction in Children with Hemophilia A, in Times of Emicizumab: A Real Life Experience

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Background: Neutralizing inhibitors against factor VIII has been a challenge in hemophilia due to inappropriate control of bleedings with bypass agents, development of arthropathy and need of central venous access.
An emergent monoclonal antibody "emicizumab" has arrived as a technology that provides prothrombinase activity, binding Factor IXa and Factor X with bispecific domains and solving unmet medical needs like subcutaneous administration and long acting efficacy. The only intervention identified for inhibitor eradication has been immunotolerance and even in the times of emicizumab remains as an important approach to be considered in real life medical practice.
Aims: To describe patients with Hemophilia A under 18 years old with high inhibitors titers in therapy with emicizumab plus immunotolerance (Adapted Regimen: FIT - Future of Immunotolerance Treatment Group).

Methods: Clinical data of patients were obtained and analyzed from registry of the hemophilia center "IPS Especializada", informed consent was obtained for every patient and approval of institutional medical ethics committee was provided.

Results: Three male patients with Hemophilia A and high inhibitors titers were included, once patients tracing started, their age were 1, 4 and 15 years old, theirs inhibitor levels were 35, 16 and 100 UBethesda respectively, all of them used bypass agents as prophylaxis and two of them were receiving immunotolerance induction, additional population characteristics Table 1. Every patient was proposed to change therapy to emicizumab plus immunotolerance with Factor VIII (50 Ui/kg thrice a week), two of them accepted the new scheme without develop new bleedings, one of them reached negative inhibitors five months later and the other one has remained with high inhibitor titer. No adverse events have been reported.

Conclusions: New scheme of therapy with emicizumab and immunotolerance is feasible in children with Hemophilia A plus inhibitors, achieving the improvement of bleeding phenotype and committed with inhibitors eradication.

HEMOPHILIA GENE THERAPY

PB1087 | Long-Term Vector Genome Outcomes and Immunogenicity of AAV FVIII Gene Transfer in the Hemophilia A Dog Model

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Background: Gene therapy using adeno-associated viral (AAV) vectors has demonstrated durable Factor VIII (FVIII) expression in patients and animal models of severe hemophilia A. The mechanisms mediating long-term expression and capsid immune responses are not known.

Aims: To evaluate the site of expression, vector DNA forms and capsid immunogenicity to AAV-FVIII in a hemophilia dog model.

Methods: Hemophilia A dogs received a single canine-FVIII AAV vector infusion (AAV-BDD-cFVIII, AAV2=4, AAV6=3 and AAV8=1). FVIII activity (FVIII:C) was measured using pooled canine plasma as a standard. AAV-BDD-cFVIII vector genomes and BDD-cFVIII mRNA were quantified by qPCR and qRT-PCR. Circular full-length AAV episomes were quantified using droplet-digital PCR. Anti-AAV capsid neutralizing antibody (nAb) titres were measured using a cell-based *in-vitro* transduction inhibition assay.

Results: Eight dogs received a median dose of $1.25x10^{13}$ vg/kg AAV-BDD-cFVIII by portal vein infusion (Table 1). After 10.8 years (8.2-12.0 years), persistent FVIII:C (median one-stage=12.7%, chromogenic=7.2%) was seen in all responding dogs (n=6), with improvement in bleeding phenotype. AAV-BDD-cFVIII DNA was detected in the liver of all dogs (median=30.5 vg/ng), with lower levels in the spleen in 4 dogs (median=1.0 vg/ng). cFVIII mRNA was only detected in the liver of responding dogs (median=7.3 copies/ng). Hepatic cFVIII mRNA copies were significantly associated with FVIII:C. Full-length linear and circular AAV-BDD-cFVIII was detected in the liver of all animals. Anti-AAV capsid nAbs were detected throughout the study: mean earliest available peak post-treatment titre 6485. Most samples demonstrated limited cross-reactivity to other capsids. Although a decline in nAb titre was seen with time (coefficient= -314, $p< 0.001$), substantial nAbs remained detectable (mean final titre: 809).

Conclusions: Persistent liver-derived FVIII expression was seen 10 years after a single AAV-BDD-cFVIII infusion, with detectable full-length linear and circular AAV-FVIII forms. Although anti-capsid humoral immune responses decrease with time, these remain at levels potentially preventing redosing with the same capsid.

TABLE 1 FVIII expression, vector genomes and capsid immune response post AAV-FVIII. nd=not detected

ID (Sex)	AAV Serotype (dose vg/kg)	Final Chromogenic FVIII:C (years)	Liver BDD-cFVIII vg/ng DNA	Spleen BDD-cFVIII vg/ng DNA	Liver BDD-cFVIII copies/ng mRNA	Spleen BDD-cFVIII copies/ng mRNA	Liver Full-length Circular AAV-FVIII vg/diploid genome	Peak AAV nAb titre (years)	Late AAV nAb titre (years)
EL (F)	2 (6.0e12)	n/a (8.2)	8.9	0.3	6.2	nd	0.014	22068 (3.6)	n/a
VC (M)	2 (1.5e13)	2.9% (11.0)	120.4	nd	8.3	nd	0.096	6110 (2.4)	220 (11.0)
ANG (M)	2 (1.5e13)	<1% (11.4)	10.7	1.0	nd	nd	0.007	5631 (7.9)	1445 (11.5)
JU (M)	2 (2.7e13)	7.2% (11.9)	50.2	1.0	15.9	nd	0.003	5092 (3.2)	1864 (11.9)
ALX (F)	6 (1.0e13)	8.6% (10.5)	36.0	nd	26.4	nd	0.089	4130 (1.5)	293 (10.5)
MZ (M)	6 (1.0e13)	7.9% (10.1)	35.1	nd	5.9	nd	0.054	4805 (1.2)	634 (10.1)
MG (F)	6 (1.7e13)	<1% (11.3)	2.8	nd	nd	nd	0.002	761 (8.0)	647 (11.5)
FLO (F)	8 (1.0e13)	1.8% (9.8)	25.9	6.8	4.4	nd	0.018	3280 (1.0)	559 (10.5)

PB1088 | Frequency, Location and Nature of AAV Vector Insertions After Long-Term Follow up of FVIII Transgene Delivery in a Hemophilia A Dog Model

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Background: Liver-derived Factor VIII (FVIII) expression has been described in the canine hemophilia A model for more than 10 years following adeno-associated viral (AAV) gene therapy. Improved understanding of AAV integration may provide insights into the long-term safety of AAV based gene therapy.

Aims: To evaluate frequency and locations of AAV-FVIII integration sites in a canine hemophilia model.

Methods: Eight severe hemophilia A dogs received a portal vein infusion of canine-FVIII-AAV vector (AAV-BDD-cFVIII). Genomic DNA was isolated from post-mortem liver samples. AAV-BDD-cFVIII vector genomes (vg/ng) were quantified by qPCR. Vector integration sites (IS) were analysed by linear amplification-mediated (LAM)-PCR and target-enrichment sequencing (TES) coupled to Illumina MiSeq sequencing and customised bioinformatical analysis.

Results: AAV-BDD-cFVIII DNA was detected in the liver of all dogs, 10.8 years after a single vector infusion (Table 1). LAM-PCR and TES analyses demonstrated overlapping IS and low integration frequencies in all animals: median=9.55e-4 IS/cell by TES and 4.50e-04 IS/cell by LAM-PCR. IS number correlated with vg/ng copies, but not with AAV dose or gender. Most integration events (93.8%) occurred in intergenic regions of the dog genome (upstream=49.0%, downstream=44.2%, in-gene=6.3% and unannotated=0.6%). The most frequent in-gene integration events occurred within the F8 (all animals, intronic=59, exonic=143) and albumin loci (7 animals, intronic=22, exonic=1). All other exonic insertions (n=8) were random appearing single gene events. Analysis of the top 10 IS in each sample, revealed IS in proximity to 81 genes, with the most frequent recurrent sites being MIR1296 (downstream=18), KCNIP2 (downstream=16), ABCB1 (upstream=13), CLIC2 (upstream=12), F8 (in-gene=6) and MET (upstream=5). Despite integration events occurring in all animals, no tumors were found within the liver at post-mortem.

Conclusions: Low hepatic AAV vector integration frequencies, at rates lower than the spontaneous natural human mutation rate, with some apparent recurrent loci were detected 10 years after a single dose of AAV-FVIII.

TABLE 1 Targeted Enrichment Sequencing of Integration Events in a Canine Hemophilia. nr=no response

ID	Dose Capsid cFVIII:C	Mean vg/ng (vg/cell)	TES Mean IS/vg	TES Mean IS/cell	TES Mean IS	Mean Upstream IS (%)	Mean In-gene IS (%)	Mean Downstream IS (%)	Mean Unannotated IS (%)
VC	1.5e13 AAV2 2.9%	120.4 (7.94e-1)	4.38e-3	3.48e-3	665.5	315 (47.3%)	47 (7.1%)	300.5 (45.2%)	3 (0.5%)
JU	2.7e13 AAV2 7.2%	50.2 (3.31e-1)	5.48e-3	1.82e-3	542	225 (41.5%)	40.5 (7.5%)	273 (50.4%)	3.5 (0.6%)
ALX	1.0e13 AAV6 8.6%	36.0 (2.38e-1)	3.32e-3	7.89e-4	336.5	162.5 (48.3%)	16.5 (4.9%)	155.5 (46.2%)	2 (0.6%)
MZ	1.0e13 AAV6 7.9%	35.1 (2.32e-1)	3.46e-3	8.02e-4	298	176.5 (59.2%)	17.5 (5.9%)	100.5 (33.7%)	3.5 (1.2%)
FLO	1.0e13 AAV8 1.8%	25.9 (1.71e-1)	7.00e-3	1.19e-3	387.5	204.5 (52.8%)	19 (4.9%)	162 (41.8%)	2 (0.5%)
ELI	6.0e12 AAV2 n/a	8.9 (5.87e-2)	1.51e-2	8.88e-4	382.5	187 (48.9%)	22.5 (5.9%)	171.5 (44.8%)	1.5 (0.4%)
ANG	1.5e13 AAV2 <1% (nr)	10.7 (7.03e-2)	1.46e-2	1.02e-3	184	98.5 (53.5%)	10.5 (5.7%)	74 (40.2%)	1 (0.5%)
MG	1.7e13 AAV6 <1% (nr)	2.8 (1.82e-2)	8.91e-3	1.62e-4	102	52 (51.0%)	6.5 (6.4%)	43 (42.2%)	0.5 (0.5%)

PB1089 | In the Future, Will ADSCs Become the Target Cells for Hemophilia A Gene Therapy?

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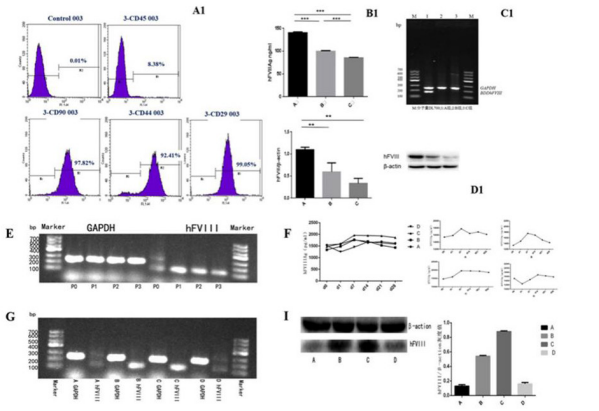
Background: Adipose-derived stem cells (ADSCs) are easy to obtain and have low immunogenicity,expected to be a target cell for hemophilia A gene therapy.
Aims: To explore the possibility of successful transfection of ADSCs as target cells for hemophilia A (HA) gene therapy by recombinant adenoviral vector containing B-region deleted human factor VIII (BDDhFVIII).
Methods: ADSCs were isolated and cultured. A recombinant adenovirus vector carrying BDDhFVIII was transfected into ADSCs: Group A was Ad-BDDhFVIII-GFP-infected ADSCs group, Group B was Ad-GFP-infected ADSCs group, and Group C was normal cultured ADSCs group,and stably transfected to the third generation ADSCs (P3)(Fig 1 A1-E). Ad-GFP and Ad-BDDhFVIII-GFP were transfected into ADSCs (HA A group, HA B group) and Ad-BDDhFVIII-GFP, Ad-GFP (HA C group, HA D group), respectively, into the HA mice via the tail vein. One week before the injection, blood was collected on days 1, 7, 14, 21, and 28 after the injection, and the liver was collected on the 28th day. ELISA, RT-PCR and Western blot were used to detect plasma hFVIII antigen and liver hFVIII gene and protein expression. (Fig 2 F-I).
Results: ADSCs were successfully extracted. The expression of hFVIII antigen, genes and proteins was detected in ADSCs after 72 hours of transfection with the recombinant adenovirus vector, and it could be stably transfected into the third generation ADSCs. The results of ELISA showed that hFVIIIAg increased in both HA B group and HA C group. RT-PCR and Western blot showed that hFVIII gene and protein expression could be detected in HA B group and HA C group. Compared with the HA D group, the difference was statistically significant ($P < 0.05$).
Conclusions: Adenoviral vector carrying BDDhF? gene can efficiently transduce ADSCs and secrete human hF?, which can be continuously expressed in HA rats. ADSCs can be used as one of the target cells for hemophilia A gene therapy.



A1: Cultivated to the third generation of ADSCs in FCM instrument testing surface antigen; B1: hFⅧAg levels after 72 hours of infection in ADSCs; C1:hFⅧ gene expression after three groups of infected ADSCs; D1:hFⅧ protein levels after 72 hours of infection in ADSCs. **P<0.01,***P<0.001（n=3） E: BDDhFVIII gene expression in four groups of cells; F: Detection of plasma hFVIIIAg in four groups of HA mice by ELISA;G:Detection of hFVIII gene expression in liver by RT-PCR; I:Detection of hFVIII protein levels in liver by Western blot..

FIGURE 1 Experimental study on adenovirus-mediated gene therapy for hemophilia A

PB1090 | Increase in the Treatment Efficacy of Genome Editing for Hemophilia B by Codon-Optimization of SaCas9 in Mice

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Background: We have previously reported that clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9-mediated genome editing using an adeno-associated virus (AAV) vector to supplement ectopic FIX sequence at the target site could be an effective strategy for hemophilia B therapy. However, the increase in the FIX activity (FIX:C) was limited to 10%-20% in our previous report.
Aims: We attempted to improve the therapeutic effect of CRISPR/Cas9-mediated genome editing by amendments in Cas9 and donor sequences.
Methods: We used an AAV8 vector to deliver gene-editing enzyme and donor sequence into mouse liver hepatocytes for *in vivo*genome editing. Phenotypic corrections were assessed using plasma FIX:C.
Results: *Staphylococcus aureus* Cas9 (SaCas9) nickases (D10A or N580A) and dead SaCas9 (D10A plus N580A) did not induce double strand break (DSB), and could not increase FIX:C after AAV vector administration. The application of homology-independent targeted insertion (HITI) or microhomology-medicated end-joining-dependent integration (PITCh) into the donor sequence also failed to improve the FIX:C. However, codon-optimization (CO) of SaCas9 resulted in higher activity to induce DSB at the target site; reducing the requirement of vector doses. Further, introducing Padua mutation (correspond to human R338L) into the donor significantly increased the FIX:C (control: 8.9% ± 0.7%; Padua: 33.3% ± 11.1%). The combination of the CO of SaCas9 and the donor sequence with Padua mutation further increased the FIX:C after genome editing (control: 13.7% ± 2.9%; CO: 34. 8% ± 5.5%; CO plus Padua: 70.0% ± 4.1%).
Conclusions: Our results suggest that the genome editing approach to insert donor sequence at a target site requires DSB induction via nuclease in genome editing. Further, CO of Cas9 enhances DSB induction, increasing the treatment efficacy of CRISPR/Cas9-mediated genome editing.

PB1091 | Multi-Centre Field Study of One-Stage and Chromogenic Factor IX Assays in Samples Containing the Factor IX Padua Variant

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Background: FIX-Padua is a thrombophilic FIX variant with an 8-fold higher specific activity than wild-type FIX (FIXwt). FIX-Padua has been incorporated into AAV gene therapy vectors and has had the expected effect of boosting FIX activity (FIX:C) by 8-fold over similar vectors expressing FIXwt. We and others have shown in limited sample sets that FIX-Padua activity measured by one-stage and chromogenic assays yield discrepant results.

Aims: We performed a multicentre field study to evaluate the performance and intra- and inter-lab variability of one-stage and chromogenic FIX assays in measuring FIX-Padua activity.

Methods: The specific activity of the FLT180a gene product (recombinant FIX-Padua) was determined by the SynthASil™ method. FIX-Padua was spiked into haemophilia B plasma at five nominal concentrations: 150, 100, 50, 20 and 5% FIX:C (S1-S5, respectively). FIX:C was analysed in samples and FIXwt controls (C1, C2) using 13 one-stage clotting and 2 chromogenic FIX assays across 38 laboratories in 11 countries.

Results: The mean FIX:C result was calculated for all one-stage and chromogenic assays (Tables 1 and 2, respectively). On average, chromogenic FIX:C results were only 43% of mean one-stage FIX:C levels. FIX:C results from one-stage assays appeared to be influenced by APTT reagent used with silica-based one-stage reagents yielding the highest FIX:C levels while ellagic acid-based reagents tended to yield the lowest FIX:C levels. The FIX:C measured in control samples did not differ significantly regardless of type of assay or one-stage reagent used.

Conclusions: Our FIX-Padua field study shows that routinely used FIX assays can yield FIX results that vary over ~3-fold while controls showed relatively little variation. Further work is needed to understand the mechanisms causing FIX-Padua assay discrepancy so that patients can be appropriately monitored and managed in the event of traumatic or breakthrough bleeding, surgery and even in routine care should gene therapy expression not achieve normal FIX:C levels.

TABLE 1 Mean one-stage FIX activity (IU/dl) in centres using a given APTT reagent

Reagent	n	S1	S2	S3	S4	S5	C1	C2
Actin FS	9	141.4	100	50.9	18.5	3.5	124.8	114.5
Actin FSL	5	99.9	71.2	37.3	14.0	2.8	118.7	105.2
Cephascreen	3	169.3	119.4	64.1	23.2	4.6	140.2	127.9
CK Prest	4	146.7	106.5	57.0	20.8	3.8	132.3	122.5
Pathromtin	7	109.5	78.0	43.0	17.0	3.6	119.2	112.3
Synthafax	9	103.8	73.4	38.7	14.8	2.8	109.3	107.5
Synthasil	10	138.2	98.5	50.2	18.2	3.5	121.6	108.5
TrinCLOT APTT HS	4	170.5	123.8	64.1	22.7	3.9	123.2	111.9
Mean FIX:C, all reagents (%CV)	-	140.8 (17.8)	99.3 (17.6)	52.7 (18.0)	19.4 (16.7)	3.4 (16.4)	124.0 (7.1)	114.0 (6.3)

TABLE 2 Mean chromogenic FIX activity (IU/dl) in centres using the specified kits. One-stage and chromogenic FIX:C ratios are also shown

Chromogenic FIX Kit	n	S1	S2	S3	S4	S5	C1	C2
Biophen Factor IX™	8	58.1 (13.2)	39.7 (15.1)	18.1 (14.9)	6.2 (11.5)	0.8 (29.7)*	115.2 (7.6)	105.6 (6.7)
Rossix FIX™	9	64.0 (24.0)	42.1 (22.1)	19.4 (24.2)	6.7 (29.8)	1.8 (138.5)	116.0 (11.8)	104.0 (7.6)
Mean Chromogenic FIX:C	-	61.0	40.9	18.8	6.4	1.3	115.6	104.8
One-stage:Chromogenic ratio	-	2.3	2.4	2.8	3.0	2.6	1.1	1.1

PB1092 | Consistent Structural Kinetics of Valoctocogene Roxaparvovec DNA in Human Blood Samples

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Background: Valoctocogene roxaparvovec is a rAAV5-FVIII-SQ gene therapy investigated for treatment of hemophilia A. While the target organ for expression of FVIII is liver, small amounts of vector DNA in blood provide insight into kinetics of vector DNA structures in recipients' cells.

Aims: Evaluate valoctocogene roxaparvovec genome kinetics in blood.

Methods: DNA was extracted from whole blood or fractionated blood, from participants in valoctocogene roxaparvovec phase 1/2 and phase 3 studies. Analyses with multicolor ddPCR, qPCR, and ITR-fusion ddPCR assays were used to investigate vector DNA presence, contiguity, and circularization.

Results: Total vector DNA in blood decreased five orders of magnitude over the first 26 weeks post-administration, with distinct phases corresponding to lifespans of granulocytes and red blood cells, while very small amounts remained in the peripheral blood mononuclear cell (PBMC) fraction. Post-administration, only small amounts of vector DNA could be found unassociated with cells in plasma, and was largely fragmented. At six months, remaining vector DNA in the PBMC fraction (the only long-lived, nucleated cells) had largely undergone processing into assembled full-length transgenes, and ITR fusion consistent with stable episomal structures, while DNA in other cell fractions was either unprocessed or fragmented. Low level vector DNA was detectable past two years in the phase 1/2 study, consistent with long-lived PBMC lifespans. Moreover, DNA structural kinetics were consistent between recipients across phase 1/2 and phase 3 studies, within the first year of available samples.

Conclusions: Blood offers an accessible and relatively non-invasive window into rAAV vector molecular processing in human cells. Vector DNA in blood, outside of PBMCs, became largely degraded confirming negligible risk of horizontal transmission from blood. In the blood, nucleated PBMCs processed valoctocogene roxaparvovec vector into full-length transgenes, and maintained stable, durable forms for long periods of time. Reproducible results across clinical phase studies help support comparability of product throughout clinical development.

PB1093 | Adenovirus Mediates Expression of Human Coagulation Factor IX Gene in Adipose-Derived Mesenchymal Stem Cells

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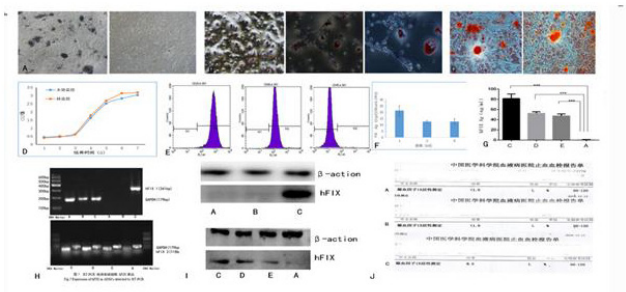
Background: Adipose stem cells have many advantages,such as easy to obtain,low immunogenicity,transformed in multiple directions,and can be used as one of the target cells for hemophilia B gene therapy.

Aims: To explore the possibility of successful transfection of adipose-derived stem cells (ADSCs) as target cells for hemophilia B (HB) gene therapy by recombinant adenoviral vector containing human coagulation factor IX gene (FIX).

Methods: ADSCs were isolated and cultured. A recombinant adenovirus vector carrying FIX was transfected into ADSCs: group A:(DMEM was used instead of adenovirus to transfect ADSCs),group B:(Ad-GFP was used to transfect ADSCs), and group C: (Ad-hFIX was used to transfect ADSCs),group D:(one generation after passage of Ad-hFIX was used to transfect ADSCs),group E:(two generations after passage of Ad-hFIX was used to transfect ADSCs).The expression of hFIX gene was detected by RT-PCR. The expression of hFIX protein in ADSCs was detected by Western blot. The hFIX protein amount in the transfected ADSCs was measured by ELISA, the coagulation activity in culture supernatant was measured by one-stage method.

Results: ADSCs were successfully extracted. the expression of hFIX-antigen, gene and protein was detected in ADSCs after transfection of recombinant adenovirus vector, and the expression of hFIXantigen, gene and protein was able to be stably transfected to the third



A:Morphology of primary adipose-derived stem cells; B:Lipid induced lipid droplets formation in ADSCs; C:ADSCs induced calcium salt formation after osteogenesis; D:Growth curve of ADSCs; E:Cell phenotype of P3 ADSCs; F:The FIX:Ag of the supernatant at different time after ADSCs transfection tested by ELISA;
G:The expression of cell supernatant FIX: Ag by ELISA; H:The expression of hFIX gene was detected by RT-PCR;
I:The expression of hFIX protein in ADSCs detected by Western blot; J:The expression of cell supernatant FIX: C by one-stage method.

FIGURE 1 Adenovirus mediates expression of human coagulation factor IX gene in adipose-derived mesenchymal stem cells

generation adscs. compared with the blank control group, the difference was statistically significant ($p < 0.05$).

Conclusions: 1.Adenovirus carrying hFIX gene can effectively transfect ADSCs. 2.ADSCs modified by hFIX gene can secrete hFIX protein with coagulation activity.3.Recombinant adenovirus-transfected ADSCs were cultured after passage and still express hFIX. And ADSCs could serve as vector cells for hemophilia B gene therapy.

PB1094 | Clinical Immunogenicity of Valoctocogene Roxaparvovec in GENEr8-1, a Phase 3 Study of AAV5-Mediated Gene Therapy Encoding Human FVIII for the Treatment of Hemophilia A

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Background: GENEr8-1 is an ongoing phase 3 clinical study evaluating the safety and efficacy of valoctocogene roxaparvovec, an AAV5-mediated gene therapy encoding human B-domain-deleted FVIII (hFVIII-SQ) for the treatment of hemophilia A. Following administration, substantial increases in FVIII activity levels were observed with an acceptable safety profile. The most common adverse event was a mild, transient increase in ALT, which was managed using glucocorticoids.

Aims: Evaluate humoral and cellular immune responses following administration of valoctocogene roxaparvovec and assess impact on patient safety and therapeutic efficacy.

Methods: Plasma and PBMCs were collected at specified timepoints prior to and following administration of valoctocogene roxaparvovec from the first 16 patients with ≥26 weeks follow-up. FVIII inhibitors were assessed using the Nijmegen-modified Bethesda assay. FVIII and AAV5 total binding antibody (TAb) titers were determined by ECLA. Patients testing positive at screening for AAV5 TAb, or with a history of FVIII inhibitors, were excluded from the study. PBMCs were analyzed by IFN-γ ELISpot assay for capsid-specific and hFVIII-SQ-specific cellular immune responses.

Results: No patients developed FVIII inhibitors after dosing. All patients were AAV5 TAb positive by week 8 and remained positive at all subsequent timepoints. TAb titers were not associated with ALT elevations or FVIII activity measures. Most patients tested positive for cellular immune responses to AAV5; positivity was largely transient, self-limiting, and not consistently associated with changes in ALT or FVIII activity measures. Detection of FVIII-specific cellular immune responses was rare. Responses to both AAV5 and FVIII could be detected during and outside periods of glucocorticoid use.

Conclusions: The immune response to valoctocogene roxaparvovec is primarily directed toward the AAV5 capsid with only sporadic, transient responses toward hFVIII-SQ. These responses have not been associated with safety or efficacy outcomes. FVIII inhibitor responses have not been detected in any valoctocogene roxaparvovec recipient to date.

PB1095 | Mechanistic Evaluation of Factor IX-Padua Activity in Chromogenic FIX and Thrombin Generation Assays

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Background: Our gene therapy trial featuring AAVS3 FIX-Padua (FLT180a) is targeting FIX-Padua expression levels that functionally cure haemophilia B. Recent data shows that FIX-Padua activity (FIXp:C) assay results can vary by up to 3-fold depending on the assay used. Gene therapy clinical outcomes can vary substantially over a 3-fold FIX:C range emphasizing the need to understand mechanisms causing FIX assay discrepancy and how FIXp:C maps on to wild-type FIX activity (FIXwt:C). A thorough understanding of FIXp:C will help identify the appropriate expression target in clinical trials and inform on which assays are most suitable for monitoring gene therapy patients.

Aims: We scrutinized one-stage and chromogenic FIX assays to identify mechanisms causing assay discrepancy and sought to determine how FIXp:C relates to FIXwt:C.

Methods: We spiked FIXwt or FIX-Padua into haemophilia B plasma to yield various FIX activities. Samples were used to evaluate the impact of increasing FX on chromogenic FIX results and the difference between FIXp:C or FIXwt:C in tissue factor-initiated thrombin generation assays.

Results: Chromogenic assays contain normal FVIII levels, but sub-physiological concentrations of FX. Increasing FX in chromogenic assays dose-dependently increases FIXp:C up to 2-fold but has no effect on FIXwt:C levels. Interestingly, after normalizing FX in chromogenic assays, FIXp:C results are similar to one-stage assay results. In thrombin generation assays, given FIX:C levels, whether supplied by FIX-Padua or FIXwt, yield similar thrombin generation parameters.

Conclusions: Our data indicate that FX is limiting in chromogenic assays when measuring FIXp:C and supplementing FX can restore FIXp:C to levels measured with one-stage clotting assays. Altogether, our data suggests that one-stage FIX assays may provide a better estimate of FIXp:C and this activity is similar to FIXwt:C in driving physiologically relevant thrombin generation.

PB1096 | Surgical Experience with Fidanacogene Elaparvovec

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Background: Fidanacogene elaparvovec, an adeno-associated viral (AAV) vector for treatment of haemophilia B, utilises a bioengineered hepatotropic AAV capsid and a transgene cassette that expresses a high-activity variant of human factor IX (FIX)-Padua transgene via a liver-specific promoter.

Aims: To assess the safety and efficacy of fidanacogene elaparvovec in patients with haemophilia B undergoing surgery.

Methods: Fifteen patients with moderately severe to severe haemophilia B at baseline (FIX activity ≤2%) who provided informed consent received fidanacogene elaparvovec in an ethics committee-approved phase 1/2a study and ongoing long-term follow-up study.

Results: Fidanacogene elaparvovec demonstrated sustained efficacy over a period of up to 4 years, as assessed by steady-state FIX activity, annualised bleeding rate (ABR), and annualised infusion rate (AIR). It has been well tolerated, with no related serious adverse events reported to date. Two patients had non-related SAEs of appendicitis and emergent lumbar discectomy. Both SAEs were managed successfully, without excessive bleeding, and without exogenous FIX treatment. FIX activity levels were in the mild range (26.3% and 11.8%) at last measurement available before the event (measured via a central laboratory 1-stage assay). The full surgical and traumatic experience post fidanacogene elaparvovec treatment will be presented.

Conclusions: These are the first reported cases of surgical procedures in patients with haemophilia B who received FIX-Padua via AAV-based gene therapy. This experience heralds the efficacy of FIX-Padua in the setting of haemostatic challenge at levels in the mild range.

PB1097 | A Novel Human Factor VIII Variant Exhibits Increased Factor VIII Expression after AAV Gene Therapy in a Unique Hemophilia A Dog Model that is Tolerant to Human Factor VIII

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Background: Hemophilia A (HA) dogs remain a valuable large animal model for the study of long-term efficacy and safety of gene therapy, especially for translation of novel human factor VIII (hFVIII) variants.

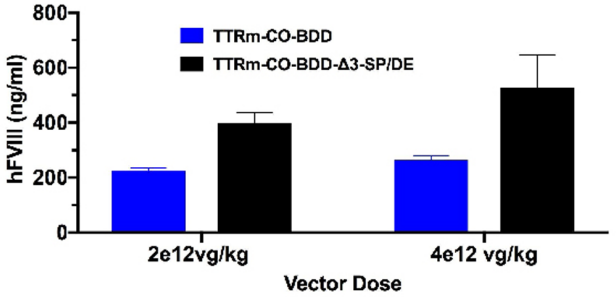

FIGURE 1 hFVIII expression in HA-CD4KO mice after AAV administration

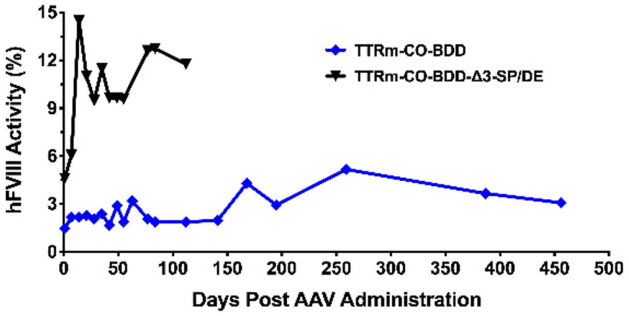

FIGURE 2 hFVIII expression after AAV administration in HA dogs tolerized to hFVIII

Due to differences between the human and canine (c) FVIII proteins, hFVIII provokes an anti-hFVIII immune response when tested in HA dogs, while using cFVIII in dogs would not be predictive of AAV dosage of a novel hFVIII variant in humans.

Aims: HA dogs were tolerized to hFVIII to evaluate a novel hFVIII variant with enhanced secretion in this model after AAV administration.

Methods: Puppies (d2) were treated with a retroviral (RV) vector expressing hFVIII-BDD. At 6 months old, the RV-treated dogs express 1-2% hFVIII and were challenged with hFVIII-BDD protein (25U/kg, 6 weekly I.V) and exhibited tolerance. Our novel variant lacks the first three amino acids (Δ3) of the furin cleavage site (1645-RHQR-1648) and contains modifications at the second major processing site in acidic region 3 (S1657P/D1658E)(SP/DE). The variant was incorporated into a liver-specific codon-optimized (CO) expression cassette with an enhanced promoter, TTRm.

Results: After AAV delivery in HA-CD4KO mice, TTRm-CO-BDD-Δ3-SP/DE exhibits a 2-fold increase in expression over TTRm-CO-BDD at both vector doses (Figure 1). The Δ3-SP/DE variant with altered processing sites is secreted more efficiently and almost exclusively in a single chain form both *in vitro* and *in vivo*, while hFVIII-BDD forms a heterodimer. One tolerized dog was treated with TTRm-CO-BDD (2e12vg/kg) and expressed around 4% hFVIII. A tolerized littermate was administered TTRm-CO-Δ3-SP/DE at the same AAV dose and expressed ~12% hFVIII

(Figure 2). Both dogs exhibit no increase in anti-hFVIII IgG1, IgG2, or total IgG.

Conclusions: These studies suggest that Δ3-SP/DE leads to ~3-fold increase in expression compared to hFVIII-BDD in the HA dog, providing a unique approach to lower the AAV dose for HA gene therapy.

PB1098 | The World Federation of Hemophilia Gene Therapy Registry

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Background: Novel genetic therapies, such as gene therapy for hemophilia, raise many questions that will not be fully answered at the completion of clinical trial programs, and when the first product(s) receive regulatory approval.

Aims: The World Federation of Hemophilia (WFH) Gene Therapy Registry (GTR) is being developed to collect long-term data on safety, and variability and durability of efficacy on all people with hemophilia (PWH) who receive gene therapy.

Methods: The registry is being developed by the WFH, in collaboration with professional societies, regulatory authorities and industry. A Steering Committee (SC), composed of health care professionals, patient advocates, industry representatives, and regulatory agency liaisons oversees the development of the project. The WFH GTR is a prospective, observational, and longitudinal registry. All PWH who receive gene therapy, via clinical trial or post-approval, will be invited to participate, with the goal of enrolling 100% of eligible PWH globally. Data will be captured over the patient's lifetime, directly via participating hemophilia treatment centres or through data linkage with existing hemophilia registries who meet criteria.

Results: The WFH GTR SC is developing the core data set through an iterative process. The core data set includes data on demographics, vector infusion details, safety, efficacy and quality of life. Exact data fields and measurement are being determined in conjunction with regulatory agencies, ISTH, other patient and physician organizations, and the pharmaceutical companies responsible for ensuring collection of long-term data upon licensure. Policies governing data usage will be developed by an executive committee comprised of relevant patient and physician advocates.

Conclusions: The WFH GTR will ensure that rare adverse events, in a small patient population over a large geographical area, will be detected. The many unknowns of gene therapy for hemophilia make it critical that the bleeding disorders community ensures all patients are followed over their lifetimes.

PB1099 | Expectations and Concerns of Patients with Haemophilia towards Gene Therapy - Results of Patients and Expert Focus Groups

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Background: Gene therapy (GT) is a rapidly evolving new therapeutic approach demonstrating improvement in endogenous factor levels over sustained periods, significant reduction in annualised bleed rates and lower exogenous factor usage. Little is known about perceptions of patients with haemophilia (PWH) concerning gene therapy. Focus-group interviews are useful to collect information in health research.

Aims: To gain better insights in expectations and concerns of PWH towards GT.

Methods: In a qualitative study a focus group with PWH and an expert group with haemophilia treaters (HCP) were conducted in Germany. These group discussions were performed as semi-structured interviews and audio-recorded. Answers were transcribed verbatim, and qualitative findings were coded using content analysis methods.

Results: Five adult haemophilia patients (range 34-58 years) participated in the focus group, all had severe haemophilia A and two had an inhibitor. Three leading HCP from Germany participated in the expert group. PWH mentioned that they don't understand the concept of GT and did not feel well-informed. PWH expected that they would have to inject less, have less bleeds and pain and that they will be cured by GT. They wished information on side effects, logistics of follow-up and additional value of GT, preferably from their HCP. HCP identified treatment burden as an unmet need of PWH; especially non-compliant PWH and frequent bleeders could benefit from GT. HCP thought that PWH would switch if they would not have to inject themselves any longer, have no bleeds and their steady state factor level would be "good" (unspecified factor level).

Conclusions: In a next step, a patient survey will be developed based on the findings of the group discussions covering aspects such as: demographic and clinical data of PWH, satisfaction/problems with current therapy, attitudes and expectation towards GT, motivation to use GT, concerns towards GT and required information for decision making.

PB1100 | Ultrasound Mediated Gene Delivery Targets Liver Sinusoidal Endothelial Cells to Achieve Endothelial-specific Factor VIII Expression

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Background: We have shown that ultrasound (US) mediated gene delivery (UMGD) combined with microbubbles (MBs) successfully transfected mouse hepatocytes and achieved therapeutic levels of factor VIII (FVIII) using a hepatocyte-specific FVIII construct (pLP-hF8-X10). However recent studies showed liver sinusoidal endothelial cells (LSECs) to be the natural site of FVIII production. We hypothesize that lower US energy may be sufficient for efficient UMGD targeting LSECs since no endothelial membrane destruction is required.

Aims: In this study, we investigated manipulating the UMGD protocol to target LSECs and induce FVIII expression.

Methods: Plasmid DNA was injected in combination with RN18 MBs into mouse liver via portal vein injection. Simultaneously, therapeutic US was applied to the surface of the liver to enhance gene transfer using a one-minute pulsed US treatment with 1.1MHz frequency and 14 Hz PRF. We used a GFP plasmid to test a matrix of US conditions and selected an endothelial-targeting condition for UMGD of FVIII gene into endothelial cells in combination with a new endothelial-specific hFVIII plasmid (pUCOE-ICAM2-hF8). A previously established hepatocyte-targeting condition (1.5 MPa PNP, 150 us PD) was used with pLP-hF8-X10 as a control. APTT was utilized to assess activity levels in plasma.

Results: One condition (1.1 MPa, 150 us) from the matrix tested specifically targeted LSECs (Figure 1). Activity levels following UMGD with this condition resulted in comparable FVIII expression to the hepatocyte-targeting condition on day 3 with average levels of 17.4% and 24.1% respectively. The endothelial-specific FVIII expression maintained an average of ~21% at day 10 (Figure 2).

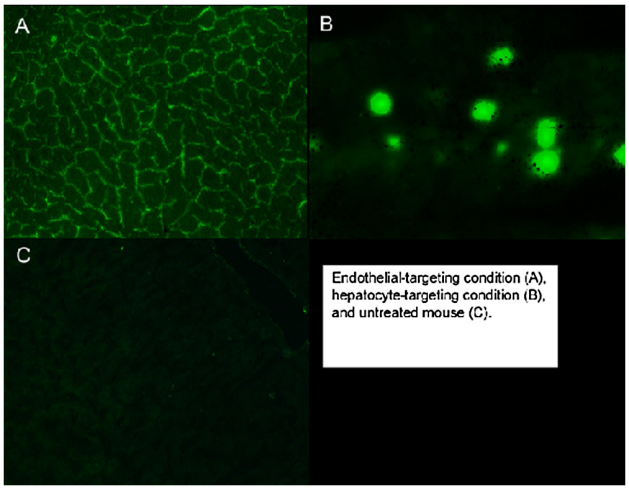

FIGURE 1 GFP Expression in Liver Sinusoidal Endothelial Cells or Hepatocytes

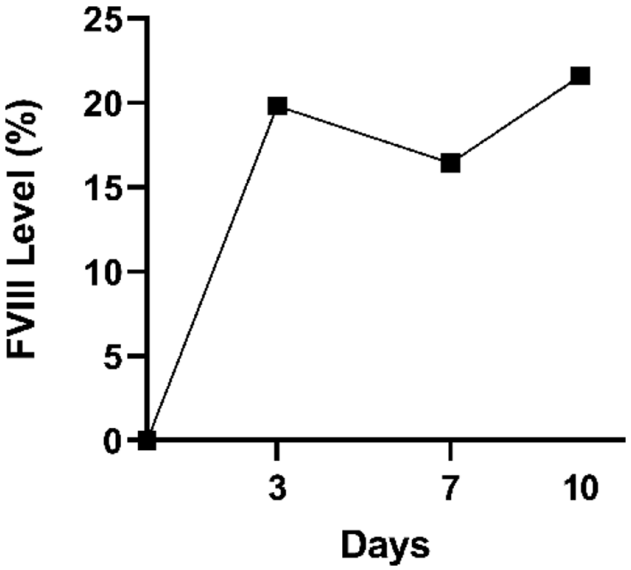

FIGURE 2 Factor VIII Expression Following UMGD Targeting Liver Sinusoidal Endothelial Cells

Conclusions: We were able to selectively target LSECs using UMGD, which resulted in stable and enduring expression levels compared to a previously-established hepatocyte condition. Efficient delivery of the FVIII gene into LSECs was achieved by lower energy US conditions, leading to better functional FVIII expression and reduced tissue damage.

PB1101 | Prevalence and Affinity/Avidity Assessment of Pre-Existing Neutralizing Antibodies (NABs) against Adeno-Associated Virus (AAV) Vector Serotypes 2, 5 and 8 Analyzed in the Serum of 300 Healthy Donors

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Background: It has been reported in preclinical and clinical studies for AAV2 or AAV8 serotypes that pre-existing anti-AAV NABs titers impact negatively transgene expression. Therefore, individuals with pre-existing NABs against AAV above titer of 5 are excluded from most systemic AAV-based clinical trials. We have previously reported that anti-AAV5 NABs titers up to 340 in humans and as high as 1030 in primates did not interfere with the therapeutic efficacy of intravenously administered AAV5 vector. Hence, patients entering the AMT-061 (AAV5-hFIX-Padua) trial are not excluded based on anti-AAV5 NABs levels.

Aims: Overall, those contradictory observations suggest that the neutralization ability of anti-AAV pre-existing NABs *in vivo* might be different between AAV serotypes.

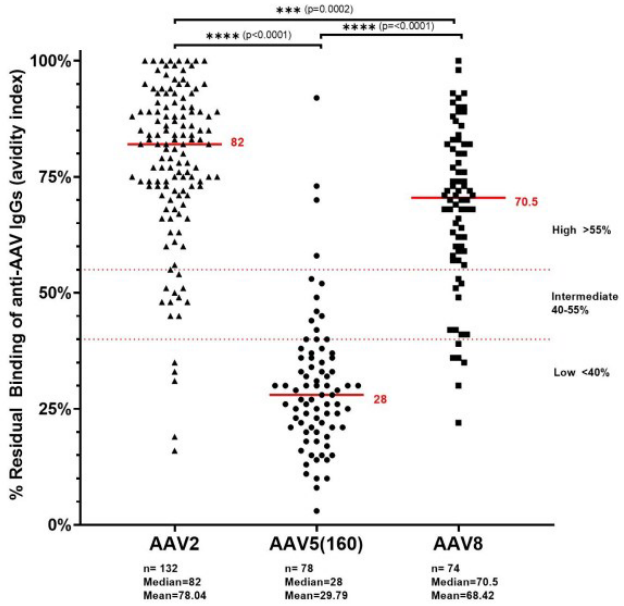
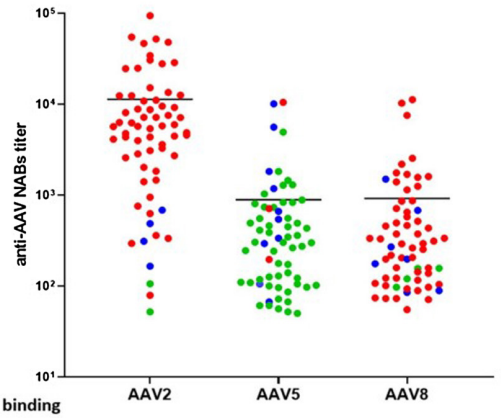


FIGURE 1 Avidity of AAV-specific IgG antibodies in healthy human population. Significant difference was observed between the different serotypes.



IgG residual binding	AAV2	AAV5	AAV8
Low [<40%]	3%	80%	6%
Intermediate [40-55%]	6%	15%	11%
High [>55%]	91%	5%	83%

FIGURE 2 NABs titers against different AAV serotypes. Plotted donors are the ones that tested triple positive, for NABs against AAV2, 5 and 8

The aim of this study was to assess the binding characteristics of the pre-existing NABs directed against AAV serotypes 1, 2, 5 and 8.
Methods: Serum samples of 300 healthy donors were analyzed to determine pre-existing anti-AAV NABs titers and avidity (residual binding) of anti-AAV IgGs to specific AAV serotypes.
Results: The results obtained demonstrate that the avidity of pre-existing AAV-specific IgG antibodies in the healthy human population is significantly different between AAV serotypes. Pre-existing anti-AAV2 or anti-AAV8 IgGs form stronger antibody-antigen complexes with respectively AAV2 and AAV8 antigens, while pre-existing anti-AAV5 IgGs generate significantly weaker antibody-antigen complexes with the AAV5 antigen (Figure 1).
Additionally, when analyzing donors that were positive for pre-existing anti-AAV NABs against all three tested serotypes (2, 5 and 8), avidity of pre-existing anti-AAV5 IgGs in 80% of those donors was low. However, avidities of pre-existing anti-AAV2 IgGs or anti-AAV8 IgGs in 91% and 83% of those donors respectively, were high (Figure 2).
Conclusions: These observations support the lack of impact of pre-existing anti-AAV5 NABs measured *in vitro* on the efficacy of AAV5-based transduction *in vivo* when compared to pre-existing anti-AAV NABs against serotypes 2 or 8.

PB1102 | Investigating the Stability of Lentiviral Vector Targeted Liver Cells during Post-Natal Growth for in *Vivo* Gene Therapy of Hemophilia

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Background: Liver-directed gene therapy with adeno-associated viral (AAV) vectors delivering a clotting factor transgene into hepatocytes has shown successful results in adults with hemophilia. However, because of their non-integrating nature, AAV are diluted during liver growth, thus challenging their use in pediatric patients. We developed lentiviral vectors (LV), which integrate in the host cell genome, and showed efficient *in vivo* hepatocyte gene transfer in preclinical models (mice, dogs, non-human primates).
Aims: In view of a potential use of LV in pediatric patients, we treated 3 2-4 month-old hemophilia B puppies by systemic administration of LV and showed long-term efficacy for up to 3.5 years, after an initial decrease of factor IX (FIX) activity.
Methods: Longitudinal studies in mice treated as newborns with LV-luciferase showed stable total signal over time, which decreases

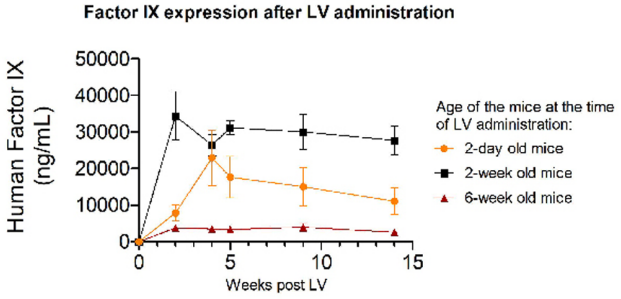

FIGURE 1 Human Factor IX expression after intravenous administration of lentiviral vector at the indicated age

around 3 weeks if normalized on mouse weight, similarly to what observed in dogs.

Results: By contrast, newborn mice treated with LV-GFP showed a continuous increase in size of GFP+ clusters of cells by 3D imaging, showing local proliferation and long-term maintenance of transduced hepatocytes, thus suggesting a decrease in transgene expression *per* cell rather than reduction of targeted hepatocytes during growth. We confirmed the same pattern in LV-FIX treated newborn mice, while administration in 2-week old mice resulted in stable and 3-fold higher FIX output compared to mice treated as neonates. We evaluated clonal proliferation of LV-transduced and untransduced hepatocytes in Alb-Cre/Rosa26-Confetti mice during growth and showed that only a fraction of cells generates continuously growing clusters, while the others appear quiescent.

Conclusions: Our data show that timing of LV administration impacts on gene therapy efficacy and suggest that different hepatocyte subpopulations contribute to liver growth. Our work will provide a rationale for the application of LV-mediated liver gene therapy to pediatric patients in order to guarantee life-long transgene maintenance.

PB1103 | Activity Measurement of Factor IX Padua against the 4th International Standard for FIX, Plasma

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Background: Factor IX Padua (FIXP), a gain-of-function factor IX (FIX) variant is the preferred transgene product for adeno-associated virus gene therapy of haemophilia B patients. One-stage clotting assay (OSCA) and Chromogenic Assay (CHA) discrepancies have been reported for FIXP.

Aims: This study aimed to investigate the validity of measurement of FIXP activity against the 4th International Standard for FIX, Plasma (IS) and to explore reagent-dependent assay discrepancies for FIXP.

Methods: Recombinant FIXP was produced and purified from HEK293 cells and Replenine, a plasma-derived FIX was used as a comparator. Both preparations were spiked into FIX immunodepleted plasma. FIX:C was estimated relative to the IS by parallel line bioassay model and statistical validity was assessed by equivalency testing. Thirteen APTT reagents and 2 chromogenic kits were included in the study.

Results: All assays were statistically valid, indicating that FIXP activity can be determined relative to the Plasma IS and expressed in International Unit (IU). Assay discrepancies were not observed for Replenine. Assay results and overall geometric mean (GM) and geometric coefficient of variation (GCV) are shown in fig 1 and table 1, respectively. Higher inter-assay variabilities were obtained for FIXP

TABLE 1 GM and % GCV for one stage clotting assays (OSCA) and chromogenic assays (CHA) for FIX Padua and Replenine

	Padua		Replenine	
	GM (IU/mL)	%GCV	GM (IU/mL)	%GCV
OSCA	7.4	17.6	4.5	5.9
CHA	3.4	27.5	4.9	9.8
OSCA/CHA ratio	2.2		0.92	

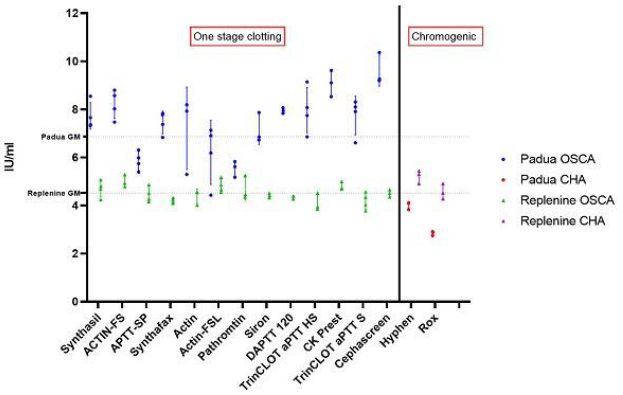

FIGURE 1 Individual assay results

than for Replenine for both OSCA and CHA. Over 60% differences in estimates by OSCA were observed, with no correlation with activators or phospholipid within the reagents. Rossix CHA gave 40% lower results than the Hyphen CHA kit. There was substantial OSCA and CHA disparity with an average OSCA/CHA ratio of 2.2. The results of this study correlated well with those of an international, multi-centre field study assessing FIXP activity.

Conclusions: Assay discrepancies exist when FIXP is assayed against Plasma Standard for FIX. Careful selection of OSCA and CHA is of paramount importance to obtain accurate activity estimates of gene expressed FIXP in patients.

PB1104 | Surgical Management of a Hemophilia B Gene Transfer Clinical Trial Participant: A Case Report Following Etranacogene Dezaparvovec (AMT-061) Gene Therapy

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Background: Clinical trials evaluating gene transfer for hemophilia B (HemB) have shown endogenous factor IX (FIX) expression in severe patients, changing their phenotype to mild/moderate or normal with a decrease in annual bleeding rate and factor usage and improvements in quality of life.

Aims: Here we report a case of unplanned orthopedic surgery in a patient with severe HemB treated with etranacogene dezaparvovec (AMT-061), an investigational gene therapy for HemB comprising an adeno-associated virus serotype 5 vector containing a codon-optimized Padua variant human FIX gene, with liver-specific promoter.

Methods: Retrospective chart review of a patient with severe HemB enrolled in a phase 2b trial of etranacogene dezaparvovec (NCT03489291) who underwent surgery.

Results: A 47 year old male with severe HemB with pre-existing stable bilateral femoral head avascular necrosis received a single intravenous dose of etranacogene dezaparvovec ($2x10^{13}$ gc/kg). At week 21 the patient reported increasing bilateral hip pain, deemed unrelated to etranacogene dezaparvovec. At week 28 he was hospitalized for surgical bilateral femoral head coring. Immediately prior to surgery he was treated, per trial protocol, with recombinant FIX (BeneFIX) 3710U (50U/kg) and 1695U (25U/kg) at 24 hours post-operative. After discharge self-administered doses (25U/kg) of BeneFIX were administered (2000U at 48 and 72hrs; 1930U at 96 hours). There were no reports of bleeds, thrombosis or other complications related to this procedure. Endogenous FIX activity at the most proximal timepoints assessed pre-surgery (week 26) and post-surgery (week 36) were 57.0% and 50.9% respectively and ranged from 51.2% at week 12 to 40.8% at week 52.

Conclusions: Gene therapy with etranacogene dezaparvovec has potential to change the phenotype of patients with severe HemB allowing a decrease or elimination of infused FIX during surgical intervention. This report demonstrates safe reduced intensity and dose FIX supplementation in the context of surgery following HemB gene therapy.

PB1105 | Exploring RNA Editing System as Innovative Correction Strategy for Hemophilia A

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Background: The emerging type VI CRISPR-Cas systems Cas13 ortholog to perform RNA editing by driving catalytically inactive Cas13 (dCas13) to direct adenosine-to-inosine deaminase activity by ADAR2 (adenosine deaminase acting on RNA type 2) to specific mutated transcripts would represent a new technology to correct G to A mutations, which represent a big portion of the Hemophilia A causing missense mutations (19%).

Aims: To correct the missense mutations in mature RNA of *F8* increasing the coagulation FVIII protein production, secretion and activity.

Methods: Transient expression of *F8* cDNA containing missense mutation to evaluate the secretion and activity in presence or in absence of the mutations. To assess the increase of protein production and corrected FVIII activity, Activated Partial Thromboplastin Time (aPTT) activity assays and ELISA on cells culture media was performed in presence and in absence of the specific guide RNA and the Cas13b-ADAR2 correction system.

Results: We selected three severe missense mutations with G to A change impairing the residual activity in patient's plasma. The selection of the missense mutants was based on CHAMPS and EAHAD databases, taking in consideration the clinical severity and number of affected patients reported (>30 patients). *In vitro* transient expression studies indicated that the selected mutations (p.R1800H; p.R2182H; p.R2228Q) impair both FVIII expression and activity (≤1% of pooled normal plasma). We assayed in transient transfection the designed single guide RNAs able to target the specific mutation. Among the guides used two out of three demonstrated to be effective for the p.R1800H (1.6-fold; p value: 0,0248) and p.R2182Q (2-fold; p value: 0,0129).

Conclusions: These preliminary results contributed to elucidate the molecular basis of FVIII deregulation in presence of missense mutations in Hemophilia A patients, providing the first proof of concept on the usage of RNA Editing system applied in Hemophilia that confirmed FVIII protein increase in vitro.

PB1106 | Combined Gene and Cell Therapy for the Treatment of Hemophilia A within an Implantable Therapeutic Device

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Background: Hemophilia A (HA) is a rare bleeding disorder caused by absence or dysfunction of FVIII protein. New regenerative medicine approaches to treat HA require insights into cell compartments capable of producing FVIII.

Aims: To develop a novel *ex vivo* cell-based therapy using a medical device (Cell Pouch™, Sernova Corp.) leading to an improvement in patient quality of life.

Methods: Blood outgrowth endothelial cells (BOECs) were isolated from healthy and HA patients. HA BOECs were transduced with a lentiviral vector carrying the B-deleted form of FVIII under the control of an endothelial promoter (LV-VEC.FVIII). BOECs were characterized for endothelial phenotype, functionality, number of integrated LV copies/cell, FVIII expression and secretion. LV-VEC.FVIII-BOECs were transplanted in association with cytodex®3-microcarrier-beads

in NOD/SCID g-null HA mice (n=6). Afterwards, cells were implanted in a prevascularized, scalable medical device (Cell Pouch™), optimized for sustained secretion of therapeutic FVIII. Finally, chromosomal aberration, senescence markers, tumorigenic potential and integration analysis were performed at different procedure timepoints.

Results: BOECs expressed endothelial markers, maintained ECs functionality and, after transduction, the number of integrated LV copies/cell was ~3.

FVIII expression in LV-VEC.FVIII-BOECs was ~80% and was efficiently secreted in the supernatant (24 ng/ml LV-VEC.FVIII-BOECs vs 4.5 ng/ml HA BOECs). LV-VEC.FVIII-BOECs transplanted in HA mice with microcarrier beads, survived and secreted FVIII at therapeutic levels (12%) up to 18 weeks ameliorating the bleeding phenotype of HA mice. Subsequently safety tests were performed after large-scale expansion showing a safe profile and suggesting that BOECs can be expanded without negative consequences. Furthermore, LV-VEC.FVIII-BOECs were transplanted in the Cell Pouch™, showing engraftment up to 12 weeks. Finally, genomic integration profile of LV-VEC.FVIII-BOECs showed the classic integration pattern of LVs, without enrichment for oncogenes.

Conclusions: Overall, these results pave the way for future human clinical testing in HA patients by transplantation of GMP produced autologous gene corrected BOECs within implanted device.

PB1107 | Platelet-targeted Hyperactive Factor IX (FIX) Gene Therapy for Hemophilia B Mice with Pre-existing Anti-FIX Immunity

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Background: Data from current clinical trials using AAV-mediated liver-targeted FIX gene therapy for hemophilia B (HB) patients are very encouraging, but this protocol can only be applied to adults without liver disease or anti-AAV or FIX antibodies.

Aims: To evaluate the potential of platelet-specific gene therapy for HB with pre-existing immunity.

Methods: Platelet-FIX (Plt-F9) expression in $F9^{null}$ mice was introduced by lentivirus transduction followed by transplantation into rhF9-primed $F9^{null}$ mice preconditioned with either 11 Gy or 6.6 Gy TBI. Lentivirus were engineered to harbor a codon-optimized hyperfunctional FIX Padua expression cassette driven by the αIIb promoter (2bCoF9R338L). The treated animals were analyzed by FIX antigen (FIX:Ag), FIX activity (FIX:C), and Bethesda assays. The phenotypic correction was assessed by a 6-hour tail bleeding test.

Results: Plt-F9:C levels in the 11 Gy and 6.6 Gy groups were 18.68±8.61 and 9.17±12.22 mU/10^8 platelets, respectively. Plt-F9:Ag levels in the two groups were 3.96±1.57 and 1.70±1.64 mU/10^8 platelets, respectively. Of note, no anaphylaxis occurred in primed HB mice after platelet gene therapy. The inhibitor titers declined to undetectable with time in recipients after treatment with a half-life of inhibitor reduction in the 11 Gy group of 49.2±3.9 days, which was significantly shorter than in the 6.6 Gy group (82.6±32.6 days) and in the untransduced 6.6 Gy control group (77.7±8.7 days). Since the clinical efficacy of plt-F9 is limited in the presence of anti-F9 inhibitors, we did not assess the bleeding phenotype in recipients until inhibitor titers dropped to an undetectable level. The remaining hemoglobin levels after the tail bleeding test were 79.5±15.5% and 64.7±23.4% in the 11 Gy and 6.6 Gy groups, respectively, which were not significantly different compared to the wild-type group, but significantly higher than the $F9^{null}$ control (38.8±6.7%).

Conclusions: Our data suggest that platelet-specific hyperfunctional FIX gene therapy can be a promising protocol for all HB patients, including patients with inhibitors.

PB1108 | Influence of N-linked Glycosylation in the A and C Domains on the Immunogenicity of Factor VIII

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Background: Previous research has highlighted differences in glycosylation patterns between recombinant and plasma derived Factor VIII proteins making glycosylation of high interest in the study of its immunogenic properties. Specifically, we are interested in exploring the role of N-glycosylation sites in the A and C domains in relation to the immunogenicity of FVIII. Based on previous research suggesting the role of sialylation in moderating the immune response and mannosylation in eliciting an increased immune response, we have focused on the N-glycosylation sites N41 and N239 of the A1 domain, N1810 in the A3 domain, and N2118 of the C1 domain.

Aims: To investigate the impact of N-glycosylation on inhibitor development to FVIII.

Methods: Through site-directed mutagenesis, we have introduced a missense mutation substituting Asparagine for Glutamine thus eliminating the specified sites ability to become N-glycosylated. To observe and measure the immune response to these modified plasmids, we performed hydrodynamic injection in male SV129/HA mice and collected plasma for analysis via retro-orbital bleed. FVIII activity was measured via APTT assay and inhibitor titers were analyzed via ELISA and Bethesda Assay.

Results: Through our gene transfer experiments, we observed an increased immune response in N41Q, N239Q, and N1810Q treated mice and a decreased immune response in N2118Q treated mice compared to mice treated with wild-type B-domain-deleted-FVIII. Specific activity of the mutated proteins remained similar between all treatment groups.

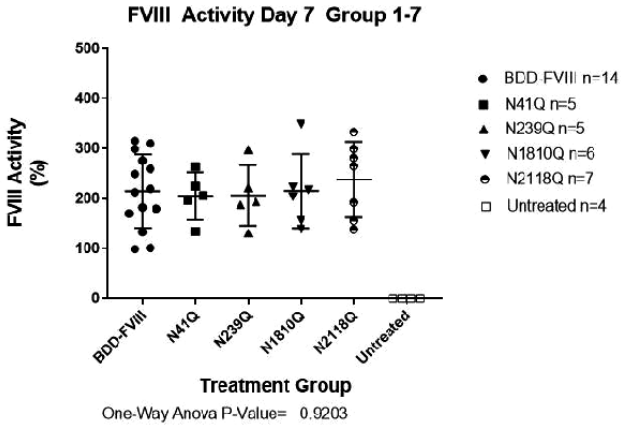

FIGURE 1 FVIII activity levels as determined by APTT analysis

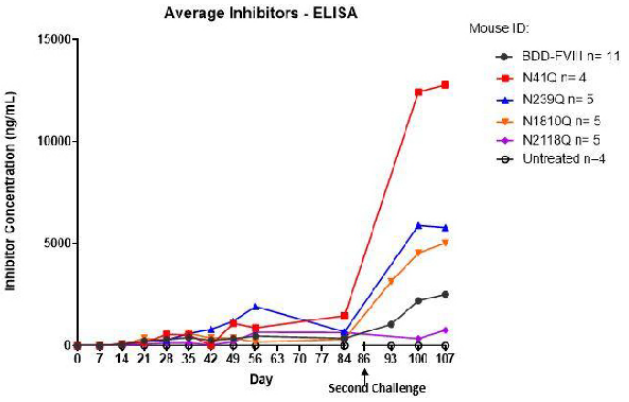

FIGURE 2 FVIII-specific inhibitor levels as measured by IgG ELISA

Conclusions: Our data suggests that N-glycans play an important role in modulating or stimulating the immune response to FVIII. The removal of high mannose glycan N2118 resulted in a decreased inhibitory response while removal of sialic-acid terminating glycan N41 resulted in an increased inhibitory response which agrees with previous research. Our study has helped in the identification of immune markers of FVIII thus improving upon current knowledge regarding the development of safe FVIII replacement therapies.

PB1109 | Assessment of Vector Integration of AAV5-hFIX in Mice and Non-human Primates Indicates No Association with Tumorigenic Risk

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Background: Integration studies have shown that rAAV vector genomes remain mainly episomal after transduction of the target cells, with minimal integration into the host genome and with no association with oncogenicity or signs of malignant transformation.

Aims: The goal of our study was to investigate the integration profile of rAAV5 vector DNA into the host genome of livers collected from mice and Non-Human Primates (NHP) following IV administration of AAV5-hFIX.

Methods: Liver tissue samples from mice and cynomolgus macaques injected with AAV5-hFIX were collected 6 months after a single intravenous AAV administration. The profile of integration of the vector DNA into the host genome was analyzed by means of linear-amplification mediated polymerase chain reaction (LAM-PCR) and non-restrictive (nr) LAM-PCR. Episomal (concatemeric) and integrated forms of AAV5-hFIX derived vector DNA sequence were further characterized by deep-sequencing. Liver tissue samples from the same animals were also assessed by microscopic evaluations by a board-certified pathologist.

Results: The AAV vector sequences retrieved after (nr)LAM-PCR followed by high throughput sequencing were found to be present almost exclusively as non-integrated episomal forms. The low level of integrants was randomly distributed throughout the host genome. No specific clustering was seen in cynomolgus macaque genome, while some level of clustering around active genes was seen in the mouse but without signs of in vivo clone selection (an indicator of tumorigenic risk) in the animals. None of the animals showed any adverse findings during microscopic evaluation of the liver and no signs of malignant transformation were found.

Conclusions: Administration of AAV5-hFIX to mice and NHPs is associated with a low level of random integration into the livers. This integration profile does not raise any specific tumorigenic concern.

MANAGEMENT OF BLEEDING AND TRAUMA

PB1111 | Activated Prothrombin Complex Concentrates for the Management of Direct Oral Anticoagulant-Associated Bleeding Events and Pre-Operative Administration for Urgent Surgery: Hemostatic and Thrombotic Outcomes

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Background: Prothrombin complex concentrates (PCCs) correct quantitative thrombin generation parameters but their impact on kinetic parameters are inconsistent. Activated PCC (aPCCs) have been shown to enhance both kinetic and quantitative thrombin generation parameters. Clinical studies evaluating aPCC for DOAC-associated bleeding are sparse.

TABLE 1 Thromboembolic Events

Demographics	Vascular Risk Factors	Type of Thromboembolic Event	Timing of Thromboembolic Event	aPCC Dose (IU)	Anticoagulation Re-Initiated	Thrombotic Death
98M	CAD, T2DM, HTN	NSTEMI + DVT	Day 10 + Day 20	3186	No	No
72M	CAD, CABG, T2DM, CKD, AF (CHADS2 = 1)	Bilateral femoral artery emboli	Day 0	2500 + 1000	No	No
92M	HTN, CHF, T2DM, CVA, bioprosthetic AV, AF (CHADS2 = 6)	TIA	Day 0	2718	No	No
69M	AF (CHADS2 = 4), CHF, CVA within 3 months, T2DM, PVD, recurrent VTE, IVC filter in situ, chronic atrial thrombus	Systemic embolism	Day 0	1782	No	Yes
83M	HTN, CHF, DLD, PVD, pulmonary HTN, AF (CHADS2 = 3), active cancer	STEMI	Day 7	3418	No	Yes

aPCC: activated prothrombin complex concentrate, TE: thromboembolic event; CAD: coronary artery disease; T2DM: type 2 diabetes mellitus; HTN: hypertension; CABG: coronary artery bypass grafting; CKD: chronic kidney disease; AF: atrial fibrillation; CVA: cerebrovascular event; AV: aortic valve; PVD: peripheral vascular disease; VTE: venous thromboembolic event; IVC: inferior vena cava; DLD: dyslipidemia.

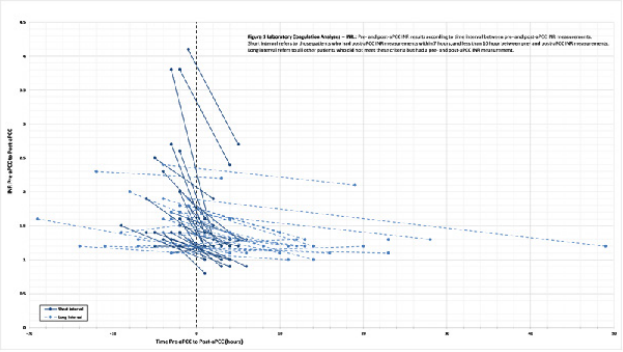

FIGURE 1 Laboratory Coagulation Analyses - INR

Aims: Evaluate the impact of aPCC on clinical hemostasis and laboratory coagulation parameters in patients with DOAC-associated bleeding or prior to urgent surgery.

Methods: We conducted a retrospective study of patients receiving aPCC for DOAC-associated major bleeding or prior to urgent surgery. The primary efficacy outcome was hemostatic efficacy by modified ISTH and Sarode criteria. Surgical hemostasis was assessed using a binary "normal" or "abnormal" scale. The primary safety outcome was the 30-day thromboembolic rate. Coagulation study results (international normalized ratio [INR] and activated partial thromboplastin time [aPTT]) were analyzed in an exploratory fashion.

Results: Eighty-two patients were included in the analysis; 54 patients received aPCC for major bleeding and 28 patients prior to urgent surgery; 14 patients on dabigatran, 39 on rivaroxaban, 29 on apixaban. Mean weight-based aPCC dosing was 38 IU/kg (SD ± 13.2 IU/kg). Hemostasis was deemed effective by ISTH criteria in 50% of cases and "Good" or "Moderate" by Sarode criteria in 45.2% and 14.3% of cases, respectively. Surgical hemostasis was rated "Normal" in 84% of cases when given prior to urgent surgery. Median pre-aPCC INR was 1.6 (interquartile range, IQR 0.5) and median post-aPCC INR was 1.2 (IQR 0.2) ($p < 0.00001$, Figure 1). Median pre-aPCC aPTT was 36 seconds (IQR 12.8), median post-aPCC aPTT was 29 seconds (IQR 9.8) ($p = 0.0001$). The 30-day thromboembolic rate was 6.1% (Table 1) and the 30-day overall mortality rate was 31.7%.

Conclusions: aPCC appears to result in a significant correction in coagulation parameters and provides effective hemostasis in patients with DOAC-associated bleeding or requiring urgent surgery.

PB1112 | Reversal of Direct Oral Anticoagulants with Three-Factor Prothrombin Complex Concentrate: Real World Experience from a Tertiary Centre in Hong Kong

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Background: Direct oral anticoagulants (DOACs) including direct thrombin inhibitors (DTIs) and factor Xa (fXa) inhibitors are extensively used. Idarucizumab is available in our centre for reversal of DTIs while three-factor prothrombin complex concentrate (3F-PCC) remains the choice of reversal for major bleeding associated with fXa inhibitors.

Aims: Our study aims to analyze the risk of thrombosis, bleeding and mortality for patients on DOACs with 3F-PCC reversal for major bleeding or emergency operations.

Methods: This was a retrospective study conducted at Tuen Mun Hospital, a tertiary trauma centre in Hong Kong. Patients aged ≥ 18 years old on DOACs who received reversal by 3F-PCC for major bleeding or emergency operation from January 2014 to October 2019 were recruited. Outcome measures included risk of 7-day thrombosis, 7-day haemorrhage and 30-day morality after 3F-PCC reversal.

TABLE 1 Details of major bleeding and emergency operation

Type of major bleeding			Type of emergency operation		
Nature of bleeding	**Number of patients**	**Percentage**	**Nature of operation**	**Number of patients**	**Percentage**
Intracranial haemorrhage	23/42	54.7%	Abdominal surgery	5/10	50%
Gastrointestinal bleeding	8/42	19%	Vascular surgery	1/10	10%
Intra-abdominal bleeding	3/42	7.1%	Neurosurgical surgery	1/10	10%
Haemoptysis	2/42	4.8%	Cardiothoracic surgery or procedure	1/10	10%
Others	6/42	14.4%	Others	2/10	20%

TABLE 2 Post-reversal thrombotic and haemorrhagic risk and mortality

DOAC	7-day thrombotic risk after reversal by 3F-PCC	7-day haemorrhagic risk after reversal by 3F-PCC
Overall	3.8%	19.2%
Dabigatran	0%	20%
Rivaroxaban	4.8%	19%
Apixaban	5%	20%
Edoxaban	0%	0%

Results: Fifty-two patients (male to female ratio: 1:1) were recruited. Rivaroxaban (40.4%) was the most commonly-used DOACs, followed by Apxiaban (38.4%), Dabigatran (19.2%) and Edoxaban (2%). The median age of our cohort was 79 years old (51-101 years old). Atrial fibrillation (AF, 88.5%) was the main indication for anticoagulation. The median CHA_2DS_2-VASc score for AF patients was 5 (0-9). Forty-two patients (80.8%) received 3F-PCC as reversal for major bleeding and 10 patients (19.2%) for emergency operation (Table 1). Intracranial haemorrhage (ICH) accounted for 54.7% of major bleeding and the median Glasgow Coma Scale upon reversal was 13 (3-15) for ICH patients.

The overall risk of 7-day thrombosis and 7-day haemorrhage after reversal were 3.8% and 19.2% respectively (Table 2). All post-reversal thrombosis occurred in major bleeding group while 20% of post-reversal haemorrhage occurred in emergency operation group. The overall 30-day mortality was 32.7%. Death due to thrombosis or bleeding in major bleeding group accounted for 62.5% of cases.

Conclusions: Our study signifies the thrombotic and haemorrhagic risk after reversal of DOACs by 3F-PCC.

PB1113 | Effect of Oral Anticoagulant Use on Surgical Delay and Outcomes in Hip Fracture Patients: A Systematic Review and Meta-Analysis

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Background: Peri-procedural management of oral anticoagulants (OACs) in surgical hip fracture patients requires navigating the competing risks associated with surgical delay, anesthetic risks and operative hemostasis. Guidelines recommend surgery within 48 hours among patients presenting with hip fractures; however, management of surgical timing for patients on OACs remains unclear. Individual studies report small sample sizes and heterogeneous outcomes.

Aims: To conduct a systematic review and meta-analysis to summarize the effect of pre-hospital OAC use on time to surgery and all-cause mortality among older adults with hip fracture treated surgically.

Methods: We searched MEDLINE, EMBASE and CENTRAL (from inception to 14 October 2019) to identify studies directly comparing outcomes among hip fracture patients receiving direct oral anticoagulants (DOAC) or vitamin K antagonists (VKA) prior to admission with hip fracture patients not on OACs. Random effects were used

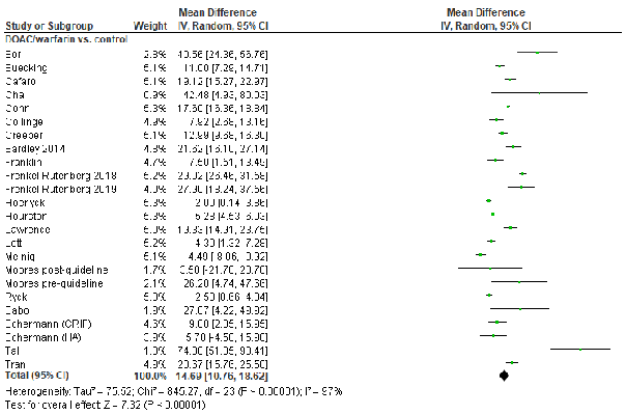

FIGURE 1 Difference in time-to-surgery among hip fracture patients on anticoagulants compared to controls

to pool all outcomes of interest (time-to-surgery and in-hospital mortality).

Results: Thirty four studies (involving 39,446 patients) were included in our systematic review. Time-to-surgery was 14.7 hours longer (95% CI 10.8 - 18.6) among hip fracture patients on OACs compared to those not on antithrombotics (Figure). Anticoagulated patients had a three-fold higher odds of having surgery beyond the recommended 48 hours from admission (odds ratio 3.0, 95% CI 2.1-4.3). In-hospital mortality was higher (odds ratio 1.4, 95% CI 1.1-1.7) among anticoagulated patients. Among studies comparing anticoagulants, we observed a trend towards longer time-to-surgery among patients taking DOACs compared to VKA (7.1 hours, 95% CI -1.1 - 15.4).

Conclusions: Patients presenting with hip fracture who were taking OACs prior to injury experience delay in time-to-surgery and higher mortality than non-anticoagulated patients. Patients on DOACs may be at risk of extended delays. Evaluating expedited surgical protocols in hip fracture patients on OACs is an urgent priority, with the potential to decrease morbidity and mortality in this group of high-risk patients.

PB1115 | Low-Factor Consumption and Cost Effectiveness for Surgeries in Hemophilia B Patients with N9-GP

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Background: Surgery in patients with hemophilia B carries a high risk of excessive bleeding and requires adequate hemostatic control for extended periods of time. Nonacog beta pegol (N9-GP) is a recombinant FIX molecule and a 40-kilodalton polyethylene glycol (PEG) moiety which is attached to the FIX activation peptide enables to generate native-activated FIX. A single-dose PK trial have shown a five-fold increase in terminal half-life as compared with standard FIX products. Development of N9-GP allowed for not only regular once-weekly prophylaxis but also reduced interval of intra/postoperative infusion in hemophilia B patients.

Aims: We report the experiences of major/minor surgeries in 4 hemophilia B patients and show the reduced consumption FIX concentrates to achieve good hemostasis.

Methods: Four male patients with hemophilia B who were scheduled for surgery were included (Table). There were no history of FIX inhibitors. The selection of the perioperative dosing regimen was based on PK modelling of data obtained in the first human dose trial where a single dose of N9-GP had been injected in a non-bleeding state (Collins, et al., 2012). All patients received total dose from 102.4 to 188.7 IU/kg of N9-GP throughout the perioperative periods of which every dosing was a single bolus injection.

Results: Intraoperative hemostasis was rated as 'excellent' as was defined previously (Escobar, et al., 2017). No transfusions were required and all patients discharged safely from the hospital and did not need surgery-related FIX replacement. No patients developed FIX inhibitors and no thromboembolic events occurred. In case 1, total cost of replacement therapy for left knee arthroplasty by using N9-GP was 1/3 of that for the previous right knee arthroplasty by using continuous infusion of a recombinant FIX.

Conclusions: Compared with standard FIX products, N9-GP was safe and effective as replacement therapy to manage surgeries in our patients and effectively reduced the consumption of FIX concentrates.

TABLE 1 Patient demographics and results

	Case 1	Case 2	Case 3	Case 4
Age and weight on surgery	57 and 75 kg	1y3 m and 8.6 kg	1y1 m and 9.8 kg	68 and 47.7 kg
Severity	Mild	Severe	Severe	Severe
History of treatment	rFIX, on demand	rFIX, on demand→ rFIX-FC, qw PPX	PUPS	pdFIX, bw PPX→rFIX, bw PPX→rFIX-FC, qw PPX→rFIX-FP, qw PPX
Surgery	Left knee arthroplasty	Port insertion	Port insertion	Release of diaphragmatic hernia incarceration + hernia repair
Surgery time (min)	166	43	64	110
Bleeding volume (ml)	11	3	Small amount	130
Total number of N9-GP infusions	3	2	2	4
Total infused units	10000 IU (131.93 IU/kg)	1000 IU (116.55 IU/kg)	1000 IU (102.4 IU/kg)	9000 IU (188.68 IU/kg)

PB1116 | Effect of rFVIIa on Thromboelastogram Parameters in Haemophilia A Patients with Inhibitor

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Background: The possibility of TEG to control the treatment of bypassing agents and the individualization of treatment of patients with hemophilia patients with inhibitor remains an issue.
Aims: To investigate the changes in TEG parameters during hemostatic therapy of rFVIIa and find out how this method can be used to control the treatment of haemophilia A patients with inhibitor.
Methods: 6 patients with severe hemophilia A with an inhibitor (median 5.5 BU/ml) received rFVIIa at a dose of 75 U/kg for stopping bleeding. All patients underwent coagulological studies before and after administration of rFVIIa and TEG was performed. The 9 TEG parameters (R, K, α-Angle, MA, CI, SP, G, TPI/c, LY30,) were analyzed.
Results: Before administration of rFVIIa to all patients, the R response time was significantly extended with a median of 80.2 min compared to healthy subjects (4.0-11.0 min). 15 min after the administration of rFVIIa in 2 patients, the TAG indices characterizing coagulation hemostasis were normalized, in one of them all parameters were not different from healthy subjects. In the other 3 patients, R, K, α-Angle and CI remained impaired after administration. In all hemophilia A patients with inhibitor, after 15 min of rFVIIa administration, the parameters characterizing the quality, stability and hemostatic properties of the clot (MA, SP, G, TPI / c, LY30) were normalized.
Conclusions: In the six severe haemophilia A patients with inhibitor treated with rFVIIa, the changes in TEG were individual. Normalization of TEG indicators occurs 15 min after agent administration. The effect of rFVIIa can be observed at the final stage of the blood clotting process - thrombin and clot formation. TEG may be a fairly reliable method of controlling the rFVIIa during hemostatic therapy of haemophilia A patients with inhibitor.

PB1117 | Continuous Infusion with Kovaltry (Octocog Alfa) during Cardiac Surgery for Tetralogy of Fallot in an Hemophilic Child

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Background: In patients with hemophilia episodes of bleeding or the bleeding' prevention (for example during surgery) are treated with replacement of the deficient factor. Surgical hemostatic management guidelines for infants with congenital heart disease and hemophilia are limited to case reports. Continuous infusion of FVIII, instead bolus infusion, is usually administered in these patients to manage perioperative and major bleeding. Continuous infusion guarantees an optimal hemostatic efficacy by maintaining stable FVIII levels.
Aims: This report broadens knowledge about the therapeutic management of patients suffering from hemophilia, who underwent cardiac surgery.
Methods: We report the hemostatic management with FVIII (Kovaltry®) continuous infusion in a 1 year-old child with severe hemophilia A who required cardiac surgery for repair cardiac tetralogy of Fallot.
Results: The patient before surgery received only 2 Exposure Days to Factor VIII. The inhibitor titers were negative. The patient received a preoperative bolus dose of recombinant factor VIII (100 IU/kg), followed by continuous infusion of recombinant factor VIII (4 IU/kg/hr) through a femoral vein, after which general anesthesia was induced. Intraoperative factor VIII levels could not be measured because of high heparin concentration. Postoperatively, the patient required adjustment of his continuous factor VIII infusion, from between 4 and 10 IU/kg per hour, to keep factor VIII levels between 55% and 100%. One week after cardiac bypass the patient was dismissed to home and treated twice a week with recombinant factor VIII bolus infusion (50 U/kg/dose) starting primitive prophylaxis. Subsequent follow-up showed good healing from both surgical procedures. Factor VIII inhibitor is still negative at six months after surgery.
Conclusions: Our report highlights that Continuous Infusion of BAY 81- 8973 for the management of cardiac surgery or in pediatric hemophilia is safe and effective with no observed adverse events.

PB1118 | Comparison of Coagulation Parameters Associated with Fibrinogen Concentrate and Cryoprecipitate for Treatment of Bleeding in Patients Undergoing Cytoreductive Surgery for Pseudomyxoma Peritonei: Results from the FORMA-05 Study

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Background: Cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy for pseudomyxoma peritonei (PMP) is associated with excessive bleeding and acquired fibrinogen deficiency. The FORMA-05 study indicated that human fibrinogen concentrate (HFC) is non-inferior to cryoprecipitate for achieving hemostatic efficacy in bleeding patients with acquired fibrinogen deficiency undergoing CRS for PMP.
Aims: This sub-analysis of the FORMA-05 study explores interoperative and postoperative coagulation profiles in patients undergoing CRS for PMP.

TABLE 1

Parameter	Treatment group	Baseline mean (SD)	Intra-operative 2 h after surgery start, mean (SD)	Intra-operative 6 h after surgery start, mean (SD)	End of surgery mean, (SD)	2 days after end of surgery, mean (SD)	10 days after end of surgery, mean (SD)
Endogenous thrombin potential (nmol/L/min)	HFC	1514.5 (430.6)	1673.5 (340.1)	1310.7 (171.2)	1225.4 (220.7)	1263.4 (176.9)	1426.2 (260.4)
Endogenous thrombin potential (nmol/L/min)	Cryoprecipitate	1639.1 (339.1)	1690.2 (320.2)	1260.7 (342.2)	1346.4 (243.6)	1385.6 (301.6)	1357.0 (349.5)
Factor XIII:C (%)	HFC	121.86 (27.32)	80.09 (27.60)	53.31 (16.16)	55.28 (22.28)	48.05 (16.86)	65.54 (18.58)
Factor XIII:C (%)	Cryoprecipitate	115.55 (29.52)	71.63 (23.63)	60.27 (15.26)	62.97 (13.42)	50.60 (13.44)	65.00 (14.31)
Factor VIII:C (%)	HFC	152.21 (53.61)	155.50 (90.60)	130.70 (50.14)	121.89 (54.50)	278.84 (57.74)	313.88 (84.16)
Factor VIII:C (%)	Cryoprecipitate	136.73 (40.90)	123.61 (50.68)	120.63 (48.36)	116.99 (39.50)	242.78 (54.59)	331.19 (102.22)
Von Willebrand factor antigen (IU/dL)	HFC	144.43 (65.58)	144.07 (61.05)	145.01 (50.99)	138.52 (54.51)	253.97 (59.91)	320.41 (88.32)
Von Willebrand factor antigen (IU/dL)	Cryoprecipitate	140.95 (49.59)	137.56 (52.21)	167.56 (52.61)	174.21 (56.23)	260.46 (72.54)	301.54 (92.71)

TABLE 2

Parameter	Treatment group	Baseline mean (SD)	Intra-operative 2 h after surgery start, mean (SD)	Intra-operative 6 h after surgery start, mean (SD)	End of surgery mean, (SD)	2 days after end of surgery, mean (SD)	10 days after end of surgery, mean (SD)
FIBTEM A20 (mm)	HFC	29.77 (10.65)	18.59 (6.05)	13.06 (3.80)	11.67 (2.69)	28.10 (4.28)	50.10 (11.70)
FIBTEM A20 (mm)	Cryoprecipitate	26.04 (12.09)	15.30 (6.94)	10.60 (4.21)	11.48 (3.78)	29.32 (5.89)	54.27 (14.23)
Plasma fibrinogen concentration (g/L)	HFC	4.79 (1.31)	3.20 (0.72)	2.34 (0.61)	2.21 (0.52)	5.47 (0.83)	6.62 (1.48)
Plasma fibrinogen concentration (g/L)	Cryoprecipitate	4.50 (1.50)	2.67 (0.98)	1.91 (0.54)	2.08 (0.56)	5.24 (0.82)	6.48 (1.69)

Methods: FORMA-05 was a single-center, prospective, randomized, controlled Phase 2 study. Patients undergoing CRS with predicted intraoperative blood loss ≥2L received either HFC (4 g), or cryoprecipitate (2 pools of 5 units, approximately 4.0-4.6 g fibrinogen), repeated as needed. Plasma fibrinogen concentration (Clauss assay) and FIBTEM A20 were measured hourly intraoperatively, while factor XIII (FXIII), factor VIII (FVIII), von Willebrand Factor (VWF) and endogenous thrombin potential (ETP) were measured every two hours. Post-surgery, all parameters were measured at 6, 12, 24 and 48 hours, and 10 days.

Results: The analysis included 45 patients randomized to receive HFC (n=22) or cryoprecipitate (n=23). Table 1 shows the intraoperative and postoperative changes in ETP, FXIII, FVIII and VWF. Table 2 shows the changes in FIBTEM A20 and fibrinogen concentration. Both groups had comparable plasma fibrinogen levels at baseline and 1 hour after surgery start, while from 2 hours after surgery start, intraoperative and postoperative mean values appeared numerically higher with HFC than cryoprecipitate. Activated partial thromboplastin time, prothrombin time and platelet count were maintained throughout surgery in both groups.

Conclusions: The FORMA-05 coagulation parameter analyses showed broad overlap between HFC and cryoprecipitate with satisfactory maintenance of clot quality, FXIII and thrombin generation parameters in both treatment groups. Together with the FORMA-05 efficacy results, this demonstrates HFC can correct for loss, consumption and dilution of fibrinogen in patients undergoing CRS for PMP.

PB1119 | Andexanet and Prothrombin Complex Concentrate Give Comparable Haemostatic Efficacy for Major Bleeding in Patients on Factor Xa Inhibitors

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Background: Prothrombin Complex Concentrate (PCC) is considered the standard of care for patients on apixaban and rivaroxaban requiring reversal in life-threatening or uncontrolled bleeding. Andexanet alfa (short: andexanet) has recently been approved by the FDA and EMA for this indication.

Aims: To systematically compare the efficacy and safety of andexanet and PCC for reversal of factor Xa-inhibitors in patients presenting with major bleeding.

Methods: A systematic literature search was conducted using Pubmed up to December 2019. Studies with patients on rivaroxaban or apixaban who needed urgent reversal with an antidote for a major bleed were included.

Outcome parameters were haemostatic efficacy and thromboembolic event rate as assessed in each study. The quality of the studies was scored by the MINORS and RoBANS methodologies.

Results: Of 360 citations identified, 7 studies were included. PCC was evaluated in 6 studies and andexanet in one study. All studies had an observational design and no comparative studies were found. Two PCC studies were graded as good quality with a low risk of bias, the andexanet study was graded as moderate with a high risk of bias. Different haemostatic efficacy assessment methods were used across the studies.

Haemostasis was achieved in 69-85% of the PCC treated patients and in 82% of the andexanet treated patients. Thromboembolic events occurred in 10% of andexanet treated patients and 0-7.6% in the PCC studies. Overall mortality showed great heterogeneity in the reviewed publications.

Conclusions: In this systemic review PCC and andexanet showed a comparable efficacy profile in patients on factor Xa-inhibitors presenting with major bleeding. Thromboembolic complications differed, probably as a result of patient selection. Due to methodological shortcomings in all studies, more high quality research, including a comparative study of andexanet in relation to PCC, is urgently needed.

TABLE 1 Results [1]Criteria as defined by ISTH (Khorsand 2016); [2]criteria by Sarode et al. (2013); [3] no standard criteria

Citation	Antidote	Design	No. patients treated with fXa-inhibitor	Quality (Minors)	Risk of bias (RoBans)	Haemostatic efficacy (%)	Mortality (%)	Thromboembolic events (%)
Connolly et al. 2019	Andexanet	Retrospective observational	352 for safety analysis; 254 for efficacy analysis	Moderate	High	81.9 [3]	13.9	9.7
Arachchillage et al. 2019	PCC	Retrospective observational	80	Moderate	Moderate	73.4 [3]	33.0	3.8
Smith et al. 2019	PCC	Retrospective observational	31	Moderate	Moderate	80.6 [2]	16.1	0
Majeed et al. 2017	PCC	Retrospective observational	84	Good	Low	69.1 [1]	32.0	2.4
Sheikh-Taha et al. 2019	PCC	Retrospective observational	29	Moderate	Moderate	72.4 [1]	27.6	3.4
Testa et al. 2018	PCC	Retrospective observational	85	Good	High	81.5 [3]	17.6	unknown
Schulman et al. 2018	PCC	Retrospective observational	66	Good	Low	84.8 [2]	13.6	7.6

PB1120 | Treatment Options in Severe Hemophilia A Patients from Romania: What Role did the Intron 22 Inversion and Age Play?

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Background: During the past decades, hemophilia A (HA) treatment has evolved tremendously, with a significant decrease in treatment-associated risks and side effects.

Aims: To investigate medical/orthopedic/surgical treatment options in Romanian severe HA patients.

Methods: Retrospective analysis of the hospitalization charts of severe HA patients, diagnosed in the Hematology Department, Fundeni Clinical Institute, Bucharest, Romania. All patients agreeing to partake in the study signed an informed consent and were tested for the presence of the intron 22 inversion (inv22) by inverse-shifting polymerase chain reaction. The approval of the Ethics Council was granted (approval number 4203/31.01.2018). We respected the regulations imposed by the national law and Helsinki Declaration (1975, revised in 5/2008).

Results: The study group involved 33 patients with severe HA (mean age=40.64±14.92 years): 48.48% (n=16) < 35 years and 51.52% (n=17) ≥35 years. All patients received FVIII substitution and 60.61% (n=20) received intermittent FVIII prophylaxis (x3 times/week), the latter more likely employed in patients < 35 years vs. ≥35 years (81.25% vs. 41.18%, P=0.0324). Eptacog alpha (recombinant FVIIa) was prescribed in 3 patients who developed inhibitors (9.09%). There were no significant differences in terms of inhibitor development among inv22-positive vs. inv22-negative or < 35 years vs. ≥35 years (P=0.2273). Patients with arthropathy required synovectomy in 6.06% (n=2) of cases and total arthroplasty in 15.15% (n=5) of cases. Physiotherapy was employed in 48.48% (n=16) of severe HA patients, particularly in inv22-positive vs. inv22-negative cases (75.00% vs. 33.33%, P=0.0299). Detailed results are depicted in Table 1 and Table 2.

Conclusions: Management of severe HA is complex, requiring a collaborative effort between specialists in hematology, orthopedics and physical&rehabilitation medicine. In our study, inv22-positivity was linked to a higher need for physiotherapy, probably due to the severity of bleeding episodes. Patients ≥35 had higher needs for intermittent FVIII prophylaxis.

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Treatment	inv22+	inv22-	P-value
Intermittent FVIII prophylaxis (x3 times/week)	50.00%	66.67%	0.465
Eptacog alpha	8.33%	9.52%	1.000
Synovectomy	16.67%	0.00%	0.125
Total arthroplasty	25.00%	9.52%	0.328
Physiotherapy	75.00%	33.33%	**0.030**

TABLE 1 Treatment Differences between Hemophilia A Patients Positive and Negative for the Intron 22 Inversion

Treatment	<35 yo	≥35 yo	P-value
Intermittent FVIII prophylaxis (x3 times/week)	81.25%	41.18%	**0.032**
Eptacog alpha	0.00%	17.65%	0.227
Synovectomy	6.25%	4.88%	1.000
Total arthroplasty	6.25%	23.53%	0.335
Physiotherapy	62.50%	35.29%	0.169

TABLE 2 Treatment Differences between Hemophilia A Patients <35 and ≥35 Years Old

PB1121 | Idarucizumab to Neutralize Dabigatran in a University Hospital: The Relevance of Renal Function

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Background: The standard Idarucizumab (Praxbind®, Boehringer-Ingelheim, Germany) dosage is reported to neutralize the activity of 99% of the peak dabigatran values observed in the RE-LY study. However, 5 mgs may be insufficient for some patients.

Aims: To analyze the effectiveness of Idarucizumab in patients with impairment of the renal function.

Methods: Starting 2017, seven patients with NVAF on dabigtran etexilate (2 women and 5 men, 45-91 years old) received the antidote because of either major bleeding (3), severe trauma (3), or emergent surgery. Four patients died in-hospital and two survived.

Results: Serum creatinine, PT ratio, and aPTT ratio ranged from 0.86 to 7.28 mg/dl, 1.72 to 9.92, and 2.07 to 6.81, respectively. Pre-treatment dabigatran plasma levels ranged from 200 to ≥900 ng/ml. In six patients, dabigatran levels in blood collected 5-10 min after idarucizumab infusion were < 10 ng/ml, with normalization of PT and aPTT ratios. Post-antidote dabigatran level was 214 ng/ml in an 88 years-old patient with melena and increasing s-creatinine. Ten hours later, dabigatran level was again >900 ng/ml, and increased to 332 ng/ml 30 hours after an additional standard dose of the antidote. To safely undergo endoscopy, an additional 2.5 mg dose of the antidote was infused.

A 45 years-old patient with acute post-renal failure requiring surgery, had 352 ng/ml plasma dabigatran 24 hours after surgery and 101 ng/ml 4 days after. A 76 years-old patient presented with marked PT and aPTT prolongations and severe melena. Plasma dabigatran was >900 ng/ml one day after antidote administration, decreasing to 715, 442, 204 and 74 ng/ml over a period of 9 days.

Conclusions: Impaired renal function may require higher than standard Idarucizumab dosing. We suggest repeated measurement of plasma dabigatran before and over the days following antidote administration.

PB1122 | Methodological and Reporting Quality in Case Series of Four-Factor Prothrombin Complex Concentrate in the Management of Oral Factor Xa Inhibitor-Associated Bleeding

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Background: As oral factor Xa inhibitor (oFXaI) use has increased, so has publication of case series describing 4-factor prothrombin complex concentrate (4F-PCC) use to manage oFXaI-related bleeding.
Aims: To identify case series describing 4F-PCC to manage oFXaI-related bleeding and appraise their methodological and reporting quality.
Methods: We performed searches of Medline and Embase (01/01/2011-11/08/2019) to identify case series of ≥10-patients who experienced oFXaI-related major bleeding managed with 4F-PCC. Case series' evaluation was performed using a validated tool adapted for this topic. The tool included items addressing patient selection (S1-5), bleed/outcome ascertainment (A1-8), causal/temporal association (C1-6) and reporting (R1-15). The percentage of series meeting each criterion (yes/+) is reported.
Results: We identified 11 case series. No series had ≥100-patients (range=13-84), three were performed prospectively, two detailed specific inclusion criteria (anticoagulant(s), time since anticoagulant dose, major bleeding restriction) and three noted consecutive patient inclusion (Figure 1). While nine series provided clear/appropriate methods for diagnosis of intracranial hemorrhage (ICH); none did so for extracranial bleeds, nor was it clear whether bleeding was

	Arachchillage 2019	Dybdahl 2019	Frontera 2019	Smith 2019	Allison 2018	Harrison 2018	Schenk 2018	Schulman 2018	Sheikh-Taha 2018	Majeed 2017	Grandhi 2015
S1. Key inclusion criteria			+							+	
S2. Consecutive inclusion	+	?	?	?	?	?	?	+	?	+	?
S3. Complete follow-up	+	+	+	+		+		+	+	+	+
S4. Adequate sample size											
S5. Prospective collection						?	+	+		+	
A1a. Diagnosis of ICH	+	+	+	+	+	+	?	+	+		+
A1b. Diagnosis of GI	?	NA	?	?	?	NA	?	?	?	?	NA
A1c. Diagnosis of other	?	NA	NA	?	?	NA	?	?	?	?	NA
A2. Adjudication of diagnosis	?	?	?	?	?	?	?	?	?	?	?
A3. Hemostasis, mortality, thrombosis	+			+	+	+	+	+	+	+	+
A4a. Ascertainment of ICH hemostasis	?	NA	+	+	?	?	?	+	+		?
A4b. Ascertainment of GI hemostasis	?	NA	+	+	?	NA	?	+	+	+	NA
A4c Ascertainment of other hemostasis	?	NA	NA	+	?	NA	?	+	+	+	NA
A5. Ascertainment of thrombosis	?	?	?	?		?	+	?	?		?
A6. Ascertainment of neurologic function	?	NA	NA	NA	NA	NA	NA	?	+	+	NA
A7. Adjudication of hemostasis		NA	?	?	?	?	?	?	?	+	?
A8. Adjudication of thrombosis	?	?	?	?	?	?	?	+	?	+	?
C1. Follow-up for hemostasis		NA	+	+	?	?	?	+	+	+	?
C2. Follow-up for mortality	+		NA				+	+		+	+
C3. Follow-up for thrombosis	+		+			+	+	+		+	
C4. Follow-up for neurologic	?	NA	NA	NA	NA	NA	NA	+	+	+	NA
C5. Lack of prior reversal agent	+	?	+	?	?	+	+	+	?	+	?
C6. Anticoagulant measured	+	?	?	?	?	?	+	+			?

FIGURE 1 Methodological quality domains (assessed as yes, no, unclear or not applicable)

	Arachchillage 2019	Dybdahl 2019	Frontera 2019	Smith 2019	Allison 2018	Harrison 2018	Schenk 2018	Schulman 2018	Sheikh-Taha 2018	Majeed 2017	Grandhi 2015
R1. Anticoagulant agent and dose				+				+			
R2. Reversal agent and dose	+		+	+	+	+	+	+	+	+	+
R3. Time since last anticoagulant dose			+					+	+	+	
R4. Time to reversal agent				+	+	+		+		+	
R5. Use of antiplatelets at presentation	+	+	+	+	+		+	+	+	+	+
R6. Renal function at presentation	+			+		+		+	+	+	
R7. Neurologic function at presentation		+		+	+	+					+
R8. Description of study site	+	+	+	+	+				+	+	
R9. Change of neurologic function											
R10. Surgery or invasive procedures	+	+		+	+			+		+	+
R11. Transfusions	+			+	+		+	+	+	+	+
R12. Additional hemostatic agents	+			+	+		+	+		+	
R13. Hemostatic effectiveness	+		+	+	+	+	+	+	+	+	+
R14. Thrombosis	+	+	+	+	+	+	+	+	+	+	+
R15. Mortality	+	+		+	+	+	+	+	+	+	+

FIGURE 2 Reporting quality domains (assessed as yes or no)

adjudicated in any series. Hemostatic effectiveness, thrombosis and mortality were together evaluated in nine series, but only four used appropriate methods to evaluate/diagnosis hemostasis in ICH, five in gastrointestinal bleeds, four in other bleeds and one in thrombosis. Independent adjudication of hemostasis (n=1 series) and thrombosis (n=2 series) was infrequent. Longer-term ascertainment (≥30-days) for mortality and thrombosis was noted in five and six series, respectively. Anticoagulation measurement/levels in at least some patients were conveyed in three series. Few case series provided data on anticoagulant agent/dose (n=2), time from anticoagulant dose (n=4), time-to-reversal (n=5) or baseline (n=5) or change (n=0) in neurologic function (Figure 2).
Conclusions: Although many case series describing 4F-PCC for reversing oFXaI-related bleeding have been published, the presence of methodological flaws and/or poor reporting necessitates caution in interpretation.
Note: Funding by Portola Pharmaceuticals

PB1123 | The Obstetric Experience of Carriers of Haemophilia

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Background: Women who are carriers of haemophilia along with their offspring face a significant haemostatic challenge during the peripartum period.
Aims: Review obstetric management and pregnancy outcomes in carriers of haemophilia.
Methods: Retrospective cohort analysis of data collected from multidisciplinary (MDT) clinic and electronic maternity notes of carriers of haemophilia, registered at the Katharine Dormandy Haemophilia and Thrombosis Centre and delivered at Royal Free Hospital.
Results: 53 carriers of haemophilia (45 A and 8 B) were identified; 17 mild, 8 moderate and 28 severe cases. Obstetric care was provided in 65 pregnancies resulting in 42 male and 23 female live-births.
Fetal gender was established in all cases by free-fetal DNA from maternal blood in the first trimester.
Invasive prenatal diagnosis (PND) was taken up by 14%. There were five affected male fetuses diagnosed and one was terminated subsequently.

Third-trimester Factor VIII and IX levels increased by 2.4 and 1.5 times, respectively (ranges: FVIII= 45-251, FIX= 53-171 iU/DL).
78% received intrapartum haemostatic cover with Tranexamic Acid (TXA). Three cases received Factor IX concentrate and another one given DDAVP in addition to TXA. Regional analgesia/anaesthesia was administered in 66% of cases.
There were no cases of fetal scalp-electrode application, fetal blood sampling or ventouse delivery.
76% of male fetuses delivered by elective LSCS; one was complicated by a non-life-threatening subdural haematoma in an affected male fetus. 22% of female fetuses delivered by LSCS, all but one were emergencies due to failure to progress or fetal distress. There were no adverse haemostatic sequale in the female neonates. Mean birth-weight was 3287 g.
There were two cases of postpartum haemorrhage; both were primary due to perineal trauma and uterine angle extension at LSCS.
Conclusions: Carriers of haemophilia require special MDT obstetric care. Management guidelines should be available and observed. Antenatal knowledge of fetal gender is essential in formulating birth plans.

PB1125 | The Need for Red Cell Support during Surgery in a Tertiary Care Hospital Is Associated to Pretransfusion Levels of FXIII and the Platelet Count

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Background: Factor XIII (FXIII) deficiency and thrombocytopenia are the most prevalent modulators of clot firmness very early postoperatively (v.Rappard, TMH, 2017).
Aims: We evaluated whether levels of FXIII, fibrinogen and/or platelet count are associated with the probability for intraoperative red blood cell transfusion.
Methods: Look back analysis (2 years) in routine surgeries (without cardiac surgery). We identified 1023 surgical patients with first time blood products order to the OR during the respective hospital stay. Transfusions were initiated at the discretion of the senior anesthesiologist in charge. Draws for platelet count, fibrinogen concentration and factor XIII activity were routinely done immediately before transfusion in the patients.
Results: According to reference intervals, FXIII deficiency (50% of all observations) and thrombocytopenia (49% of all observations) were similarly (very) frequent. In contrast, fibrinogen deficiency was rare (9% of all observations). FXIII deficiency was associated with a significantly increased probability to receive red cell transfusions (OR 4.58, 95% CI 3.46 - 6.05) as was thrombocytopenia (OR 1.94, 95% CI 1.47 - 2.56), but not fibrinogen deficiency (OR 1.09, 95% CI 0.67 - 1.76). Similarly, ROC curves (continuously distributed variables) showed an AUC of red cell transfusion for FXIII activity of 0.744 (95% CI 0.716 - 0.770), 0.632 (95% CI 0.601 - 0.661) for the platelet count and 0.578 (95% CI 0.547 - 0.609) for fibrinogen concentration. All AUCs were significantly different from each other ($p< 0.0001$ and $p=0.0106$, respectively).
Conclusions: This large retrospective cohort study in routine surgical interventions (without cardiac surgery) suggests that pretransfusion FXIII deficiency and, to a lesser extent, thrombocytopenia influence the need for intraoperative blood cell transfusion. This was not the case for hypofibrinogenemia. These results suggest that modyfing F. XIII activity and/or platelet count might influence the need for blood product support, which needs confirmation in future studies.

PB1127 | Endovascular Abdominal Aortic Aneurysm Repair in a Mild Haemophilia A Patient: A Case Report

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Background: Haemophilia A is a genetic disorder that had have an improvement in the life expectancy. We herein report the strategies used in mild haemophilia A patient successfully underwent endovascular aortic aneurysm repair (EVAR).
Aims: A 64-year-old man HIV + with mild hemophilia (F8:C 6.9%), without inhibitor was referred for treatment of symptomatic infrarenal aortic aneurysm that enlarged from 5.6 cm to 6 cm in diameter in last 6 months. He had traumatic amputation of right lower limb.
Methods: -
Results: He was successfully treated with EVAR, Femoro-femoral bypass and right common iliac artery embolization with Coils (Endurant 32*14 mm) associated with perioperative administration of titrated doses of Octocog Alfa (Advate®, Takeda Lab), administered on bolus; it was made under anticoagulation with bolus of unfractionated heparin (UFH 5000 IU), inmediately before surgery and after perioperative administration of a bolus of 55 IU by kilogram of Advate® (F8 presurgery 105%). 3 hours after the first bolus, an intraoperative control of F8 was performed (chromogenic technique) and new bolus of F8 Concentrate was administered (20 IU by kilogram). At the end the UFH was reversed with protamine (1:1 ratio (50 mg). To reduce the risk of hematoma, local dry weight and cold were applied. He received treatment on bolus 14 days, performing antithrombotic prophylaxis (LMWH) and antiaggregant therapy(Acetylsalicylic acid 100 mg / day, 1 month). The determination of the postoperative levels of F8 was carried out by one stage coagulative assay (ACL Top). There were no perioperative complications; inhibitor test at 15 days and 2 months after surgery, were negative.
Conclusions: We show that EVAR is a relatively safe procedure in haemophilic patients and believe, like other authors previously, that EVAR should be considered as first-line therapy in patients with clinically relevant haemorrhagic disorders. No contraindication to intraoperative anticoagulant treatment and long-term antiplatelet therapy.

PB1128 | Direct Oral Anticoagulants Bleeding Events in Patients with Atrial Fibrillation Vs Venous Thromboembolism Admitted to an Emergency Department: Real-Life Study

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Background: Direct oral anticoagulants (AOD) are prescribed for both atrial fibrillation (AF) and venous thromboembolic disease (VTE). In addition, like any anticoagulant treatment, their use may be associated with the occurrence of haemorrhagic complications. To our knowledge, no study compared hemorrhagic events between these two indications.

Aims: The aim of our study is to compare these two populations in terms of hemorrhagic incidence and clinical-biological characteristics.

Methods: We performed a retrospective and monocentric study from RATED database (NCT02706080) in our emergency department. Major patients under DOACs, for AF or VTE, were included. Patients treated for dual VTE and AF indication were excluded

Results: Of 525 patients included, 100 patients (19%) were on Dabigatran, 282 (54%) on Rivaroxaban and 143 (27%) on Apixaban with 149 patients (28.4%) treated with VTE and 376 (71.6%) with AF. Of the 95 patients who had hemorrhage, 27 patients (28.4%) were treated for VTE and 68 (71.6%) for AF, or 18.4% hemorrhages in the VTE group versus 18.3% in the AF group. Patients in the AF group were older (77.4 ± 11.0 vs 62.8 ± 19.6, p < 0.001), had more arterial hypertension (51.9% vs 33.6%, p < 0.001), more stroke (21.1% vs 8.7%, p < 0.001), more myocardial infarction (21.8% vs 13.1%, p = 0.029), more associated treatments (3.7 versus 3, p < 0.001), and higher hemorrhagic risk scores. Paradoxically, when analyzing subgroups "AF with haemorrhage" versus "VTE with bleeding", apart from the persistent age difference (78.6 ± 10.2 versus 60.6 ± 21.1, p < 0.001), the medical history became comparable in the two groups.

Conclusions: Our study showed that during the occurrence of a haemorrhagic event differences between AF and VTE patients faded, suggesting that patients with bleeding do not differ, regardless of the therapeutic indication.

PB1129 | The Age Dependent Effects of Female Sex on Platelet Behavior after Injury

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Background: Altered post-injury platelet behavior is recognized in the pathophysiology of trauma-induced coagulopathy, however our mechanistic understandings are derived primarily from male trauma cohorts. In uninjured patients, female sex confers enhanced platelet activation and aggregation, but the effect of sex on platelet hemostatic functions after injury is incompletely described.

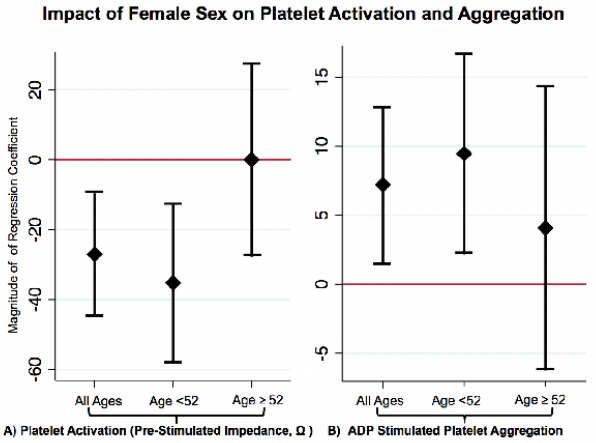

Figure. *Impact of female sex on platelet activation (pre-stimulated impedance, Ω) and ADP-stimulated platelet aggregation (area under the aggregation curve [AUC]), stratified by median age of menopause.* ◆ = point estimates with surrounding 95% confidence intervals for regression coefficients of female sex on platelet activation (pre-stimulated impedance, *Ω) (panel A) and* ADP-stimulated platelet aggregation (panel B), controlling for age, mechanism, injury severity, shock (base excess), and platelet count, and stratified by pre and post-menopausal age groups. Coefficients for female sex: Panel A) Platelet activation (pre-stimulated impedance, *Ω)*: all ages, -27.1, *p*=0.003; Age<52, -35.2, *p*=0.003; Age>52, 0.01, *p*=0.99. Panel B) ADP-stimulated platelet aggregation- all ages, 7.20 *p*=0.013; Age<52, 9.46, *p*=0.011; Age>52, 4.08, *p*=0.43.

FIGURE 1

Aims: We aimed to characterize sex specific effects on platelet activation and aggregation in trauma patients.

Methods: Blood from 558 injured patients was prospectively collected (2010-2019). Post-injury platelet activation (pre-stimulated impedance, Ω) and aggregation stimulated by adenosine diphosphate (ADP), collagen, thrombin, and arachidonic acid were measured by multiple electrode platelet aggregometry. Multivariate regressions tested the association of sex with platelet activation and aggregation, and results were stratified by median age of menopause (52y).

Results: Despite being older (median 48y vs 35y) and having higher rates of brain injury (37% vs 25%) and mortality (15% vs 10%), women had no difference in platelet counts or rates of impaired platelet aggregation on presentation. However, when controlling for known confounders, post-injury platelet activation was decreased (by 27Ω, *p*=0.003), but ADP-stimulated platelet aggregation was increased (by 7AUC, *p*=0.013) in women. However, these sex differences were only significant for injured patients younger than 52 years (median age of menopause) (Figure). Furthermore, the effect of female sex on platelet activation was mediated by differences in hematocrit, which was not observed for platelet aggregation.

Conclusions: Sex specific effects on platelet hemostatic functions after trauma remain underappreciated. Compared to similarly aged men, injured women of pre-menopausal age have decreased levels of platelet activation, but increased platelet aggregation when stimulated by ADP. These findings have implications for precision resuscitation efforts as sex may impact response to platelet transfusions, and attention to age, menopausal status, and hematocrit deserve further investigation to optimize platelet-dependent hemostasis in trauma patients.

PB1130 | Improved Sensitivity of Global Hemostasis Assays Using Different Combinations of Phospholipid Reagent and Tissue Factor

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Background: Conventional coagulation assays are associated with certain limitations, as they lack to depict the clinical heterogeneity of bleeding in hemophilic patients. Global hemostasis assays (GHA) like thromboelastometry (ROTEM), thrombin generation assays (TGA) or clot waveform analysis (CWA) are helpful which can mimic and reflect the major physiological aspects of hemostasis process. However, these methods are still not well standardised and do not reflect correctly to the individual patient response to treatment with bypassing agents or novel factor concentrates. So, there is a need to make the GHAs like ROTEM and CWA more sensitive to correlate with the gold standard assay i.e. Thrombin generation assays.
Aims: To standardize and increase the sensitivity of ROTEM and CWA by using different combinations of phospholipid and tissue factor reagents.
Methods: Plasma samples of different factor levels (< 1 IU/ml, 1 IU/ml, 5 IU/ml, 30 IU/ml, 35 IU/ml, 40 IU/ml, 100 IU/ml) were prepared by using different dilutions of factor concentrate in FVIII deficient plasma to mimic samples with different ETP values. The parameters used were ETP, Peak Height (ST-Genesia, Stago); 2nd Derivative +: CWA (ACL-TOP CTS 300, IL): ROTEM (ROTEM® delta Thromboelastometry System). The TGA was done in fully automated TGA (ST-Genesia, Stago). Phospholipid vesicles consisted of 20 mol% phosphatidylserine, 20 mol% phosphatidylethanolamine and 60 mol% phosphatidylcholine and recombinant relipidated tissue factor not containing polybrene or Ca^{2+} from Dade Behring.
Results: In CWA, 2nd Derivative (+) shows statistically significant correlation with ETP when 1:2 Actin FS (Siemens Healthcare Diagnostics) was used as the triggering reagent (P< 0.05). ROTEM showed statistically significant correlation with ETP in both Whole blood and Platelet poor plasma (PPP) by using 0.5pM TF+2 μM phospholipid and 0.2pM TF+0.8 μM phospholipid as triggering reagent respectively (P< 0.05) [Table 1].
Conclusions: In the current study, GHAs like CWA and ROTEM were made sensitive to ETP with a combination of PL and TF reagents.

PB1132 | Extended Half-life FIX and Disruptive Therapies in Carriers with FVIII and FIX Deficiencies: Preliminary Experience

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Background: Extended half-life FVIII and FIX concentrates as well as disruptive therapies such as monoclonal antibodies mimicking the action of FVIII have recently been extensively validated and are increasingly adopted by the hemophilia community. However, female carriers with clotting factor deficiency have been excluded from these trials and have very limited or no access to these innovative therapies.
Aims: Multiple research initiatives have shown that a significant proportion of carriers present with clotting factor FVIII or FIX deficiency, mostly in the mild hemophilia range. While DDAVP represents a treatment of choice for carriers of HA with mild FVIII deficiency, mainly if not exclusively short-acting concentrates are used in most carriers with FIX deficiency and severe to moderate FVIII deficiency requiring replacement.
Methods: We here report our experience with new treatments in carriers with clotting factor deficiency. Five carriers with FIX deficiency ranging from 1 to 27% were treated with EHL-FIX for surgery (n=3) (mastectomy, tonsillectomy, uterine curettage, one bolus required), vaginal delivery (n=1) (1 bolus pre-delivery and one on day 4) or prophylactically (n=1) (infusions every 14 days).
Results: There was no immediate or delayed postoperative bleeding complication and prophylaxis was very effective with a spontaneous

TABLE 1 Difference in varying level of FVIII (IU/ml) and correlation with different GHAs

		FVIII levels (IU/ml)			
		Difference between different FVIII levels: (Median)			
GHA	Parameter	30-35	35-40	30-40	40-100
CWA	2nd Derivative +	141	49	93	117.7
ROTEM	Clotting Time (CT)	88.5	50	103.5	141
TGA Genesia (Gold Std)	Endogenous Thrombin Potential (ETP)	69.9	69.16	139.09	114.83
	Peak Ht	13.5	18.4	41.8	56.6

joint bleeding rate of 0. One carrier of HA with severe FVIII deficiency secondary to double inheritance of the int22 inversion was recently started on replacement with Hemlibra (6 mg/kg ¼ weeks) with an excellent haemostatic control and tolerance.

Conclusions: New therapies should be considered in carriers with clotting factor deficiency requiring replacement therapy for invasive procedures or prophylaxis. Although very little experience in this setting has been reported so far, initiatives should be encouraged to collect data and promote access to innovative therapies in carriers.

PB1133 | Desmopressin Response and Clinical Outcome in Bleeding Disorders: A 30 Year Retrospective Analysis

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Background: Desmopressin (DDVAP) is an effective and well-tolerated haemostatic drug used for prevention and management of bleeding in patients with inherited bleeding disorders. We provide a single centre experience spanning 30 years.

Aims: Retrospective evaluation of the use of DDAVP in patients with inherited bleeding disorders.

Methods: A retrospective review of the patients registered at the centre with a diagnosis of inherited bleeding disorder and treated with DDAVP between 1989 to December 2019 was undertaken. Information on baseline diagnosis, indication for treatment, laboratory response to treatment and mode of administration of DDAVP reviewed. Treatment response was monitored by observation of factor VIII (FVIII), and von Willebrand factor (VWF) levels (VWF:RCof) prior and 90 minutes post-administration.

Results: A total of 520 eligible patients were identified. The following patients groups were seen; Mild haemophilia A (HA) (n=124), HA carriers (n=61), von Willebrand disease (n=239) platelet disorders (n=77) and patients with combined bleeding disorders (n=16). VWD patients included; type 1 (n=173) (mild, n=126; severe, n=47), type2 (n=71) which included type 2A (n=12), type 2B (n=20), type 2M (n-34), type 2N (n-5). A total of 785 minor procedures were covered with DDVAP. In mild HA, the median FVIII increased from 22 IU/dL to 58 IU/dL, 65% increase. In HA carriers median FVIII increased from 48 IU/dL to 130 IU/dL, increment of 63%. Patients with VWD, mild type 1 had an increment of FVIII and VWF:RCof levels of 67.6% and 74.3% respectively (median baseline VIII 66 IU/dL and Ricof 30 IU/dL, respectively).

Conclusions: DDVAP was used regularly in our patients with mild bleeding disorders; the responses appear to be higher in HA carriers compared to mild HA and this may be related to the higher baseline levels. Further characterisation of the absolute response to the procedure and bleeding enables development of clinical algorithms.

PB1134 | Glycocalyx Shedding as a Potential Driver of Trauma-induced Coagulopathy in Severe Trauma Patients: Insights from the TRAUCC Trial

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Background: Massive bleeding is a major cause of early death after trauma. One third of bleeding patients have trauma-induced coagulopathy, which increases severity of bleeding and mortality. Trauma-induced endotheliopathy consists of a glycocalyx degradation and shedding and an activation of endothelium leading to endothelial secretion. It can contribute to massive bleeding and death.

Aims: To assess the presence of glycocalyx shedding and endothelial damage on admission of trauma patients and determine the association of these endothelial dysfunctions with coagulopathy, severity of traumatic injury and 30-day mortality.

Methods: Endothelial markers were explored in 41 trauma patients from the TRAUCC trial, using blood samples collected at hospital admission. Glycocalyx shedding was quantified by soluble Syndecan-1 (sCD138). Endothelial secretion was quantified by Angiopoitein-2 (Ang-2). Injury Severity Score (ISS) estimated severity of traumatic injury, and ISS>25 defined the most severe patients. Prothrombin ratio (PTr) >1.2 defined coagulopathy. Relationship between sCD138, aPTT, and thrombin time (TT) were investigated to explore its anticoagulant effect.

Results: Patients who died within 30 days (n=11) have a higher concentration of Ang-2 (p=0.034) (Table I). Patients with an ISS>25 (n=20) have increased concentrations of sCD138 (p=0.001) and Ang-2 (p=0.002) when compared to those with ISS≤25 (n=21). A coagulopathy was present at admission in 26 patients. The levels of sCD138, but not the levels of Ang-2 were increased (p=0.011) in the presence of coagulopathy. aPTT and TT were closely correlated with sCD138 (spearman r_s=0.73 and r_s=0.72 respectively) (Figure I). We found no relation between coagulation parameters and Ang-2.

Conclusions: Trauma patients had endothelial dysfunctions correlated to injury severity at admission. The secretion of Ang-2 after trauma was correlated to mortality regardless of the presence of a coagulopathy. Glycocalyx shedding seems deeply associated with

TABLE 1 sCD138, Ang-2 levels according to mortality, severity of trauma and presence of coagulopathy

	Survival (n=30)	Death (n=11)	*p value*	ISS≤25 (n=21)	ISS>25 (n=20)	*p value*	PTr≤1,2 (n=15)	PTr>1,2 (n=26)	*p value*
Severity of trauma									
ISS	22 (16-28)	36 (27-45)	***<0.0001***	17 (14-22)	33 (28-38)		16 (14-29)	27 (22-34)	***0.022***
Coagulopathy									
TQr	1.4 (1.1-1.5)	1.5 (1.3-1.9)	***0.027***	1.2 (1.1-1.4)	1.6 (1.3-1.9)	***0.001***	1.1 (1.1-1.2)	1.5 (1.4-1.8)	
Endotheliopathy									
sCD138 (ng/mL)	62.4 (33.3-118.3)	74.1 (44.9-574.3)	***0.26***	40.1 (24.2-67.1)	106.7 (57.6-182.0)	***0.001***	33.6 (23.2-74.1)	89.5 (47.8-168.4)	***0.011***
Ang-2 (pg/mL)	263 (152-391)	482 (316-776)	***0.034***	232 (126-338)	441 (298-746)	***0.002***	242 (154-405)	352 (154-519)	***0.19***

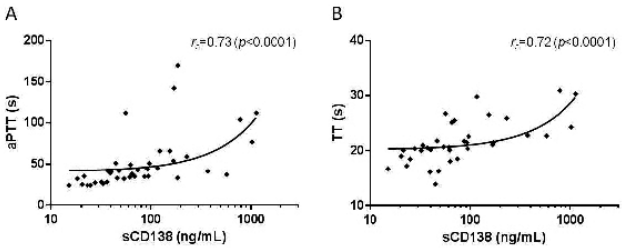

FIGURE 1 Correlations between sCD138 and clotting times; A: aPTT (rs=0.73; p<0.0001) (n=41); B: TT (rs=0.72; p<0.0001) (n=36)

coagulopathy and this could suggest a causal relation between alteration of coagulation and endothelial damage.

PB1136 | Biomarkers of Fibrinolysis Predict the Need for Massive Transfusion in Trauma

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Background: The relevance of fibrinolysis in major trauma was highlighted by the CRASH-2 study which demonstrated that early administration of tranexamic acid reduced the risk of death and need for transfusion. The thromboelastogram (TEG) Ly30 parameter has been proposed to stratify patients by fibrinolysis phenotypes. While TEG has some major advantages (point-of-care, whole blood etc.), its sensitivity to fibrinolysis is not optimal. Alternative approaches to assess global fibrinolysis in plasma include the euglobulin clot lysis time (ECLT) or turbidity clot lysis in response to tPA assays (Ilich, RPTH 2020).
Aims: Our aim was to test the predictive capacities of TEG Ly30, as well as ECLT, tPA-mediated CLT, free tPA, and D-dimer for massive transfusion.
Methods: Citrated plasmas from 175 trauma patients collected on admission to the Mayo Clinic Trauma Center were evaluated. All samples were obtained before any antifibrinolytic therapy or transfusion. The critical administration threshold (CAT, defined as the administration ≥3 red cell units over any 1-hour period, during first 24 hours) was used to define massive transfusion. For 133 of these patients, concurrent whole blood TEG data were available. All 175 samples were tested for ECLT, tPA-mediated clot lysis, as well as D-dimer, free tPA by ELISA.
Results: Of 175 patients, 16 reached CAT criteria. In global assays, ECLT demonstrated the best performance; of 16 CAT individuals, 13 were hyperfibrinolytic by ECLT. The c-statistic (area under the ROC curve) to predict massive transfusion for ECLT was 0.704, compared to TEG Ly30 of 0.493 and 0.503 for the tPA clot lysis time. D-dimer, but not free tPA, demonstrated a good predictive value for massive transfusion, with AUC ROC values 0.720 and 0.572, respectively.
Conclusions: These data suggest that hyperfibrinolysis defined by the ECLT and D-dimer predict the need for massive transfusion, with both assays outperforming TEG in this regard.

PB1138 | The Effect of Bypassing Agents in Combination with Emicizumab-treatment

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Background: Emicizumab is a relatively new treatment for haemophilia A. It is a bispecific monoclonal antibody that binds to both factor IXa (FIXa) and X (FX), thus mimicking FVIII activating FXa. Although it is shown to be effective reducing the frequency of bleeding episodes in haemophilia A patients, there is still need for bypassing agents (BPA) in case of breakthrough bleedings or need for surgery in inhibitor patients. The HAVEN-1 study showed an increased risk of thromboembolic complications with simultaneous

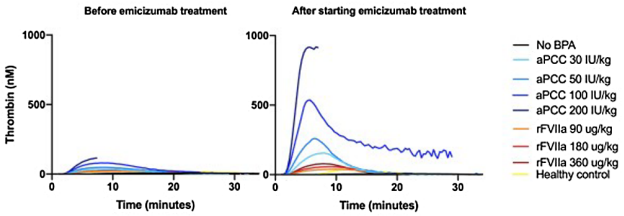

FIGURE 1 Thrombin generation before and after starting treatment with emicizumab

treatment with emicizumab and activated prothrombin complex concentrate (aPCC) in high doses (>100 IU/kg daily) for more than one day. It is suspected that aPCC and emicizumab have a synergetic haemostatic effect.

Aims: To assess and compare the haemostatic effect of the bypassing agents, aPCC and recombinant activated FVII (rFVIIa) in haemophilia patients before and after starting treatment with emicizumab.

Methods: We collected blood from patients before and after starting treatment with emicizumab and spiked the samples with aPCC and rFVIIa in different concentrations. The haemostatic effect of aPCC and rFVIIa was assessed by thrombin generation (TG) and thromboelastometry.

Results: Four patients (two adults and two children) with haemophilia A were included. Both aPCC and rFVIIa showed a concentration-dependent effect on TG and thromboelastometry clotting time (CT). However, aPCC increased TG to greater extent after starting emicizumab than before. The enhancement of effect was weaker for rFVIIa after starting emicizumab-treatment (Figure 1). Furthermore, aPCC had a stronger effect reducing CT after starting emicizumab treatment than before starting this treatment. CT was not shortened more markedly by rFVIIa after starting emicizumab- treatment compared with before starting this treatment.

Conclusions: Combining emicizumab with aPCC seems to induce a stronger synergistic pro-haemostatic effect than combining emicizumab with rFVIIa.

PB1139 | Intravenous Synthetic Platelet Nanotechnology for the Treatment of Bleeding Complications

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Background: Platelet transfusions are clinically used in thrombocytopenia, surgery, trauma etc. However, platelets have limited availability, portability, high risk of contamination, and short shelf-life (~ 5 days), which severely limit their use. Therefore, there is a significant need for an intravenous hemostat with easy manufacturability, portability and long shelf-life, that enables platelet-mimetic hemostasis when platelets are unavailable. To this end, we have developed a synthetic platelet nanotechnology.

Aims: Evaluate hemostatic efficacy of synthetic platelets.

Methods: Synthetic platelets were manufactured on a liposomal template to mimic platelet's hemostasis-relevant adhesive and aggregatory mechanisms by decoration with VWF-binding, collagen-binding and active platelet GPIIb-IIIa-binding peptides. The particles were sterilized and stored at room temperature in suspension or lyophilized powder, and particle stability was assessed by DLS. Their platelet-mimetic bioactivity was assessed by aggregometry, microfluidics and ROTEM. The hemostasis-augmenting procoagulant and secretory mechanisms of platelet were further engineered into the particle by modifying the surface with phosphatidylserine (PS) or loading them with polyphosphate (PolyP). Their hemostatic efficacy was evaluated in vitro and in vivo.

Results: Synthetic platelets showed high stability in long-term storage as suspension or powder, and maintained platelet-mimetic bioactivity. Aggregometry showed that they could specifically enhance aggregation of active platelets but did not affect resting platelets. ROTEM studies showed that they improved clot kinetics and firmness. In vivo, they did not show systemic toxicity, immune activation or off-target thrombotic risks. Incorporation of PS or PolyP components in the synthetic platelets enabled enhanced hemostatic outputs even when natural platelets were depleted or dysfunctional. In vivo, the particles reduced blood loss, stabilized blood pressure and significantly improved survival.

Conclusions: Synthetic platelet surrogates are a promising hemostatic nanotechnology for transfusion management of bleeding dysfunctions.

PB1140 | The 2020 World Federation of Hemophilia Guidelines for the Management of Hemophilia

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Background: The World Federation of Hemophilia (WFH) has developed the third edition of the WFH Guidelines for the Management of Hemophilia.

Aims: The aim of these guidelines are to provide health care professionals and people with hemophilia recommendations that will guide the management of hemophilia around the world.

Methods: This 2020 version of the WFH treatment guidelines incorporates evidence-based and Trustworthy Consensus-Based Statement (TCBS) approaches in conformance with established international standards for clinical practice guidelines. The TCBS process produces evidence-informed recommendations based on the evidence and expert clinical advice.

Results: Guided by content leads (AS, GP), a guidelines methodology expert (SZL), and 11 chapter leads, the original 7 chapters of the 2012 version of the guidelines were updated and 4 new chapters were added (Table 1). Each chapter was assigned an expert panel composed of up to 10 members, healthcare professionals with clinical expertise, and patients/caregivers, with the latter making up 25%

of each chapter panel. A total of 50 panelists were assigned to the 11 chapters, with some panelists serving on more than one panel.
Each chapter topic went through a systematic review of the published literature, and each study was screened for eligibility based on apriori defined criteria on population, interventions, comparisons, and outcomes. Data was extracted from included studies and evidence tables developed. Evidence-based recommendations were drafted by the chapter panel and each set of recommendations went through a Delphi consensus process. The threshold for achieving consensus was 80% agreement among panel voters.
Across all chapters, 344 recommendations achieved consensus. Recommendations not achieving consensus were included as discussion points in the final manuscripts.
Conclusions: This third edition of the WFH Guidelines for the Management of Hemophilia are trustworthy, reliable, evidence-informed, and expert-driven recommendations that will guide the treatment and management of hemophilia around the world.

NOVEL BIOTHERAPEUTICS IN HEMOPHILIA

PB1141 | Emicizumab in the Management of Hemophilia A: Real World Experience in East Malaysia

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Background: Hemophilia A (HA) may be inherited or arise from spontaneous mutation. The development of inhibitory alloantibodies results in impairment of quality of life (QoL). The advent of emicizumab offers the potential for a paradigm shift in hemophilia treatment. There is a paucity of real-world data on emicizumab.

TABLE 1 Baseline characteristics of patients

Patients (n)	3
Gender, n (%) Male Female	3 (100%) 0 (0%)
Age at diagnosis (months), median (range)	12 (1.0 to 132.0)
Ethnicity, n (%) Chinese Malay Sarawak native	1 (33.3%) 1 (33.3%) 1 (33.3%)
Inhibitor level (BU), median (range)	3.0 (1.6-20.0)
Type of treatment Prior to Emicizumab, n (%) On demand Prophylaxis	2 (66.7%) 1 (33.3%)

Aims: Therefore, we aimed to determine the demographic characteristics of our patients and to study their outcome.
Methods: This was a retrospective review of HA patients treated with emicizumab in East Malaysia between June 2018 and December 2019. The primary endpoint was reduction of bleeding events. The secondary endpoints included:
(a) improvement in QoL assessed using Haemo-QoL-A questionnaire; and
(b) adverse events.
Results: Three patients were started on emicizumab (Table 1). All were severe HA patients with inhibitors. The median duration of treatment was 56 weeks, ranging from 27 to 72 weeks. All patients were given 3 mg/kg every 2 weeks. Two had achieved 100 percent reduction in bleeding events after receiving emicizumab. One had one episode of minor spontaneous bleed one day prior to the scheduled dose, which fully resolved after emicizumab administration without requiring any bypassing agents. All of those who required walking aids prior to emicizumab (two patients) were able to ambulate independently after receiving emicizumab. QoL assessment was shown in Table 2. None had any adverse events. No thromboembolic events were reported.
Conclusions: The results demonstrated that emicizumab has a favourable safety profile with encouraging efficacy in patients with HA. Study limitations include a small sample size and the results were generally descriptive. Additional studies are warranted to establish clinical data on its safety and to define suitable assays for hemostatic monitoring.

TABLE 2 Health-related quality of life scores by 6 domains (Haemo-QoL-A)

	Before emicizumab	After emicizumab	Difference	p-value
Physical functioning	11.87 (±8.44)	77.77 (±3.87)	65.90 (±11.23)	0.010
Role functioning	29.03 (±13.74)	81.73 (±11.06)	52.70 (±15.86)	0.029
Worry	46.67 (±39.46)	84.00 (±10.58)	37.33 (±34.49)	0.202
Consequences of bleeding	17.13 (±15.04)	75.20 (±14.67)	58.07 (±26.52)	0.063
Emotional impact	47.80 (±23.38)	73.30 (±5.77)	25.50 (±18.92)	0.144
Treatment concern	37.80 (±42.26)	64.40 (±36.75)	26.60 (±23.04)	0.184
Overall transformed score	31.72 (±12.95)	76.10 (±2.27)	44.38 (±10.68)	0.019

PB1142 | Perioperative Management of Patients with Hemophilia Receiving Fitusiran, an Investigational RNAi Therapeutic Targeting Antithrombin for the Treatment of Hemophilia

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Background: Patients with hemophilia A or B are at risk of excess blood loss when undergoing surgical procedures and require management of hemostasis during the perioperative period. Fitusiran is a once-monthly subcutaneously administered investigational RNA interference (RNAi) therapeutic that targets antithrombin (AT) to enhance thrombin generation and rebalance hemostasis for people with hemophilia A or hemophilia B, with or without inhibitors. Successful perioperative hemostatic management of patients treated with fitusiran prophylaxis has been observed in a variety of surgical procedures, including a thoracotomy/partial lung segmentectomy, molar tooth extraction, premolar tooth extraction, nasal septoplasty, and endoscopic cholecystectomy. Data from additional surgical procedures are needed to better define the hemostatic coverage provided by fitusiran and appropriate perioperative hemostatic management plans.

Aims: To describe the hemostatic management of patients with hemophilia A or B who underwent surgical procedures while receiving fitusiran prophylaxis.

Methods: Fitusiran was evaluated in a phase 1 dose-escalation study (NCT02035605) followed by a phase 2 open-label extension (OLE) study (NCT02554773) that included patients with hemophilia A or B, with or without inhibitors. Patients who were eligible to continue in the phase 2 OLE study received monthly fixed subcutaneous doses of fitusiran 50 mg or 80 mg. Data on perioperative hemostatic therapies and hemostatic response were collected for patients undergoing surgical procedures who had lowered AT during the study.

Results: In addition to 5 previously presented surgical procedures, 1 total knee replacement and 1 total hip replacement were performed. Further details on perioperative treatment regimens and hemostatic responses in all 7 procedures will be presented.

Conclusions: Successful perioperative hemostatic management of patients in the context of AT lowering with fitusiran has now been observed in several orthopedic, thoracic, gastrointestinal, maxillofacial, and dental surgical procedures.

PB1143 | Correction of Human Hemophilia B Gene in iPSCs by Base-Editing Approach Based on Engineered Cas9 with Broad PAM Flexibility

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Background: We have shown that genome editing using CRISPR-Cas9 could repair the phenotype responsible for bleeding in mice with hemophilia B. Recently, because of the reduced potential risk for genotoxicity, the application of base-editing, "non-cut" genome editing, for the treatment of genetic diseases has attracted much attention. However, the requirement of protospacer adjacent motif (PAM) sequence for Cas9 recognition (NGG for SpCas9) may prevent its application to treat a variety of mutations.

Aims: This research was aimed at correcting the mutation of disease pattern responsible for human hemophilia B by base-editing system based on SpCas9-NG, an engineered Cas9 with broad PAM flexibility.

Methods: We generated induced pluripotent stem cells (iPSCs) from human peripheral blood mononuclear cells using non-integrative Sendai virus vector carrying human reprogramming factors. Sanger sequencing was performed to ensure the genetic mutation. We examined the genome editing by the surveyor assay and next generation sequencing. The differentiation to hepatocytes was achieved through the use of several combinations of cytokines under hypoxic conditions.

Results: We found that the C to T conversion in *F9* gene in the patient-derived iPSCs (H002 cells) could repair the disease genotype (c.947T > C). We designed 3 gRNAs near the mutation region and confirmed the broad PAM flexibility of SpCas9-NG. Next, we developed the cytidine base editors from wild type SpCas9 or SpCas9-NG combined with APOBEC1 or PmCDA1 deaminase and the most effective gene repair was observed after treatment with PmCDA1 based on Cas9-NG. We obtained the mutation-repaired patient-derived iPS clone and both original H002 clone and the repaired clone could be differentiated into their hepatocyte lineage.

Conclusions: We showed that Cas9-NG-based cytidine base-editing could effectively repair the genetic mutation in hemophilia B iPSCs without host genome digestion. The broad PAM flexibility of Cas9-NG can be applicable to treat disease mutations by using a base-editing approach.

PB1144 | A Next Generation Recombinant Factor VIII for Subcutaneous Hemophilia A Prophylaxis

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Background: Despite the availability of non-factor treatment options, subcutaneous prophylaxis with FVIII replacement therapies is highly desirable as frequent intravenous injections are a great burden for patients.
Aims: The development of a new recombinant FVIII with outstanding subcutaneous bioavailability, maintaining full prophylactic properties compared with intravenous treatment.
Methods: Biotest's novel recombinant FVIII proteins containing four albumin-binding domains (FVIII-ABD) were injected intravenously and subcutaneously into hemophilia A mice (n=10/group) and Göttingen minipigs (n=3/group) with Moroctocog alfa used as comparison. Blood samples taken at defined time points (0 - 240 h) after administration, were analyzed for chromogenic FVIII activity and/or FVIII antigen levels. Non-compartmental analysis was performed to determine half-life, and area under the curve (AUC) comparison was done to assess subcutaneous bioavailability.
Results: While a subcutaneous bioavailability of 15.3% (mean) and 18.6% (median) was determined for FVIII-ABD in hemophilia A mice, Moroctocog alpha was not detectable. In the Göttingen minipig model, which provides a dermal structure closer to the human setting, subcutaneous bioavailability of up to 50% was observed for FVIII-ABD, depending on the dose and formulation.
Conclusions: A next generation recombinant FVIII molecule fused to four albumin-binding domains resulted in a feasible treatment option allowing subcutaneous hemophilia A prophylaxis with the benefit of a substantial half-life extension.

PB1145 | New Factor VIII Function-Mimetic Bispecific Antibodies Engineered from Emicizumab for Further Improving the Treatment of Hemophilia A

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Background: Emicizumab is a factor (F)VIII-function mimetic therapeutic antibody for treating persons with hemophilia A (PwHA). Currently it plays a central role in treating PwHA, although there is some room for improvement in dosing frequency/volume and/or hemostatic activity to achieve a non-hemophilic status.
Aims: To address these points, we have further engineered emicizumab.
Methods: Emicizumab used commonized light chains to enable industrial manufacturing, but this limited further optimization. Therefore, we developed a new antibody engineering technology to enable industrial manufacture of a four-chain asymmetric bispecific antibody. We further engineered emicizumab by incorporating new non-commonized light chains with our newly established manufacturing technology and by replacing amino acids in both the variable and constant regions to enhance hemostatic activity and improve pharmacokinetics. Then, we evaluated hemostatic activity and half-life of the engineered antibodies with in vitro and in vivo studies.
Results: We successfully identified emicizumab-based, four-chain asymmetric bispecific antibodies, termed NXT004 to 007, which increased in vitro FVIII-equivalent activity of plasma thrombin generation to levels similar to those of standard FVIII (100%). The undesirable inhibitory interference of NXT004 to the coagulation cascade was not evident at a targeting therapeutic concentration in vitro. The hemostatic activity was also confirmed in non-human primates with acquired hemophilia A at low dose of the antibody. NXT004 had an approximately three week of half-life in non-human primates. From these results, we simulated that the engineered antibody may have a potency to keep a non-hemophilic range of FVIII-equivalent thrombin generation activity by subcutaneous doses every four weeks clinically.
Conclusions: The limitations for further optimization of emicizumab were overcome by the newly established antibody engineering technologies. The further optimized antibody, administered subcutaneously every four weeks, may have the potency to retain a non-hemophilic range of FVIII-equivalent hemostatic activity. Phase I/II clinical study with healthy volunteers and PwHA are on-going.

PB1146 | Thrombin Generation Potential of Modified FIX: Dalcinonacog Alfa

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Background: Dalcinonacog alfa (DalcA) is a full-length recombinant FIX with 3 amino acid substitutions that confer the product with increased specific activity, enhanced FVIIIa binding and reduced inhibition by antithrombin. Labelled potencies do not always reflect the full therapeutic potential as factor assays may not take into account the full functionality of FIX variants. Thrombin generation tests could be employed to provide a better understanding of the dynamic functionality of DalcA in plasma.
Aims: To compare thrombin generation profiles of DalcA with recombinant (RB) and plasma derived (PD) FIX products in FIX immunodepleted plasma.
Methods: Thrombin generation profiles (Thrombinoscope, Stago) of DalcA (Catalyst Biosciences), RB (Benefix, Pfizer) and PD (14/148, NIBSC) were compared at 3 concentrations: 0.01, 0.05 and 0.3

TABLE 1 Average peak thrombin (blank subtracted) + SD for the 3 FIX samples, at all concentrations tested, using 3 different triggers

	DalcA 0.3 IU/ml	DalcA 0.05 IU/ml	DalcA 0.01 IU/ml	RB 0.3 IU/ml	RB 0.05 IU/ml	RB 0.01 IU/ml	PD 0.3 IU/ml	PD 0.05 IU/ml	PD 0.01 IU/ml
High Tissue Factor (nM Thrombin)	**103.3**	**21.7**	**2.7**	**89.4**	**20.0**	**2.6**	**58.0**	**11.3**	**1.5**
Standard deviation (SD)	2.82	1.16	1.25	4.85	0.77	0.94	2.40	1.10	0.86
n	4	4	4	4	4	4	4	4	4
Low Tissue Factor (nM Thrombin)	**96**	**23.8**	**7.2**	**64.6**	**13.2**	**3.9**	**33.5**	**7.3**	**3.4**
Standard deviation (SD)	2.80	1.80	1.84	2.03	1.45	0.24	2.93	1.18	-
n	4	4	4	4	4	3	4	4	1
FXIa + very low Tissue factor (nM Thrombin)	**232.1**	**89.1**	**22.6**	**193.5**	**48.4**	**7.5**	**218.8**	**58.9**	**9.8**
Standard deviation (SD)	9.3	11.9	4.76	18.27	10.11	2.25	25.89	12.07	3.56
n	4	4	4	4	4	4	4	4	4

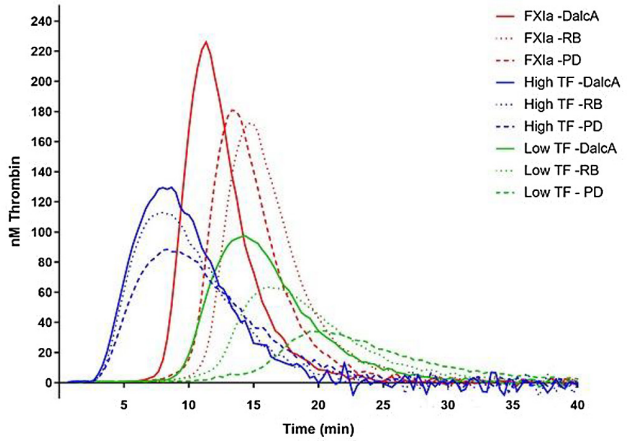

FIGURE 1 Representative thrombin generation curves for recombinant (RB), plasma derived (PD), dalcinonacog alfa (DalcA) at 0.3 IU/ml with 3 triggers

International Unit (IU)/ml, based on labelled potency, using 3 different stimuli: high tissue factor (TF) (~1pM), low TF (~0.1pM) and FXIa (6.5 mIU FXIa/ml + 0.05pM TF), each containing phospholipid (4 μM).

Results: Different thrombin generation profiles were seen when using the 3 triggers (figure 1) and peak thrombin values are presented in table 1. Lagtimes were shortest using high TF and longest with low TF. In all cases FXIa generated more thrombin than the other 2 triggers. The Endogenous Thrombin Potential followed a similar trend. FXIa was a more sensitive trigger especially for DalcA. On average, when compared at the same IU, DalcA had a shorter lagtime and was able to generate 10-300% more thrombin than RB and PD. RB was more active than PD using high TF and low TF but similar when FXIa was used.

Conclusions: On the same IU basis, DalcA had higher thrombin generation potential than RB and PD FIX and this may be due to the modifications in DalcA which enhance its binding to FVIIIa and reduced its inhibition by antithrombin. This may translate to better efficacy *in vivo*.

PB1147 | A Novel FVIIIa-Mimetic Bispecific Antibody, Mim8, Enhances FIXa Proteolytic Activity While Allowing for Efficient, Lipid-Dependent Assembly with FX

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Background: Haemophilia A (HA) is a bleeding disorder resulting from deficient Factor VIII (FVIII), which is a cofactor facilitating activation of Factor X (FX) via Factor IXa (FIXa). Here, we have developed a next generation anti-FIXa/anti-FX bispecific antibody, Mim8, which has demonstrated haemostatic properties *in vitro* as well as in bleeding models in HA mice[1]. In preclinical models, Mim8 shows

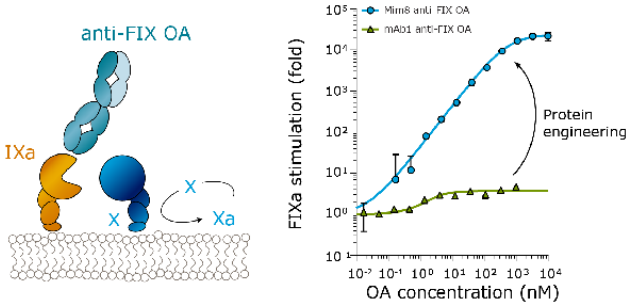

FIGURE 1 Stimulation of FIXa activity by the anti-FIX arm of Mim8 in a one-armed (OA) monovalent mAb format. mAb1 is the parental antibody

15-fold increased potency as compared to the marketed bispecific antibody emicizumab (Hemlibra®).

Aims: The aim of the present study was to investigate the mode of action of Mim8 using a biochemical *in vitro* set-up.

Methods: Biochemical and biological assays were used. FX activation assays, using various recombinant variants of FX, were employed to investigate the mode of action for Mim8 in the presence of FIXa and synthetic phospholipid vesicles.

Results: The enhanced activity of Mim8 was demonstrated to primarily derive from a strong stimulation of FIXa's proteolytic activity by the anti-FIXa arm, which increased the turnover of FX by FIXa approximately 18 × 10^3-fold (Fig 1).

Furthermore, Mim8 targets unique epitopes on FIXa and FX allowing for their simultaneous engagement with the procoagulant membrane surface. This results in an efficient assembly of the FIXa/Mim8/FX complex on phosphatidyl serine-containing vesicles with an apparent dissociation constant (K_D) of 12 nM despite binding FIXa and FX in solution with K_D-values in the low micromolar range.

Conclusions: Mim8 is a next-generation FVIII-mimetic with a potent and efficacious haemostatic effect in preclinical studies. The activity of Mim8 is derived from efficient assembly with FIXa and FX on the procoagulant membrane surface and a strong stimulation of FIXa's proteolytic activity.

[1]Kjellev et al. Blood (2019) 134 (S1): 96.

PB1148 | Comparison of Bypassing Agents in Patients on Emicizumab Using Global Hemostasis Assays

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Background: Management of breakthrough bleeding events in patients on emicizumab involves episodic treatment with the bypassing agents (BPA), activated prothrombin complex concentrate (aPCC) and recombinant activated FVII (rFVIIa). A concomitant drug reaction between emicizumab and aPCC resulting in thrombotic events was noted in the HAVEN clinical trials.

TABLE 1 Concentrations for in vitro spiking

Medication	Concentrations for in vitro spiking							
fFVIIa (mcg/ml)	0	0.5	1	1.5	2	3	4	5
FEIBA (IU/ml)	0	0.05	0.1	0.2	0.3	0.5	0.75	1

Table 2a Summary Statistics of TGA results for Severe HA + Emi

Variable	aPCC Concentration (IU/ml)								
	0	0.05	0.1	0.2	0.3	0.5	0.75	1	Pooled Normal Plasma
ETP (nM*min) [1]	258.79 (114.01) [10]	748.98 (156.66) [10]	948.02 (294.55) [10]	1429.44 (219.36) [10]	1701.94 (195.56) [10]	2242.35 (478.37) [10]	2800.72 (362.93) [6]	2034.41 [1]	617.02 (99.45) [10]
ETP (nM*min) [2]	244.84 (114.77, 415.35)	737.56 (538.48, 1086.58)	1007.67 (367.57, 1330.92)	1479.25 (846.03, 1610.93)	1725.96 (1329.87, 2020.61)	2288.07 (1402.64, 3089.41)	2884.95 (2295.77, 3419.82)	2034.41	592.93 (489.13, 750.08)
Peak Thrombin (nM) [1]	11.05 (4.90) [10]	52.51 (14.70) [10]	80.98 (33.53) [10]	157.16 (35.53) [10]	207.84 (35.12) [10]	303.68 (76.21) [10]	426.44 (42.60) [0]	495.87 [1]	55.16 (12.45) [10]
Peak Thrombin (nM) [2]	11.64 (5.42, 18.25)	51.31 (34.22, 67.86)	87.37 (25.57, 139.52)	160.94 (71.29, 198.54)	212.18 (133.36, 246.53)	328.11 (145.31, 385.38)	435.89 (259.17, 476.83)	495.87	54.79 (38.37, 69.61)

1: Arithmetic Mean (SD) [n]
2: Median (Range)

Table 2b Summary Statistics of TGA results for Severe HA + Emi

Variable	rFVIIa Concentration (mcg/ml)								
	0	0.5	1	1.5	2	3	4	5	Pooled Normal Plasma
ETP (nM*min) [1]	837.09 (44.30) [3]	951.22 (112.26) [5]	1140.43 (179.53) [7]	1221.09 (160.83) [7]	1203.44 (169.51) [7]	1415.25 (170.42) [7]	1551.05 (197.46) [7]	1305.95 (287.27) [6]	1464.12
ETP (nM*min) [2]	838.48 (792.1, 880.66)	959.50 (778.49, 1089.05)	1111.57 (969.58, 1459.13)	1255.54 (1080.65, 1457.24)	1319.76 (1033.27, 1471.76)	1447.26 (1164.43, 1668.87)	1517.27 (1201, 1754.72)	1577.62 (1219.86, 2886.58)	1464.12
Peak Thrombin (nM) [1]	29.88 (11.35) [7]	32.63 (7.64) [6]	41.64 (6.26) [7]	51.11 (7.80) [7]	55.20 (7.67) [7]	75.51 (12.99) [7]	86.74 (14.50) [7]	97.60 (14.47) [6]	107.74
Peak Thrombin (nM) [2]	30.81 (8.79, 45.54)	31.80 (24.63, 41.13)	42.24 (32.19, 50.1)	48.26 (41.85, 63.07)	56.13 (48.32, 68.71)	71.80 (60.26, 95.3)	86.88 (71.35, 109.35)	102.51 (76.14, 112.56)	107.74

1: Arithmetic Mean (SD) [n]
2: Median (Range)

TABLE 2 2A-2B

Aims: We aimed to assess the effect of spiking various concentrations of BPA on plasma taken from patients on emicizumab on thrombin generation.

Methods: Patients with severe hemophilia A (HA) with inhibitors who are currently on emicizumab and who achieved steady state were recruited to participate. Table 1 describes the concentrations of the bypassing agents and descriptive statistics are provided for the different TGA parameters.

Results: Eleven patients with severe HA and inhibitors currently on emicizumab for at least 6 weeks were enrolled in the study. The TGA parameters were assessed. The summary statistics are provided in tables 2a and 2b.

Conclusions: Due to the known thrombotic complications when emicizumab is used in conjunction with aPCC, there has been a large-scale abandonment of the use of aPCC in patients on emicizumab. However, it is possible that aPCC can be used safely with emicizumab albeit with lower doses than may typically be used and below the doses in the prescribing information. This, in turn, could suggest which doses of both rFVIIa and especially aPCC could be studied in a trial (or even used safely) when managing breakthrough bleeding or surgery in patients on emicizumab. In conclusion, we have demonstrated that lower doses of aPCC could potentially be used safely and effectively in inhibitor patients on emicizumab. It would be important to test this hypothesis in a clinical study.

PB1149 | Determining the Approximate Factor VIII Level Equivalency of Patients with Severe Hemophilia A on Emicizumab Using Global Hemostasis Assays

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Background: The treatment of patients with inhibitors currently involves using bypassing agents (BPA). Recently, the novel agent, emicizumab, has demonstrated a significant reduction in the rate of bleeding compared to BPA.

Aims: What remains unclear is the degree to which emicizumab corrects the clotting defect, and the objective of this study was to address this question.

Methods: Patients with mild/moderate hemophilia (group 1) and severe hemophilia patients with inhibitors on emicizumab (group 2) participated in the study. Blood drawn from both groups were assayed for FVIII activity (group 1) and thrombin generation (both groups). First, linear regression was utilized to model the FVIII levels as a function of the endogenous thrombin potential (ETP) and peak thrombin. Then, we used the results of the emicizumab patients (group 2) with the calibration curve to calculate their predicted FVIII level. Association between patient weight and their predicted FVIII levels were evaluated by linear regression.

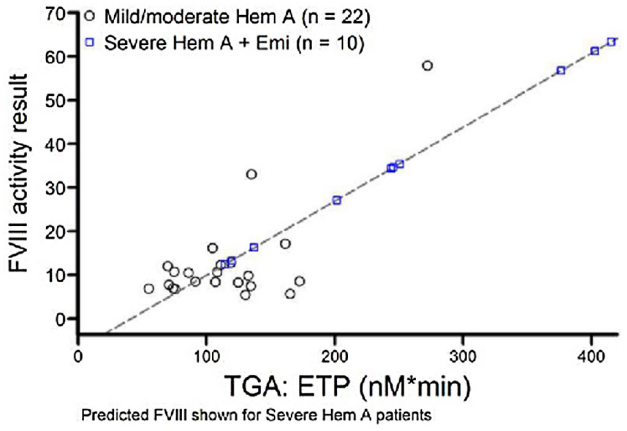

FIGURE 1 Predicted FVIII for severe Hemophilia A patients on emicizumab

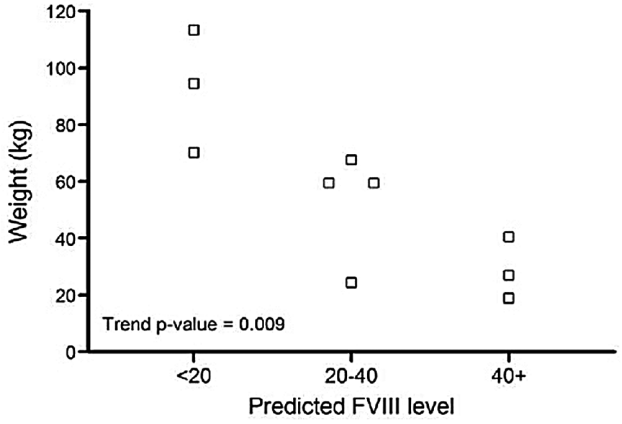

FIGURE 2 Relationship between pateints' weight and predicted FVIII level

Results: Twenty five patients with moderate and mild hemophilia and 11 patients with severe hemophilia and inhibitors on emicizumab were enrolled. Figure-1 demonstrates the raw data and the regression line for the FVIII levels versus ETP for group 1 and the squares represent the predicted FVIII levels for patients on emicizumab based on their actual ETP. Figure 2 shows the relationship between the inhibitor patient's weight and their predicted FVIII level.

Conclusions: All the patients on emicizumab had predicted FVIII levels above 10% with most having levels above 20%. The wide variability in the predicted FVIII level was tightly correlated to weight with the heaviest patients having the lowest FVIII predicted levels. It is unclear at this time why this may be the case, and further data will be collected to assess this relationship. Moving forward, understanding the correction of the clotting defect of non-replacement therapies is an important goal.

PB1150 | Assay of Emicizumab Using the Modified One-Stage Clotting Assay

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Background: Emicizumab is a factor VIII (FVIII)-bypassing agent that presents a challenge for laboratory assays. Because of cross-interference with FVIII in the one-stage clotting assay (OSC), an alternative, modified OSC has been recommended, using a product-specific emicizumab calibrator and a 1in8 dilution of samples.

Aims: This study investigates the effects of different dilutions and analysis methods on results.

Methods: Samples of emicizumab were prepared at ~50 µg/ml in FVIII-deficient plasma. These were pre-diluted 1in5, 1in8 or 1in10 in buffer prior to assay and tested neat, 1in3, 1in10 and 1in30, in duplicate per assay (n=3). A commercial emicizumab calibrator standard was used, with 5 activated partial thromboplastin time (APTT) reagents and 2 FVIII-deficient plasmas (PA and PB). Data were analysed by parallel line (PL) using at least 3 dilutions, and single point analysis at the 1in5, 1in8 or 1in10 pre-dilution only (neat). To obtain the best linearity, exclusion of the neat results for each pre-dilution was necessary during PL analysis.

Results: The results for PA (Figure 1) and PB (Figure 2) show good agreement between results for each pre-dilution and APTT reagent when analysed by PL (unstriped bars). For PA, results were similar between PL and single-point analysis (striped bars), with the

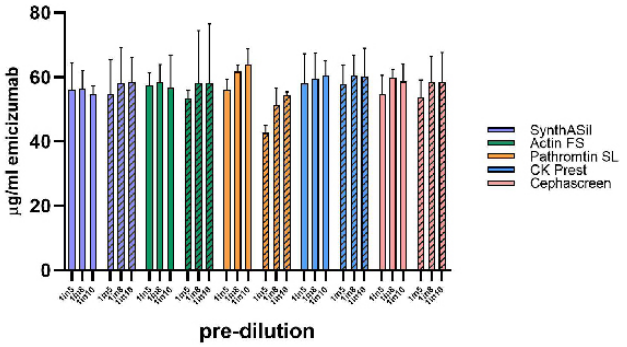

FIGURE 1 Modified OSC with plasma PA. Plain=parallel line analysis; stripes=single-point analysis. Error bars are 95% confidence limits

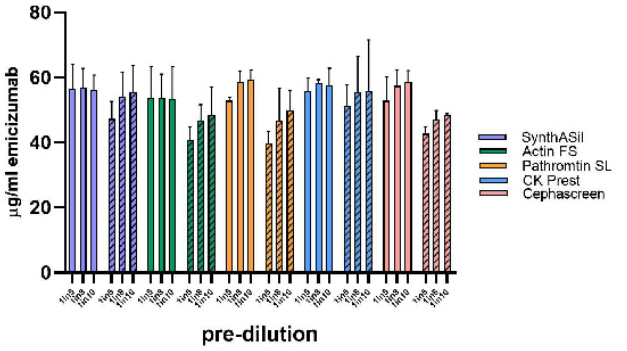

FIGURE 2 Modified OSC with plasma PB. Plain=parallel line analysis; stripes=single-point analysis. Error bars are 95% confidence limits

exception of APTT reagent Pathromtin. For PB, single-point results were usually lower than PL for each APTT reagent. Overall, the 1in5 pre-dilution yielded lower results than the other pre-dilutions, and was more marked for PB.

Conclusions: This study has shown that a routine 1in8 dilution is not suitable for all modified OSC assays, since only higher dilutions produced linear results, and that single-point estimates may be misleading. Assay of emicizumab should therefore be undertaken with careful consideration of dilution, APTT reagent and deficient plasma. For accurate results, dilutions should be carefully chosen to ensure results lie on the linear part of the standard curve.

PB1151 | Optimization of Measurement of Emicizumab, FVIII and Anti-FVIII Activity in Patients Treated with Emicizumab

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Background: Emicizumab(Hemlibra®), an antibody that bridges factor IXa(FIX) and factor X(FX), is approved as prophylactic treatment in patients with haemophilia A.

Aims: The first aim was to confirm that a one stage clotting assay(OSCA) and chromogenic assay(CA) modified with emicizumab calibrator is suited for quantification of emicizumab. Secondly, we evaluated a OSCA and a CA for the measurement of FVIII:C and anti-FVIII in patient samples with emicizumab.

Methods: Both the OSCA(C.K.PREST® reagent) and CA(BIOPHEN™ FVIII:C reagent) modified with emicizumab calibrator were applied on STA-R EVO for quantification of emicizumab(µg/mL) in a severe hemophilia patient before and during treatment with emicizumab, normal pooled plasma(NPP), FVIII deficient plasma and control samples. To explore potential interference of FVIII in the emicizumab assay, dilutions of normal pool plasma in FVIII deficient plasma(0-100%FVIII) were measured. FVIII:C and anti-FVIII were measured with OSCA(C.K.PREST reagent) and CA(TriniCHROM Factor VIII:C reagent) in the same patient before and during treatment with emicizumab.

Results: %CV for emicizumab control samples(n=6) was 1.8-2.1% for OSCA and 8.7-9.7% for CA. Emicizumab levels(16-24µg/ml) in samples from the treated patient(n=3)(FVIII< 0.3%) were similar(difference 2-17%) between both methods. A positive linear correlation was observed between FVIII:C and emicizumab for both assays. In patients under emicizumab therapy, a large overestimation of FVIII was observed using the OSCA(FVIII:C 121.0-175.8%) compared to the CA with BIOPHEN™ reagent(FVIII:C 9.3-13.6%). CA with TriniCHROM reagent measured <1.6% FVIII and 2.8-3.5BE anti-FVIII as measured with OSCA before treatment.

Conclusions: The OSCA(C.K.PREST® reagent) modified with emicizumab calibrator is more accurate compared to CA(BIOPHEN™ reagent) for the quantification of emicizumab. Prior denaturation of FVIII is necessary in samples that contain native FVIII due to interference with both emicizumab assays. The CA(TriniCHROM reagent) is the preferred method for the quantification of FVIII:C and anti-FVIII in patients treated with emicizumab, explained by the use of bovine instead of human coagulation factors.

PB1152 | Determining the Optimal Method for FVIII:C and Anti-FVIII Quantification in a Patient Treated with Adynovi® who Developed Anti-FVIII during Treatment

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Background: Adynovi® contains rurioctocog alfa pegol, a novel recombinant factor VIII (rFVIII) indicated for the treatment of haemophilia A. Since Adynovi® structurally strongly differs from other rFVIII proteins, the optimal assay for measurement of Adynovi® must be determined.

Aims: In this study, we compared two one stage clotting assays (OSCA) with two chromogenic assays (CA) for the FVIII activity measurement in patients treated with Adynovi®. Secondly, we

investigated the Bethesda assay for the quantification of anti-FVIII against Adynovi®.

Methods: Adynovi® was diluted in FVIII deficient plasma with end concentrations of 2.5%, 5%, 10%,50%, 100% and 200%. FVIII: C was measured in the dilution series and samples of a patient treated with Adynovi® therapy (N=9). Four different methods were tested, including OSCA with C.K. PREST® and PTT-A reagents, and CA with TriniCHROM Factor VIII: C and BIOPHEN™ FVIII: C on STA-R EVO. In addition, anti-FVIII inhibitor against Adynovi® was measured in samples (N=2) of a patient chronically treated with Adynovi® who developed anti-FVIII (1.3-6.2BE). The classical Bethesda assay was applied using both 100% Unicalibrator and 100% Adynovi® as reference.

Results: For FVIII:C measured with OSCA, an acceptable recovery of -13 to 9% was observed for C.K. PREST and -11 to 8% for PTT-A reagent for Adynovi® concentrations 5-200%. In contrast, we observed a large positive deviation from the expected value with both CA (14-30% with TriniCHROM Factor VIII: C reagent and 28-63% with BIOPHEN™ FVIII: C reagent). In Adynovi® patient samples, we also report a large difference between OSCA and CA (10-150% for TriniCHROM Factor VIII: C and 22-110% for BIOPHEN™ FVIII: C reagent). For quantification of anti-FVIII, only a small difference of 0.3% was observed between the application of 100% unicalibrator and 100% Adynovi®as reference for the Bethesda assay.

Conclusions: The OSCA is the preferred method to monitor FVIII:C in patients treated with Adynovi®. The Bethesda assay yields comparable results for quantification of anti-FVIII inhibitor against Adynovi® using 100% Unicalibrator and 100% Adynovi® as reference.

PB1153 | SIG-001 for Hemophilia A: Preclinical Safety and Efficacy Studies Conducted in Mice and Non-Human Primates

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Background: Current therapies for Hemophilia A have significant limitations, including breakthrough bleeds and joint disease due to suboptimal adherence, non-ideal factor kinetics, and inhibitor generation, as well as risk of thrombotic events and coagulation test interference with newer non-factor therapies.

Cell therapies with genetically modified human cells are a possible alternative. To avoid cytotoxic immune response by the host, allogeneic cells either need to be physically shielded and/or the host immunosuppressed. Various biomaterials, while serving as the physical barrier from the host immune cells, activate a foreign body response (FBR), which significantly limits cell survival and durability of cell therapies. As previously described, SIG-001 is a 1.5 mm alginate sphere encapsulating hFVIII-expressing human cells. The cells are contained in an inner compartment that is surrounded by an outer layer of alginate conjugated to a proprietary compound selected to avoid FBR. SIG-001 can produce functionally active hFVIII in a dose-dependent manner, correct the bleeding phenotype in hemophilia A mice, and maintain viable cells in explanted spheres of NSG animals sacrificed at 6 months (Carmona ASH 2019).

Aims: To assess safety of SIG-001 in non-clinical setting.

Methods: Safety studies of SIG-001 spheres in mice and non-human primates (NHPs).

Results: Six months after IP placement into NSG mice, SIG-001 spheres remained intact, with viable cells, without safety or toxicological findings. We also conducted a broken sphere study in immunocompetent mice and none of the intentionally broken sphere components raised any safety concerns for up to one month post-administration. Finally, no toxicologically relevant effects were observed 6 months after empty spheres were placed laparoscopically into the peritoneal cavity of NHPs.

Conclusions: These safety studies, combined with the *in vivo* efficacy data reported previously, indicate that SIG-001 has a clear safety/toxicology profile and results in sustained, long-lasting FVIII levels. The first-in-human trial of SIG-001 is planned to open in Q2-2020.

PB1154 | Surgical Experience with Emicizumab: A Single Centre Experience with Central Venous Access Device Removal

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Background: Emicizumab is a humanised bi-specific monoclonal antibody functionally similar to factor VIII (FVIII) with demonstrated efficacy of haemostatic prophylaxis in boys with severe haemophilia A (SHA) and FVIII inhibitor. There is limited surgical data in patients on emicizumab and the treating-community are still establishing best practice guidance.

Aims: To describe single-centre experience with central venous access device (CVAD) removal in boys with SHA and inhibitors on treatment with emicizumab.

Methods: Twenty-three boys are currently on treatment with emicizumab at Great Ormond Street Hospital. We present a case series of ten boys who had CVAD removal while on prophylaxis with emicizumab.

Results: Nine boys who underwent surgical CVAD removal were on weekly emicizumab dosing and one on monthly dosing. All had received emicizumab and tranexamic acid (TXA) the day before surgery. The median age at procedure was 7years (range 2- 14years) and median time on emicizumab at the time of procedure was 39 weeks (range 8- 80 weeks). There was no recorded blood loss during any procedure and all patients were discharged the following day with a plan to continue TXA (15mg/kg/dose three-times daily) for 10 days. None of the patients required rescue by-passing agent treatment for bleeding. One patient was readmitted within 24 hours with swelling of the CVAD site, an ultrasound of the CVAD area revealed a haematoma (8cms diameter) which responded rapidly to re-starting

tranexamic acid. This patient had erroneously stopped TXA after discharge.

Conclusions: Surgical removal of CVAD was carried out with TXA cover only in 10 patients. One had a minor bleeding complication due to early cessation of TXA which responded to re-starting the medication. This cohort demonstrates that TXA given regularly at doses of 15mg/kg three-times daily to start before surgery and continued for 10 days afterwards is successful in securing haemostasis during CVAD removal in children on emicizumab.

PB1155 | Characterization of Factor IX-FIAV: A Factor IX Variant that Displays Cofactor-Independent Activity

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Background: Previously, factor (F)IX variants have been generated that function independently of the cofactor VIIIa (FIX-IDAV, FIX-FIAV) or demonstrate FIX hyperactivity (FIX-KLW) and ameliorate the hemophilia phenotype *in vitro/vivo* [Quade-Lyssy et al. J.Thromb. Haemost. 2014].

Aims: Assess the FVIIIa-independent FIX activity of variants comprising the IDAV/FIAV and KLW modifications and evaluate the FVIII-bypass efficacy and biochemical characteristics.

Methods: Recombinant FIX variants were stably expressed, and (partially) purified variants were evaluated for specific clotting activity, kinetic tissue factor-factor VIIa (TF-FVIIa) activation, and FVIII-bypass efficacy.

Results: Specific FVIII clotting activity analysis of partially purified FIX variants demonstrated a ~2-fold increase in FVIII-like activity for FIX-IDAV-KLW and a ~2-fold reduction for FIX-FIAV-KLW relative to FIX-IDAV/FIX-FIAV. This indicates no distinct synergistic effect of the combined modifications on FVIII-like activity. Conversely, the FIX-IDAV/FIAV-KLW variants demonstrated a 9-14-fold increase in specific FIX clotting activity relative to wild-type FIX(-WT), analogous to the hyperactivity of FIX-KLW. FIX-FIAV displayed a favorable profile (high FVIII-bypass and normal FIX activity) and was selected for further evaluation following purification. TF-FVIIa activation revealed kinetic parameters like FIX-WT, and FIX-FIAV exhibited significant FVIII-like clotting activity (56±4U/mg) compared to FIX-WT (≤13U/mg). Values correlate with ≤28% of FVIII-independent activity for FIX-FIAV at FIX plasma levels (5ug/mL), confirming FIX-FIAV may enhance thrombin generation in FVIII deficiency. TF-initiated (0.5 or 1.0pM) thrombin generation in FVIII-immunodepleted plasma led to a reduced thrombin peak (88% or 81% reduction) relative to 100% FVIII. Adding FIX-FIAV (5ug/mL) partially restored these parameters, with a ~30% increase in thrombin peak and endogenous thrombin potential. FVIII-independent activity of FIX-FIAV in severe hemophilia A (HA) patient plasma w/wo an inhibitor provided up to 18% or 32% FVIII-like activity, demonstrating efficacy in the presence of FVIII inhibitors.

Conclusions: FIX-FIAV has a mechanism of activation capable of sustaining therapeutic levels of coagulation activity in FVIII deficiency, supporting its potential as a treatment for HA.

PB1156 | Rescue of the Endogenous FVIII Expression in Hemophilia A Mice Using CRISPR/Cas9 Gene Editing

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Background: The current treatment for hemophilia A (HA) patients is FVIII protein replacement. However, it needs multi-dose injections which is costly and inconvenient. CRISPR/Cas9 is used to perform gene editing in vitro and in vivo. Correction of HA patients' nonsense mutation by gene editing can regain expression of missing protein permanently. Furthermore, advancement of nanoparticle (NP) technology can provide a biocompatible and gene delivery system. We investigate if NP technology can achieve targeted gene correction in a HA mouse model.

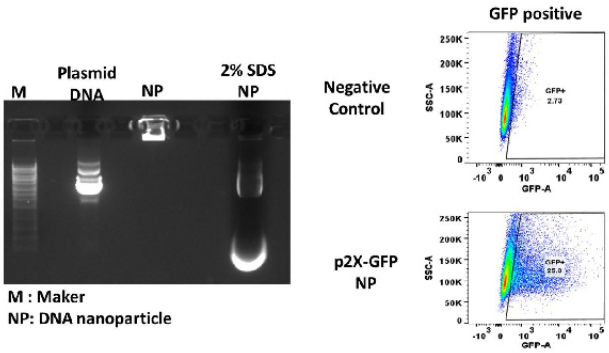

FIGURE 1 Transfection efficiency of NPs in HUVEC cells

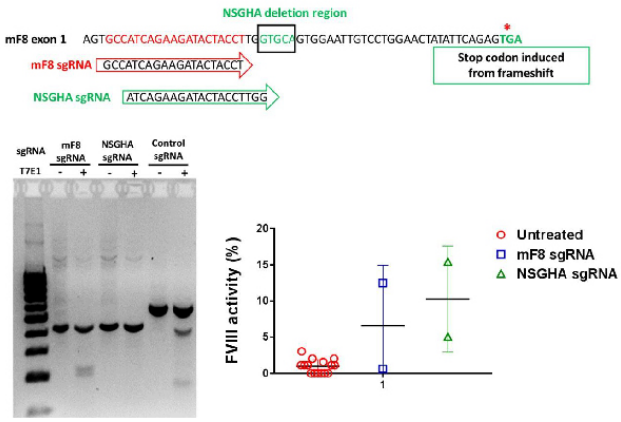

FIGURE 2 Regained FVIII activity in NSG HA mice after gene editing

Aims: To examine gene delivery efficiency of NP in vitro and regain FVIII function in HA mice using CRISPR/Cas9 in vivo.

Methods: NPs carrying p2X-GFP plasmid was examined for transfection efficiency in HUVEC cells by flow cytometry. Immunodeficient hemophilia A mice (NSG HA) with indel mutation in exon 1 of FVIII gene were used as an animal model. sgRNAs that target mouse FVIII gene (mF8sgRNA) or mutant FVIII gene in NSG HA mice (NSGHAsgRNA) were examined in vitro using T7E1 assay, respectively. Mice that were hydrodynamically injected with sgRNA and Ca9 protein expressing plasmids simultaneously. Blood was collected periodically to examine FVIII activity in plasma by aPTT assay.

Results: DNA electrophoresis showed that NPs can carry plasmid efficiently. Flow analysis suggested that NPs carrying p2X-GFP can efficiently transfect HUVEC cells compared to control. mF8sgRNA but not NSGHAsgRNA can specifically induce indel mutation in NIH3T3 cells. NSG HA mice challenged with hydrodynamic injection of mF8sgRNA or NSGHAsgRNA regained FVIII activity at day 7.

Conclusions: NPs can efficiently transfect endothelial cells as shown by GFP expression in HUVEC cells. Both mF8sgRNA and NSGHAsgRNA can induce indel mutation in frameshift site of NSG HA mice, leading to therapeutic levels of FVIII expression. Combining NP and gene editing technology has the potential to recover FVIII gene expression in HA patients and rescue them from daily infusion of recombinant proteins.

PB1157 | Design of a Novel Factor IX Albumin Fusion Protein with Enhanced Coagulant Activity and Pharmacokinetic Profile

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Background: Several approaches have been developed to prolong half-life of infused recombinant factor IX (FIX), such as genetic fusion with wild-type human albumin (HSA). However, to further widen the therapeutic window, rational engineering for improved binding to the neonatal Fc receptor (FcRn), which regulates HSA half-life, combined with the use of natural gain-of-function FIX variants, may result in products with more favourable features.

Aims: To develop a novel fusion protein with improved features conferred by the gain-of-function FIX-Padua variant and by an engineered HSA variant (QMP) with enhanced FcRn binding, and thus endowed with extended half-life.

Methods: FIX-Padua variant was fused to engineered albumin through an optimized cleavable linker. Wild-type (FIX-HSA) and improved (Padua-QMP) purified proteins were characterized for activity (chromogenic and aPTT-based assays), FcRn binding properties (SPR and ELISA-based assays) and *in vivo* plasma persistence in humanized transgenic mouse models.

Results: The hyperactive features of the FIX-Padua variant were completely preserved upon HSA fusion, with an 8-to-15-fold improved activity. The presence of the HSA QMP variant greatly enhanced FcRn binding of the engineered Padua-QMP fusion protein (K_d = 0.4 nM) in comparison with the wild-type (K_d = 200 nM). Noticeably, this translated into a more than 2-fold extended half-life of the Padua-QMP chimaera in human FcRn transgenic mice (2.5 days) compared to wild-type FIX-HSA (1.1 days), as well as to the commercially-available albutrepenonacog alfa (1.0 days) fusion proteins.

Conclusions: The combined improvements conferred by the FIX-Padua and QMP variants resulted in a novel engineered fusion protein with hyperactive features, enhanced FcRn binding and extended half-life in pre-clinical relevant human FcRn transgenic mouse models. This would translate into a widened therapeutic window and thus an amelioration of patients' quality of life.

PB1158 | Optimizing Outcomes in Hemophilia A Prophylaxis Using Recombinant Factor VIII Fc Fusion Protein (rFVIIIFc): Results from Three Portuguese Haemophilia Centers

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Background: In the last years, several extended half-life (EHL) products became available to prophylaxis in persons with Hemophilia A (pwHA) which increased *real-world* experience. EHL products might lead to higher trough levels without increasing infusion frequency or lead to reduce infusion frequency while maintaining through levels.

Aims: To evaluate outcomes after switching 35 pwHA from standard half-life (SHL) to EHL products from three Portuguese Haemophilia Centers, comparing bleeding rates (BR) and amount of FVIII used, in the same period of time before and after starting EHL.

Methods: Since august 2018, 35 males, mean age 23 yrs (range 2-66), all but one with severe HA, switched from different SHL products to rFVIIIFc. The mean period with ELH was 10.6 months (range 4-16) with 51% of patients with >12 months of observation. Comparisons were performed for BR, joint bleed rates (JBR) and amounts of clotting factor used, before and after switching. Half-lives (HL) of the SHL and EHL products (pharmacokinetics with WAPPS-Hemo platform) were also evaluated in twelve patients.

Results: 50% of patients (17) reduced injection frequency as it was aimed, two started prophylaxis with rFVIIIFc and in the others it was intended higher trough levels. The median BR was 2 (mean 3.9, range 0 - 22) with SHL products and 0 (mean 1.6; 0 - 15) with rFVIIIFc. The median JBR was 1 (mean 2.6; 0 - 13) with SHL products and 0 (mean 1.3; 0 - 14) with rFVIIIFc. The amount of FVIII decreased 18.7%: mean 177900 units/patient with SHL and 144714 units/patient with rFVIIIFc. The mean HL with EHL products (14.9 hrs, range: 8.75-22.5) increased 18.8% comparing with the mean HL with SHL products (12.1 hrs, range: 7.5-14.8).

Conclusions: Our data confirmed lower BRs and reduced injection frequency with increased HL and simultaneous reduction of the amount of FVIII administered.

PB1159 | Characterization of Neutralizing Anti-emicizumab Antibody Developed in a Hemophilia A Patient

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Background: A genetically engineered, humanized, bispecific monoclonal antibody, Emicizumab, mimicking the cofactor activity of activated Factor VIII, has been developed for treatment of Hemophilia A patients, with and without inhibitor. Among 398 patients enrolled in the Clinical Trials, 14 (3.5%) developed anti-drug antibodies (ADAs), 3 of which were classified with potential neutralizing activity. One of those patients, after 5 Exposure Days, developed a stable, high titre, neutralizing ADA, associated with loss of treatment efficacy. In this study we report the biochemical characterization of this antibody.

Aims: Biochemical characterization of the neutralizing anti-Emicizumab antibody developed in a Hemophilia A patient treated with Emicizumab.

Methods:

- Total and specific ADA purification and sequencing;
- Confirmation of ADA by Western blot;
- Binding between ADA and Emicizumab, the whole molecule and the papain digested fragments, by using Surface Plasmon Resonance (SPR) and Biolayer Interferometry (BI);

Results: The development of ADA was confirmed by Western blot on the total IgG fraction. The sequencing of specific ADA (recovery 1.3% of total IgGs content) showed an extreme polyclonality (in excess of 12 different CDR-H3 variant peptides). This was confirmed by SPR, which identified at least two populations of antibodies binding Emicizumab with different affinities (35nM and 5nM). Moreover, BI analysis carried out after papain digestion of Emicizumab, showed binding of ADA towards both Fab and Fc fragments.

Conclusions: The present study reports the biochemical characterization of the first neutralizing anti-Emicizumab antibody developed in a Hemophilia A patient with inhibitor.

Until now, about 6500 patients have been treated with Emicizumab with few data on the drug immunogenicity in clinical practice. Therefore, the incidence of the anti-Emicizumab antibodies is not clear, yet. A prospective cohort study of patients, treated with Emicizumab, will help to determine the frequency of the anti-Emicizumab antibodies, with and without neutralizing activity.

PB1160 | Raising Factor VIII's Half-life to the Next Level

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Background: The half-life of FVIII products determines not only injection frequency, but also FVIII trough level and is, therefore, a critical parameter for the treatment burden of hemophilia A patients.

Aims: The development of a novel recombinant FVIII with von-Willebrand-Factor-independent half-life prolongation while maintaining the characteristics of FVIII wild-type after its activation.

Methods: Albumin-binding domains (ABD) were incorporated into a single chain FVIII molecule construct. Human cells were transfected with several FVIII-ABD variants and supernatants were screened for FVIII expression and activity. The most promising FVIII-ABD variants were produced, purified, and investigated for von-Willebrand-Factor binding potency as well as for thrombin-mediated activation. Half-life was assessed by pharmacokinetic studies in hemophilia A mice and in albumin-deficient mice which express the human instead of the murine neonatal Fc receptor. The *in vivo* functionality was investigated in hemophilia A mice using a tail transection assay determining bleeding time and blood loss after a standardized cut.

Results: The screening of various FVIII-ABD fusion molecules demonstrated good expression and *in vitro* functionality of several candidates. However, only a few variants extended the *in vivo* half-life of FVIII significantly. An inverse correlation between half-life extension and binding of FVIII-ABD to von-Willebrand factor was found, indicating the half-life limiting effect of the latter. Finally, a FVIII-ABD variant was selected that exhibited an up to 4-fold longer half-life compared to Moroctocog alfa while retaining full *in vivo* functionality in terms of efficient stop of bleeding.

Conclusions: A next generation recombinant FVIII molecule with four albumin-binding domains was generated resulting in a half-life extension of up to 4-fold while preserving the wild type characteristics of activated FVIII with full *in vivo* functionality.

PB1161 | Emicizumab, beyond Annualized Bleeding Rates: Real World Joint Health and Physical Activity Data

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University of Colorado School of Medicine, Aurora, United States

Background: Emicizumab, a humanized bispecific antibody factor VIII mimetic, has improved convenience for hemophilia A prophylaxis with decreased annualized bleeding rates (ABR). However, the functional impact of emicizumab on joint outcomes has been less explored.

Aims: To explore joint physical exam outcomes and physical activity in a cohort of persons with hemophilia A (PwHA) on emicizumab.

Methods: Data regarding age, inhibitor status, prior treatment, physical activity, ABR, and Hemophilia Joint Health Scores (HJHS) on PwHA on emicizumab for >6 months were extracted from a consented, single-center cohort study (HemoPICS). Joint ABR (J-ABR) and HJHS progression (annualized change in 6-joint average scores) for each participant were compared before and after starting emicizumab using a related-samples Wilcoxon Signed Rank test.

Results: Data from 58 PwHA, with baseline FVIII levels < 1% to 6%, were analyzed. Median age of emicizumab start was 12.8 (range 0.6-79.8) years. Median time on emicizumab was 10.9 (range 6.2-44.2) months. Twelve school-aged children participated in 1 or more sports, including soccer, baseball, tennis, cross-country track, swimming, and alpine skiing, without regular FVIII prophylaxis. At emicizumab start, 9 participants had active inhibitors, and 49 did not, including 39 (80%) on FVIII prophylaxis. J-ABR decreased in all patients (p=0.03), including in patients without an inhibitor (80% previously on FVIII prophylaxis) (Figure 1) HJHS progression slowed in the overall group (p=0.03), but this was not significant in subgroup analyses (Figure 2). Surprisingly, neither pre-emicizumab J-ABR nor rate of HJHS progression predicted subsequent outcomes.

Conclusions: Following adoption of emicizumab by PwHA, HJHS and bleeding rates were stable or improved, including in those previously on full prophylaxis with FVIII, and including a few school-aged children who participated in sports. Emicizumab may improve bleeding and progression of arthropathy in PwHA with active inhibitors, those using episodic therapy and also in those on FVIII prophylaxis.

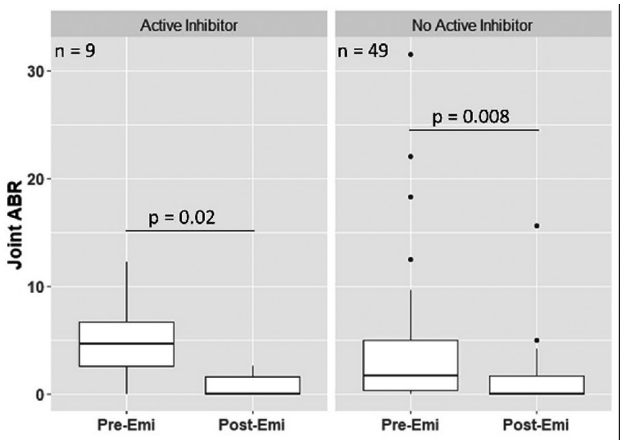

FIGURE 1 J-ABR before and after emicizumab (Emi)

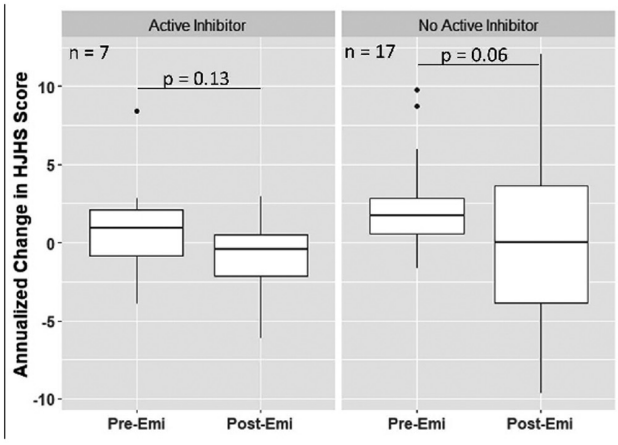

FIGURE 2 Annualized change in average HJHS before and after emicizumab (Emi)

PB1162 | Marzeptacog Alfa (Activated) Population PK: Simulations for Dose Selection in Phase 3 Trials

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Background: Marzeptacog alfa (activated) (MarzAA), is a variant human rFVIIa in clinical development for subcutaneous (SQ) administration to treat episodic bleeding or prophylaxis for haemophilia A

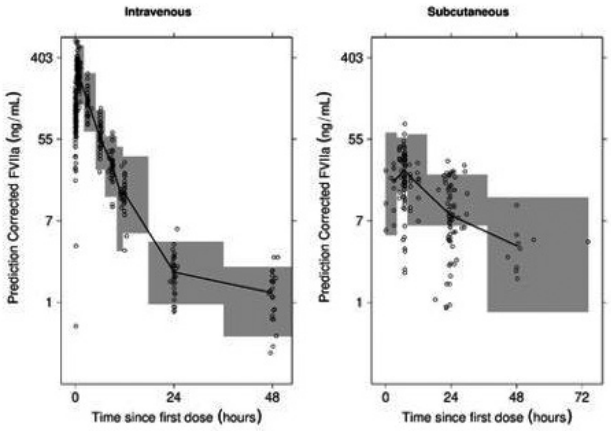

FIGURE 1 Visual predictive check for FVIIa concentrations (all doses). Solid line represents the median. Shaded area: 95%CI based on 1000 simulations

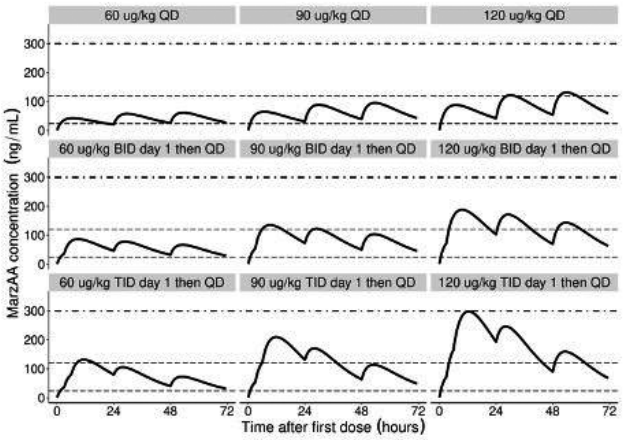

FIGURE 2 Clinical trial simulations in a 70 kg adult using 3 different MarzAA treatment regimens: target range and max desirable level indicated

(HA) or B (HB) with or without inhibitors, and other inherited bleeding disorders.

Aims: To characterize the population pharmacokinetics (PopPK) of intravenous (IV) and SQ MarzAA to support dose selection for Phase 3 trials using simulations of varying dose regimens.

Methods: MarzAA exposure data (FVIIa activity) was evaluated after single IV doses (4.5, 9, 18 and 30 ug/kg) (NCT01439971) and single IV (18 ug/kg) and SQ doses (30 and 60 ug/kg) (NCT03407651). Using nonlinear mixed-effects modeling, data was sequentially modeled describing both IV and SQ data, including nonlinearities and allometric scaling. Model discrimination was based on parameter uncertainty, plausibility and changes in the objective function value (ΔOFV=-3.84, $p < 0.05$, one degree of freedom). Simulations were conducted for a 70 kg adult subject.

Results: The final PopPK model described the clinical data well (Figure 1). Subcutaneous bioavailability was estimated at 33%, with inter-individual variability of 76%. The terminal elimination half-life for IV dosing was estimated at 3.3 hours, and the absorption and elimination SQ half-lives were both estimated to be 11.8 hours, indicating flip-flop kinetics. Simulations indicated that daily SQ dosing, as well as twice and thrice in 24 hours (0, 3 and 6 hours) at 60, 90 and 120 ug/kg achieved exposures within or above the target range. Using a 60 ug/kg dose, MarzAA may be administered SQ thrice in 24 hours without significantly exceeding the defined target range (Figure 2).

Conclusions: A PopPK model was developed for clinical trial simulations for SC MarzAA to guide dosing in Phase 3 trials and suggests target levels for haemostasis may be achieved with the 60 ug/kg SC dose with 1 to 3 doses in 24 hours.

PB1164 | Emicizumab beyond Annualized Bleeding Rate: Do Laboratory Assays Help Predict Emicizumab Effects?

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Background: Emicizumab, a humanized bispecific antibody that activates factor X in the absence of factor VIII (FVIII), has improved convenience for hemophilia A prophylaxis and decreased annualized bleeding rates (ABR); however, the use of laboratory assays to confirm hemostatic efficacy and predict outcomes has not been explored.

Aims: To measure factor Xa and thrombin generation and compare them to hemostatic efficacy of emicizumab.

Methods: Data regarding age, inhibitor status, ABR, and Hemophilia Joint Health Scores (HJHS) were extracted from a consented cohort study (HemoPICS) of persons with hemophilia A (PwHA) on emicizumab. Samples from the cohort biobank were used in a chromogenic FVIII assay (Human BIOPEN FVIIIC Hyphen, BioMed) and the Calibrated Automated Thrombogram (CAT, Stago); both assays were performed according to manufacturers' instructions. Age-stratified reference ranges were derived on 46 healthy adults and 17 children. Pre-emicizumab samples were from time of FVIII trough. Data were analyzed using correlations, related samples Wilcoxon signed rank tests (pre-to post-emicizumab), and Mann-Whitney U tests (+ vs - inhibitor on emicizumab).

Results: Data from 33 PwHA were analyzed. At emicizumab start, 7 participants had active inhibitors, 26 did not. Figure 1 shows that the CAT endogenous thrombin potential (ETP, area under the curve), thrombin peak and chromogenic FVIII all increased significantly and similarly in both inhibitor positive and negative PwHA on emicizumab. Laboratory results did not correlate with outcomes except for non-significant trends ($p = 0.05$-0.1) between CAT peak thrombin and ABR. The lack of correlation between any assay parameter

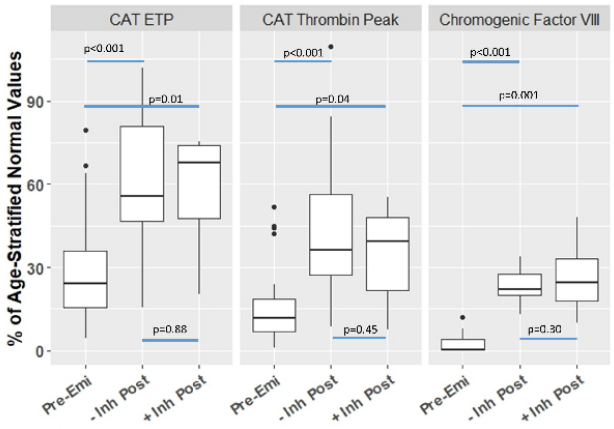

FIGURE 1 Endogenous thrombin potential, thrombin peak and chromogenic FVIII with human reagents in PwHA on Emicizumab

and ABR or HJHS is not unexpected given the similar laboratory responses, low bleeding rate, small sample size and short duration of observation.

Conclusions: Following adoption of emicizumab, samples from PwHA showed a significant increase in CAT ETP, CAT thrombin peak and chromogenic FVIII. More data are needed to determine if these assays correlate with outcomes.

PB1165 | Emicizumab beyond Annualized Bleeding Rate: How Does Emicizumab Affect Bone Biomarkers?

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[1]*University of Colorado School of Medicine, Aurora, United States;*
[2]*Oregon Health and Science University, Portland, United States*

Background: Emicizumab, a humanized bispecific antibody that activates factor X without factor VIII, has improved convenience for hemophilia A prophylaxis and decreased bleeding. Elevated osteoclastic activity and depressed osteoblastic activity have been reported in persons with hemophilia A (PwHA). The relationship of emicizumab to bone biomarkers has not been explored.

Aims: To measure the osteoclastic biomarker carboxy terminal telopeptide of collagen type 1 (CTX1) and osteoblastic osteoprotegrin (OPG) in PwHA on emicizumab in comparison to baseline results prior to emicizumab, and to controls.

Methods: Biobank samples from a consented single-center cohort study (HemoPICS) of PwHA on emicizumab were assayed by ELISA for CTX1 and OPG (R&D Systems). Comparisons of pre and post emicizumab values to age-matched controls were analyzed using independent samples t-tests. Intra-group relationships were evaluated using Pearson's correlations. Pre-emicizumab samples were from time of FVIII trough.

Results: Normal reference ranges for CTX1 and OPG were derived from 30 healthy adults and 32 children. In controls, both CTX1 and OPG were significantly lower in adults than children (p< 0.001), and OPG correlated negatively with age (r=-0.38, p=0.003). Figure 1 shows CTX1 and OPG levels in 33 PwHA prior to and on emicizumab in comparison to controls. In adults, CTX1, previously reported to be elevated in adult PwHA, was higher than controls both before and on emicizumab. In pediatric PwHA, CTX1 did not differ from controls before or on emicizumab, but OPG was lower (p=0.01). In PwHA, OPG trended towards increasing with age regardless of treatment (r=0.32, p=0.07).

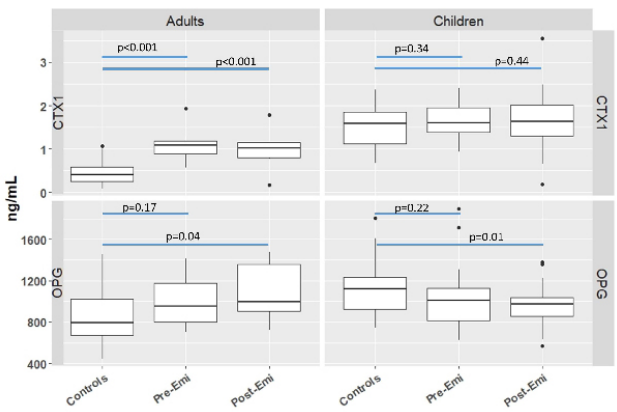

FIGURE 1 Bone biomarkers CTX1 and OPC in PwHA using Emicizumab

Conclusions: Adult PwHA showed elevated osteoclastic CTX1 both before and on emicizumab compared to controls. OPG decreased significantly with age in controls, but seemed to increase with age in PwHA. The clinical significance of these findings is unknown and requires longer observation in a larger cohort of PwHA on prophylaxis with FVIII or emicizumab.

PB1166 | A Simple Algorithm to Identify Emicizumab Interference in Samples Received in the Clinical Coagulation Laboratory

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Background: Laboratories, including our institution, often do not receive information regarding the presence of therapeutic drugs in patient blood samples. Emicizumab, a bispecific monoclonal antibody that bridges factor IXa and factor X, has unique effects on standard coagulation screening assays, causing potential confusion when interpreting coagulation work-ups leading to possible misdiagnosis. To provide guidance to clinical laboratories an algorithm was developed to identify potential Emicizumab interference.

Aims: To develop a simple algorithm to provide guidance to clinical laboratories assisting in the identification of clinical samples containing Emicizumab.

Methods: An algorithm was developed, and tested on factor VIII inhibitor panels specifically ordered for patients on Emicizumab. These inhibitor panels consisted of an aPTT and one-stage factor VIII activity assay (both Siemens Dade® Actin® FSL), a bovine-based chromogenic factor VIII activity assay (Chromogenix Coatest® SP4), and a bovine-based chromogenic inhibitor titer (Siemens FVIII Chromogenic Assay). All testing was performed on the Siemens BCS®XP. A total of 167 factor VIII inhibitor panels, resulted over a 12-month period, were reviewed.

Results: Of 167 test panels reviewed, aPTT results below the reference interval were obtained in 153 of the panels (92%) with 151 panels (90%) demonstrating one-stage factor VIII activity values above 200% (range: 75% to 945%).

Conclusions: In our institution, the identification of possible Emicizumab interference aids in the work-up of abnormal screening assays, particularly aPTT and one-stage factor VIII activity assays. Samples containing Emicizumab typically show a shortened aPTT,

an elevated one-stage factor VIII activity, and low bovine-based chromogenic FVIII activity. Although the pattern of elevated one-stage factor VIII and shortened aPTT may be occasionally seen with high native factor VIII levels, bovine chromogenic factor VIII activity will distinguish native factor VIII from Emicizumab. It is critical for technicians to consider Emicizumab when evaluating screening coagulation tests in order to appropriately interpret results and guide additional work-up.

RARE BLEEDING DISORDERS

PB1167 | Rare Bleeding Disorders in Southern Iran: Revisited and Updated Data

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Background: Rare bleeding disorders (RBDs) are defined as all hereditary coagulation disorders except Hemophilia A and B and Von Willebrand disease. In Iran, RBDs are more prevalent due to high rate of consanguineous marriage.

TABLE 1 General characteristics of the study population

	Number	Percent
Age category (years)		
0-5	18	10.9
6-18	53	32.1
19-40	76	46.1
>40	18	10.9
Sex		
Female	91	55.2
Male	74	44.8
Sign and symptoms		
Epistaxis	34	20.6
Ecchymosis/hematoma	27	16.4
Post-operation bleeding	17	10.3
Asymptomatic (screening)	15	9.1
Gum bleeding	10	6.1
Intracranial hemorrhage	8	4.8
Umbilical cord bleeding/cephalhematoma	7	4.2
Menorrhagia	7	4.2
Gastrointestinal bleeding	6	3.6
Joint bleeding	3	1.8
Hematuria	2	1.2
Others*	29	17.7
Plasma factor level**		
<1%	54	32.7
1-5%	34	20.6
>5%	66	40
Treatment protocol		
On-demand	130	78.8
Prophylaxis	35	21.2

*Others include bleeding after trauma, dental surgery or surgical biopsy or detected with pre-operation testing

**Excluding Cases with afibrinogenemia (one patient) and hypofibrinogenemia (10 patients)

TABLE 2 Characteristics of different types of rare bleeding disorders

Factor deficiency	Frequency (%)	Sex (female) Number (%)	The most prevalent symptom (%)	Prophylaxis number (%)
FVII deficiency	44 (26.7)	25 (56.8)	Epistaxis (34.1)	9 (20.5)
FX deficiency	26 (15.8)	16 (61.5)	Ecchymosis/ hematoma (19.2)	3 (11.5)
FXI deficiency	23(13.9)	12 (52.2)	Miscellaneous* (30.4)	0
FV deficiency	19 (11.5)	8 (42.1)	Miscellaneous (36.8)	0
FXIII deficiency	21 (12.7)	11 (52.4)	Umbilical cord bleeding (23.8)	20 (95.2)
Combined FV&FVIII deficiency	12 (7.3)	8 (66.7)	Epistaxis (41.7)	0
FI deficiency	11 (6.7)	7 (63.6)	Miscellaneous (45.5)	0
FII deficiency	2 (1.2)	1 (50)	Gastrointestinal bleeding (50) Cephalhematoma (50)	2 (100)
Combined FVIII & FXI deficiency	1 (0.6)	0	Epistaxis (100)	0
Combined FVII & FX deficiency	1 (0.6)	1 (100)	Epistaxis (100)	0
VKCFD**	3 (1.8)	1 (33.3)	Ecchymosis/hematoma (33.3) Gastrointestinal bleeding (33.3) Hematuria (33.3)	1 (33.3)
Total	163 (100)		Epistaxis (20.6)	35 (21.2)

*Bleeding after trauma and dental procedures, pre-operation testing

**Hereditary combined vitamin K dependent clotting factors deficiency

Aims: We aimed to update the frequency of different types of RBDs and their clinical characteristics and therapeutic aspects in Southern Iran until end of 2019.

Methods: In this cross-sectional study, 163 patients who have been registered as RBDs at Shiraz hemophilia comprehensive treatment center were evaluated. A designed questionnaire adapted from www.rbdd.org/pro-rbdd was used to gather required information.

Results: Mean age of patients was 22.9 ± 15.2 years (range: 1-75 years) and 55.2% of them were female. Most patients were in the age category of 19-40 years (Table 1). Distribution of different types of RBDs and their clinical characteristics are demonstrated in Table 2. The most frequent RBD was FVII deficiency (44 patients, 26.7%) and the most common presenting symptom was epistaxis (20.6%). Overall, most of the patients (40%) had mild factor deficiency (plasma factor concentrate >5%). Prophylaxis was given to 21.2% of all patients with satisfactory outcome.

Conclusions: RBDs are a heterogeneous group of disorders with a wide range of clinical manifestation. In contrary to hemophilia, the rate and severity of bleeding may not correlate well with plasma factor level in some types of RBDs (1). Since 2007, the frequency of patients with RBDs in Shiraz Hemophilia Center, has more than doubled (2). It may be explained by the high rate of consanguineous marriage in this region as well as higher detection rate. Prophylactic replacement therapy in a subgroup of patients especially FVII and FXIII deficiencies give rise to less bleeding episodes and higher quality of life (3).

PB1168 | Isolated Factor VII Deficiency

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Background: Rare coagulation factor deficiencies in constitutional bleeding disorders are quantitative and/or qualitative abnormalities in one or more of these factors. Constitutional factor VII deficiency is a rare inherited disorder with autosomal recessive transmission and is associated with a variable hemorrhagic syndrome.
Aims: This is a study of an isolated deficiency of factor VII found in 13 cases.
Methods: Patients are of different age groups, presenting a hemorrhagic symptomatology with low PT. The diagnosis was guided by the first-line coagulation test: prothrombin time and activated partial thromboplastin time (APTT). Factor VII deficiencies are supplemented by the prothrombin complex assay to confirm the isolated trait. These tests are carried out by automated chronometric method, scrupulously respecting the pre-analytical phase, and the disturbance is confirmed on a second sample.
Results: During this study, 13 patients presented with factor VII deficiency, in whom an exploration of hemostasis was performed as part of a preoperative check-up, or following a haemorrhagic syndrome. The average age of our patients was 21 years. The problem sex ratio of male to female was 1.6, with a factor VII level of less than 40% in 06 patients and more than 40% in 07 patients.
Conclusions: Clinical expression is highly variable and the severity of hemorrhagic syndrome is not correlated with residual FVII activity levels. Finally, there are many subjects who are totally asymptomatic despite a very low FVII rate.

PB1169 | Results from a Phase 4, Real-World Study Confirm the Safety and Effectiveness of rFXIII-A2 for the Treatment of Congenital FXIII A-Subunit Deficiency

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TABLE 1 AEs during mentor™6

Table 2 Treatment-requiring bleeds (all were traumatic) during mentor™6.

Patient	Age (years)	Sex (F/M)	Type and cause of bleed	Treated with	Dose (IU/kg)	Time since last prophylaxis dose	Haemostatic response
Patient 1	13	F	Traumatic (slipped on ice face first; no loss of consciousness, no vomiting, headache or dizziness)	rFXIII-A_2	53.28	7 days	Excellent
Patient 2	21	M	Traumatic (trauma of left knee)	rFXIII-A_2	36.02	10 days	Good
Patient 3	12	F	Traumatic (distortion)	rFXIII-A_2	36.27	16 days	Excellent
			Traumatic (fell directly on the knee)	rFXIII-A_2	37.64	23 days	Excellent
Patient 4	2	M	Traumatic (fell on to abdomen and developed haematoma to penile shaft)	rFXIII-A_2	41.65	18 days	Excellent
Patient 5	68	M	Traumatic (fell and hit leg)	Fibrogammin	13.30	36 days	Good

F, female; M, male; rFXIII-A_2, recombinant factor XIII-A_2.

TABLE 2 Treatment-requiring bleeds (all were traumatic) during mentor™6

Table 1 AEs during mentor™6.

Summary	Children <18 years (n=13) E	Adults 18–65 years (n=15) E	Elderly >65 years (n=2) E	Total (N=30) E
All AEs	16	23	5	**44**
Serious AEs	2	7	1	**10**
AEs by severity				
Mild	12	16	2	**30**
Moderate	4	6	3	**13**
Severe	–	1	–	**1**
AEs by relationship				
Probably or possibly related	3	5	3	**11**
Unlikely	13	18	2	**33**
MESIs	3	1	–	**4**

AE, adverse event; E, event; MESI, medical event of special interest; N/n, number of patients.

Background: Recombinant factor XIII-A_2 (rFXIII-A_2) is indicated for prophylaxis in patients with congenital FXIII A-subunit deficiency (FXIII-A CD). Real-world evidence on usage of rFXIII-A_2 is lacking.
Aims: Investigate the long-term, real-world rFXIII-A_2 use on effectiveness and adverse drug reactions from mentor™6 (a prospective, multinational, non-interventional, post-authorisation safety study), and effectiveness and safety outcomes from patients in the Prospective Rare Bleeding Disorders Database (PRO-RBDD) registry.
Methods: Both mentor™6 and PRO-RBDD prospectively assessed the following endpoints: annualised bleeding rate (ABR), efficacy for minor surgery and bleed treatment, serious adverse events (SAEs), medical events of special interest (MESIs), and specific adverse drug reactions (anti-FXIII antibodies, allergic reactions, embolic/thrombotic events and lack of therapeutic effect).
Results: mentor™6 enrolled 30 patients (Table 1, mean [range] age: 25.5 [2-68] years), total exposure time: 75.4 person-years.
No spontaneous treatment-requiring bleeds occurred during mentor™6. Six traumatic bleeds required treatment with a FXIII-containing product, thus the mean ABR was 0.066 bleeds/year; 5/6 treatment-requiring bleeds were treated with rFXIII-A_2 and all had a successful outcome (Table 2). Of nine minor surgeries performed during rFXIII-A_2 prophylaxis, eight reported successful haemostatic outcomes (one missing evaluation).
No neutralising anti-FXIII antibodies (inhibitors), allergic reactions, withdrawals due to AEs, or embolic/thrombotic events with causal

relation to rFXIII-A_2 were reported. Eighteen patients reported 44 AEs; 30 were mild, 13 moderate and one severe. Eleven AEs were possibly/probably related to rFXIII-A_2. Ten AEs were considered SAEs, all were unlikely related to rFXIII-A_2; of four MESIs, two were possibly/probably related to rFXIII-A_2 (non-neutralising [binding] antibodies and suspected lack of efficacy). All patients with related AEs, MESIs or SAEs recovered.

PRO-RBDD included four patients exposed for ≈9.5 person-years. No AEs, or treatment-requiring or spontaneous bleeds were reported.

Conclusions: These real-world, long-term data confirm the safety and effectiveness of rFXIII-A_2 for prophylaxis, treatment of bleeding episodes and minor surgeries in patients with FXIII-A CD.

PB1170 | Economic Burden Associated with Vaso-Occlusive Crisis Management in Patients with Sickle Cell Disease

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[2]*Analysis Group, Inc., Boston, United States*

Background: Vaso-occlusive crises (VOCs) are common complications of sickle cell disease (SCD). Limited information exists in the literature on the economic burden of VOC management, especially as it pertains to inpatient VOC management.

Aims: To estimate US healthcare resource utilization associated with VOC management.

Methods: Retrospective database analysis was conducted using the IBM Watson Health MarketScan® Commercial Claims and Encounters database utilizing data over a 5-year period (Q3 2013-Q2 2018). Patients included had ≥2 diagnoses of SCD on different dates and ≥1 VOC diagnosis at age ≥6 years (both defined by ICD-9 or ICD-10 codes). Descriptive statistics were conducted for VOC-level demographic (age, sex) and clinical (Charlson comorbidity index [CCI] and VOC-related comorbidities) characteristics and main study outcomes (duration of inpatient VOCs and VOC-related healthcare costs by clinical setting adjusted to 2018 US dollars).

Results: A total of 69,662 VOCs, contributed by 8,045 SCD patients, were analyzed. Mean age ± standard deviation (SD) at VOC was 31.3 (±14.2) years, 78.4% of VOCs occurred in patients aged ≥14-50 years, and 58.9% of VOCs occurred in females. A majority of the VOCs (77.7%) were managed in the outpatient setting. Mean (±SD) CCI was 0.2 (±0.7), with chronic pulmonary disease, renal disease, and diabetes the most common comorbidities. Overall, mean (±SD) inpatient VOC duration was 6.7 (±6.0) days with most inpatient VOCs (60.4%) having a duration of ≥5 days. Mean healthcare cost (±SD) per VOC was $1,661 (±$11,164) and $22,044 (±$45,115) for outpatient and inpatient settings, respectively.

Conclusions: Over 20% of VOCs were managed in the inpatient setting, at a considerably higher mean cost (≈13x) than the cost of outpatient VOCs. Treatments for VOCs that reduce the duration of VOC-related inpatient stays or shift management to the outpatient setting would be expected to reduce the cost burden associated with VOC management.

PB1171 | Clinical Characteristics of Patients with Rare Bleeding Disorders: Real-Life Data from the Netherlands (RBiN Study)

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Background: Patients with rare bleeding disorders (RBD) present with diverse hemorrhagic clinical symptoms and varying frequencies of bleeding episodes. The lack of a distinct correlation between factor level activity and bleeding severity, hampers diagnosis and targeted treatment.

Aims: Study aim was to determine clinical phenotype, to compare ISTH BAT with RBD BAT (Palla et al., 2016) and to validate previously determined (Peyvandi et al., 2012) threshold factor levels for bleeding severity.

Methods: The RBiN study reports results from a nation-wide cross-sectional multicenter study of patients with a known RBD including

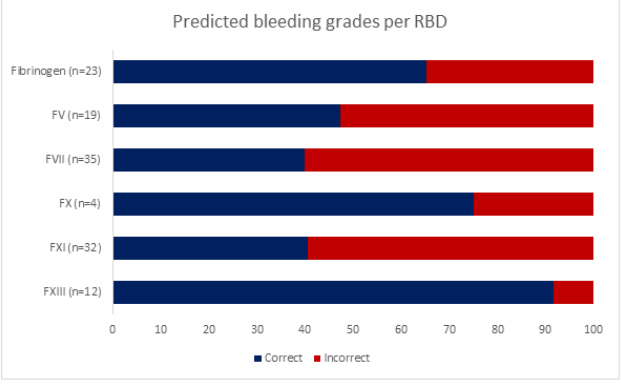

FIGURE 1 Predicted bleeding grade based on earlier RBD registry study (EN-RBD,Peyvandi et al. 2012) per RBD shown as either correctly or incorrectly predicted

rare coagulation factor deficiencies and disorders of fibrinolysis, from six Dutch Haemophilia Treatment Centers, aged 1-99 years. After written informed consent, bleeding assessment tools (BAT) were administered and clinical data extracted from patient files.

Results: Two-hundred sixty-three patients were included, of whom 197 attended a planned study visit. Most patients suffered from provoked bleeding: bleeding after dental procedures (66%), surgery (76.4%), menorrhagia (80.2%) and postpartum hemorrhage (51.2%). The median ISTH BAT score was 10. The sensitivity of the RBD BAT was better compared to the ISTH BAT (85% versus 81%, $p< 0.05$), especially for women and children. Correlations between baseline factor levels and ISTH BAT scores were strong for factor (F) II deficiency (r=-0,792) and FX deficiency (r=-0,838) and moderate for deficiencies of fibrinogen (r=-0,683), FV (r=-0,623), FVII (r=-0,516),FXIII (r=-0,516) and α2-antiplasmin (r=-0,594). There was no correlation for FXI deficiency. Forty-eight percent of patients had more severe bleeding than predicted on the basis of baseline factor activity level. Thirty-four percent of patients were assumed to be asymptomatic but experienced grade II or grade III bleeding.

Conclusions: The RBD score may be a better diagnostic tool than the ISTH BAT for RBDs. Threshold factor levels for RBD patients to remain free from bleeding are often inaccurate and should be used with caution.

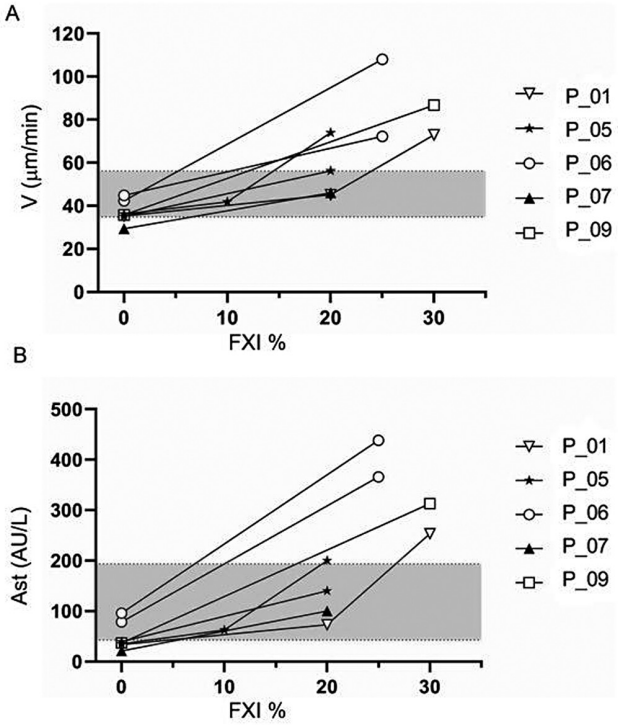

FIGURE 1 Rate of clot growth (V) and amplitude of thrombin peak (Ast) at baseline and after infusion with Hemoleven®. Shaded zone = normal values

PB1172 | Usefulness of Tissue Factor-Independent Coagulation Parameters for Monitoring FXI-Concentrate Replacement in Patients with FXI-Deficiency

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Background: In FXI-deficiency, bleeding is unpredictable and poorly correlated to FXI plasmatic levels as measured by aPTT-based clotting assays. Current treatment options for patients with FXI-deficiency include the use of commercial FXI-concentrates, which are associated with an increased thrombotic risk. In absence of a laboratory tool able to predict the bleeding risk in FXI-deficiency, clinical management of these patients remains empiric and carries the risk of under- or over-treatment. Assays able to monitor the hemostatic potential of FXI-replacement therapy would have a high clinical value.

Aims: We evaluated the utility of the recently marketed global coagulation assay Thrombodynamics [(TD), Hemacore, Moscow, Russia] for monitoring the hemostatic potential of FXI-deficient patients after FXI-replacement therapy with a commercial FXI-concentrate (Hemoleven®, LFB-Biomedicaments, Les Ulis, France).

Methods: TD analyzes the spatio-temporal propagation of tissue factor (TF) dependent and independent coagulation. We first established reference ranges for thrombin generation (TG) and fibrin clot formation (FCF) parameters analyzing plasma of 53 healthy controls. Subsequently, we investigated the effect on coagulation parameters of increasing doses of Hemoleven® spiked to FXI-deficient plasma. Finally, we measured the hemostatic potential at baseline and after FXI-replacement in five patients treated with Hemoleven® (1000 IU) prior to a planned intervention (n=7).

Results: During the TF-independent phase of coagulation, TG and FCF increased dose dependently after in vitro FXI spiking. Similarly, TG and FCF were strongly increased in patients receiving Hemoleven® already at FXI-concentrations of 20% and shifted towards hypercoagulation at FXI-levels of about 30% (see figure below).

Conclusions: TD appears to be useful for assessing the hemostatic potential of patients after administration of Hemoleven® and for monitoring replacement therapy, thus representing the first laboratory tool allowing patient-tailored management in FXI-deficiency. Our study supports the use of very low doses of Hemoleven®, aiming to FXI plasma levels ≤20%, which is lower than currently recommended (30-40%).

PB1173 | Fibrin Clot Properties in Patients with Qualitative Fibrinogen Disorders: Data from the PRO-RBDD Cohort

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Background: The clinical phenotype of patients with hereditary dysfibrinogenemia (HD) and hypodysfibrinogenemia (HHD) is heterogeneous, from mild bleeding to thrombosis, including absence of symptoms. Excluding a few fibrinogen variants, the clinical course is often unpredictable

Aims: To assess if fibrin clot properties are correlated with the clinical phenotype and/or genotype.

Methods: *Ex vivo* plasma fibrin clot properties assessed by turbidity assays, clot permeability and dynamic fibrin clot growth (thrombodynamics) were investigated in 51 patients compared to normal pooled plasma. Blood was drawn at inclusion of patients in the PRO-RBDD registry and plasma aliquots frozen at -80° until analyses

Results: Overall, 51 patients were prospectively included (Table 1). Close to half of patients were carriers of a hotspot mutation (*FGA* Arg35Cys/His or *FGG* Arg301Cys/His, 48.9%). Compared to normal pooled plasma, patients were characterized by statistically significant slower fibrin polymerization (lag time 565.8±254.1 vs 180±58.15 sec), decreased clot density (maximal absorption 0.19±0.06 vs 0.37±0.01 optical density), prolonged fibrinolysis (clot lysis time 40.09±31.58 vs 16±1.59 min), defective dynamic fibrin clot growth (rate 32.91±7.97 vs 61.48±7.64 Um/min and size 1229±170.5 vs 1525±199 at 30 min) but similar clot pore size ($4.55x10^{-9}$±$3.41x10^{-9}$ vs $4.58x10^{-9}$±$2.76x10^{-9}$), without correlation with antigen fibrinogen levels. Among all biological parameters, only the permeability was able to distinguish the clinical phenotypes at presentation; it was statistically increased in patients with a bleeding phenotype compared to asymptomatic (p=0.0001) or thrombotic patients (p=0.01). Fibrin clot properties were similar among hotspot mutations

Conclusions: Patients with a bleeding tendency have an increased permeability. Follow-up studies will help to determine if this parameter is able to predict a bleeding phenotype and thus guide patient management

TABLE 1 Patients' genetic data and clinical data at inclusion

Dysfibrinogenemia, n (%)	43 (84.3)
Hypodysfibrinogenemia, n (%)	8 (15.7)
Mutations in FGA, n (%)	17 (33.4)
Mutations in FGB, n (%)	3 (5.9)
Mutations in FGG, n (%)	27 (52.9)
Not available, n (%)	4 (7.8)
Asymptomatic, n (%)	25 (49)
Bleeding, n (%)	19 (37.3)
Thrombosis, n (%)	2 (3.9)
Both, n (%)	5 (9.8)

PB1174 | Inherited Rare Bleeding Disorders in Non-Consanguinity

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Background: Rare bleeding disorders (RBD) inherited in an autosomal recessive manner create difficulties in diagnosis and management.

Aims: The study aimed to report 39 patients with RBD using retrospective analysis.

Methods: The study included 39 patients (male 18, female 21) with inherited coagulation factor deficiencies of FVII (n=24), FV (n=4), FX (n=2), fibrinogen (n=2) and one each of FII, FV & VIII and combined deficiency of vitamin-dependent coagulation factors, and type 3 von Willebrand's disease (n=4). Consanguinity was not found among 35 parents. The median age of first bleed was 14 days (IQR 2 days to 10 months) and the presumptive diagnosis was made by screening coagulogram.

Results: All patients initially received fresh frozen plasma (FFP) or cryoprecipitate to control episodic bleeding. Upon the definite diagnosis, a combination of FFP, cryoprecipitate, factor VIII concentrate, prothrombin complex concentrate and recombinant activate factor VII was used accordingly. However, factor concentrate was seldom used due to its unaffordable high price. Genotypic analysis revealed homozygous or double heterozygous mutations while the parents were heterozygote. After managing the severe bleeding episode, prophylaxis was given to patients with deficiencies of prothrombin, factor VII and factor X using FFP (15 mL/kg) once/week to 2-3 times/week while patient with afibrinogenemia received cryoprecipitate (0.5 unit/kg) monthly. The remaining patients received episodic or early bleeding treatment. Desmopressin, antifibrinolytic agent, hormone and fibrin glue were used as adjuvant treatment. The case fatality rate was 12.8% (5/39) among patients with factor VII deficiency at severe degrees.

Conclusions: Inherited RBD of coagulation factor deficiencies were more commonly found among non-consanguinity cases. Early recognition and diagnosis are essential for the prompt replacement

therapy to control serious bleeding episodes, followed by prophylaxis for patients with severe initial bleeding. Genotypic analysis is helpful for confirmation and genetic counseling.

PB1175 | Evaluation of the Potential Utility of the Total Thrombus-Formation Analysis System (T-TAS) in Comparison to the Platelet Function Analyzer (PFA) in Subjects with Primary Hemostatic Defects

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Background: The platelet-function analyzer (PFA) which provides a simple global measure of high shear-dependent platelet function has been used for the diagnosis of primary defects. However, this system is still of limited diagnostic value in patients referred for platelet dysfunction. The total thrombus-formation analysis system (T-TAS) was recently developed for quantitative analysis of the thrombus-formation process under shear rates in a situation closure to the *in vivo* condition.

Aims: Our current study sought to evaluate the potential utility of the T-TAS in comparison to the PFA in subjects with defects in primary hemostatic ability.

Methods: Blood samples from patients referred to our centre and healthy donors were tested. Platelet-related haemostasis was evaluated using the PFA by determining closure times with the collagen/epinephrin cartridge and T-TAS microchips (collagen, PL chip) to analyze platelet thrombus formation. Cutoffs of closure time > 150 sec (PFA) and AUC < 260 (T-TAS) were used to define abnormal primary hemostatic ability. Light transmission aggregometry (LTA) was performed using several concentrations of different agonists and VWF:GPIbR levels were measured by immunoturbidimetry.

Results: In total, 73 VWF and LTA assays were performed of which 36 were pathologic with VWF:GPIbR< 50% (n=13), abnormal LTA results (n=18) or both abnormalities (n=5). The T-TAS assay was 89.2% specific and 38.5% sensitive for identifying VWF defects, whereas the PFA assay was 89.2% specific and 69.2% sensitive. On the contrary, the T-TAS assay was 86.5% specific and 72.2% sensitive for identifying abnormal LTA results, whereas the PFA was 89.2% specific, but only 22.2% sensitive.

Conclusions: Our results show that both assays seem to have different patterns regarding their ability to detect primary hemostatic defects. Indeed, PFA seems to be more sensitive for screening VWF defects, whereas T-TAS assay shows better performance for identifying platelet aggregation abnormalities. However, these results should be confirmed on larger studies to be extrapolated.

PB1176 | Efficacy and Safety of Fibrinogen Concentrate for On-Demand Treatment of Acute Bleeding and Surgical Prophylaxis in Pediatric Patients with Congenital Fibrinogen Deficiency: Results from the FORMA-04 Study

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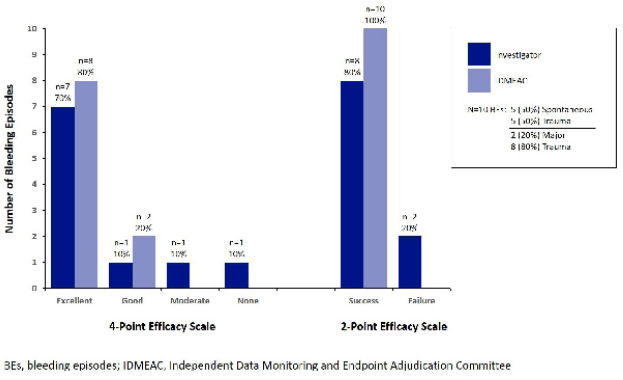
Background: Congenital fibrinogen deficiency (CFD) is a rare, inherited bleeding disorder affecting hemostasis. Patients experience severe and/or frequent bleeding episodes (BEs). Treatment with human fibrinogen concentrate (HFC) can prevent and arrest bleeding. The rarity of CFD makes it difficult to perform prospective pediatric clinical trials.

Aims: FORMA-04, was a multinational, multicenter, prospective, open-label, uncontrolled, Phase 3 study to determine the efficacy, safety and pharmacokinetics (PK) of HFC (*Fibryga*®, Octapharma AG) for treatment of acute BEs and surgical prophylaxis in pediatric patients with CFD.

Methods: Hemostatic efficacy was assessed based on an objective 4-point scale with final adjudication by an Independent Adjudication Committee (IDMEAC). PK blood samples were collected at baseline and at 1- and 3-hours on day 2, 5, 7, 10 and 14 post-infusion. Safety was assessed by monitoring adverse events (AEs).

Results: Fourteen afibrinogenemic patients (mean age of 6.0 years, range 1.0-10.0 years) were enrolled. Eight patients received HFC for the treatment of 10 BEs (8 minor, 2 major). Overall hemostatic



BEs, bleeding episodes; IDMEAC, Independent Data Monitoring and Endpoint Adjudication Committee

FIGURE 1 Efficacy assessment for treatment of all bleeding episodes according to the Investigator and the IDMEAC (N=10 BEs in 8 patients)

TABLE 1 PK Parameters for HFC Determined by Fibrinogen Antigen (N=13)

Parameter	Mean	SD	Median	Range
AUC, g*h/L	106.824	36.8896	98.365	64.665-193.045
AUCnorm h*kg*g/L/mg	1.419	0.4385	1.298	0.940-2.375
Cmax, g/L	1.559	0.3183	1.560	1.120-2.190
Incremental IVR, mg/dL/(mg/kg)	1.592	0.3224	1.599	1.155-2.259
Tmax, h	1.154	0.5547	1.000	1.000-3.000
T1/2, h	84.356	34.2658	72.233	57.456-181.027
MRT, h	114.332	37.9732	102.554	78.583-221.358
CL, mL/h/kg	0.756	0.1872	0.770	0.421-1.064
Vss, mL/kg	81.651	15.2735	74.663	62.563-113.297

efficacy for on-demand treatment of BEs was rated as 100% successful (Figure). Haemostatic efficacy of three surgeries (2 minor, 1 major) in 3 patients was rated as 100% successful. Mean (±SD) PK parameters, as determined by measuring fibrinogen activity are shown in the Table. Overall, 10 AEs occurred in 4 (28.6%) patients, 2 of which (pyrexia and portal vein thrombosis) were in the same patient and assessed as possibly related to treatment. The latter AE was classified as serious, occurring after splenectomy for a spontaneous spleen rupture, leading to discontinuation from the study. No allergic/hypersensitivity reactions and no deaths were observed.

Conclusions: This data set demonstrates that HFC treatment was efficacious and safe for the on-demand treatment of acute BEs and surgical prophylaxis in pediatric patients with congenital afibrinogenemia. PK profile was comparable to that in adults.

PB1177 | Achenbach Syndrome in a 19 Year-Old Girl: A Clinical Case

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Background: Achenbach's syndrome (AS) was first described by Walter Achenbach in 1958. It is a rare, benign, intermittent and self-limiting condition that causes paroxysmal bruising and pain in one or more fingers. Its pathophysiology has not yet been established, but as symptoms are typically triggered by everyday activities, local vascular fragility causing capillary micro-haemorrhages, possibly related to age or minor trauma, has been proposed. Its prevalence is unknown, although, according to a review published in 2017 by Jiménez et al, middle-aged women seem to be more commonly affected.

Aims: Presenting the case of a 19-year-old woman with AS.

Methods: A 19-year-old woman presented to the emergency department with acute painful and bruised fifth digit of the left hand. The patient reported the recurrent appearance of "blue fingers" for three months without any trauma and the situation usually improved spontaneously. On examination the finger was swollen to the level of the metacarpophalangeal joint with bruising over both surfaces of the hand, more intense in the palmar area. After clinical examination, a complete blood count and complete study of coagulation were requested.

Results: The diagnosis is essentially based on history and clinical examination, and knowledge of the condition facilitates the diagnosis. Blood counts, conventional radiology and coagulation, autoantibody and vascular flow studies are normal and not useful for the diagnosis, as shown in our case. The patient presented the last one. AS is triggered by stress and she was anxious due to admission exams. Besides that, the intermittent character of the "blue fingers" was crucial for the diagnosis of AS.

Conclusions: Due to the benign and self-limiting character of the syndrome, no specific treatment has been proposed. Patients should be counselled about the possibility of symptom recurrence in order to prevent anxiety, as the manifestations of the syndrome may be alarming.

PB1178 | Long-Term Anticoagulant Treatment in Patients with Factor VII Deficiency: A Single Centre's Experience

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Background: Inherited factor VII deficiency (FVIID) is the most frequent among the rare congenital coagulation disorders and clinical expression poorly correlates with FVII residual activity. Paradoxically, FVII deficiency does not protect against thrombotic events, with a reported incidence about 3-4% of thrombotic events. At present, there is limited evidence about management of the anticoagulation therapy in these patients, being an important challenge in clinical practice.

TABLE 1 FVII Deficiency patients' characteristics

Patient	Age	Sex	FVII level	ISTH-BAT	Mutation FVII gene	Thrombosis type	Anticoagulant therapy	Risk factors for thrombosis	Follow -up (years)
1	28	F	28%	3	PENDING RESULT	PULMONARY THROMBOEMBOLISM	ACENOCUMAROL	-Estrogen-progestin oral contraceptives. -Obesity.	19
2	35	F	15%	0	-F7 Gene: splicing mutation in heterozygosis (c.DNA:c803 805+1delTGGG) -PROS1 Gene: splicing mutation in heterozygosis (c.DNA:C.1919T>C, protein pMet640Thr) -G20210A POLYMORPHISM OF F2 (PROTHROMBIN) GENE (heterozygous carrier)	-DEEP VEIN AND PULMONARY THROMBOSIS -VENOUS THROMBOSIS IN INFERIOR VENA CAVA, ILIAC AND GONADAL VEINS.	1° ACENOCUMAROL 2° RIVAROXABAN 20 mg 3° DABIGATRAN 150 mg	-Abortion. -Trombofilia.	10
3	71	M	31%	3	PENDING RESULT	-	ACENOCUMAROL	-Atrial fibrillation -Lung cancer -Arterial Hypertension. -Dyslipidemia. -Diabetes	10

Aims: Providing data about management of the anticoagulant therapy in FVIID.

Methods: We report three cases of patients with FVIID receiving anticoagulant treatment followed at our hematology service.

Results: *Case 1:* A 28-year-old female with mild FVIID presents a pulmonary thromboembolism in the context of taking estrogen-progestin oral contraceptives and obesity, initiating oral antiacoagulant therapy (OAT) with acenocoumarol.

Case 2: A 35-year-old female was diagnosed with inherited protein S deficiency in 1999. In 2010, she had an abortion, developing deep venous thrombosis later, initiating acenocoumarol. Despite this, she had an extensive venous thrombosis and she was diagnosed of FVIID, starting with rivaroxaban, which was switched by dabigatran for osteopenia. Moreover, the thrombophilia study revealed that she was a heterozygous carrier of G20210A polymorphism at the prothrombin gene.

Case 3: A 71-year-old man was diagnosed of mild FVIID and he was taking acenocumarol because of atrial fibrillation (AF). In 2015, he suffered a cardiorespiratory arrest because of AF with complete heart block, and a permanent pacemaker implantation was performed with tranexamic acid treatment. Four years later, he was diagnosed with squamous cell lung cancer, after mediastinoscopy performed with acid tranexamic too.

Conclusions: We provide our institution's experience about management of anticoagulant therapy in patients with FVII deficiency. Fortunately, none of the patients present bleeding events, but these case reports reveal the need of further studies and data from large case series in order to shed light on this issue.

PB1179 | Blocking Tissue Factor Pathway Inhibitor (TFPI) Function Has a Therapeutic Benefit in Murine Models of Factor Deficiencies

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Background: Restoring the hemostatic equilibrium by targeting the anticoagulant, TFPI shows tremendous promise for hemophilia. Because of the mechanism of action, it is plausible that this strategy could also fill the unmet need for treating other rare bleeding disorders. Our *in vitro* data indicate that modulating TFPI activity is potentially effective in restoring hemostatic activity in some human factor deficient plasmas.

Aims: To explore the *in vivo* therapeutic advantage of blocking TFPI function in the background of a specific clotting factor deficiency, e.g. coagulation factor VII (FVII).

Methods: To generate FVII-deficient mice, wild-type (wt) mice were treated with a single dose of a liver targeted antisense oligonucleotide (ASO) to knockdown plasma FVII (ASO-FVII; 40 mg/kg).

Results: The almost complete lack of plasma FVII (< 1% levels of baseline) substantially increased the clot time (n=5; p< 0.0001) and impaired thrombin generation vs. wt mice. Injection of a monoclonal TFPI antibody (ab) against kunitz-2 domain (1-5 mg/kg) in ASO-FVII mice

(n=3-5; p< 0.005) resulted in a dose-dependent reduction of the prolonged clot time. To assess efficacy, we used two bleeding models and monitored blood loss in ASO-FVII mice (n=3-5) injected with PBS or TFPI ab (0.5-5 mg/kg). We found that ASO-FVII mice exhibited a ~6-fold (p< 0.005) increase in blood loss vs. wt; however, administration of TFPI ab (p< 0.005) reduced the bleeding phenotype in ASO-FVII mice in a dose-dependent manner. Intravital microcopy studies showed that ASO-FVII mice (n=3) displayed considerably impaired thrombus formation following vascular injury. In contrast, infusion of TFPI ab (2.5 mg/kg) into ASO-FVII mice produced an increase in both platelet and fibrin accumulation after injury.

Conclusions: Our data indicate that modulating TFPI activity mitigates bleeding and restores clot formation in a mouse model of bleeding disorders. TFPI inhibition thus represents a potential alternative therapeutic strategy to enhance hemostasis in some rare bleeding disorders.

PB1182 | Collagen Turnover and Plasma Ascorbic Acid Levels in Patients Suspected of Inherited Bleeding Disorders Harboring Variants in Collagen-related Genes

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Background: Variants in the collagen-related genes *COL1A1*, *COL3A1*, *COL5A1* and *COL5A2* are associated with Ehlers-Danlos syndrome (EDS), a group of heterogeneous connective tissue disorders known to be associated with increased bleeding. Following genetic screening, a relatively high proportion of patients suspected of inherited bleeding disorders were found to harbor at least one heterozygous variant of unknown significance in one of the above-mentioned collagen-related genes. For the synthesis of functional collagen molecules, ascorbic acid (AA) is required.

Aims: To investigate the functional consequences of variants in *COL1A1*, *COL3A1*, *COL5A1* and *COL5A2*, as well as plasma levels of AA in patients with incompletely explained bleeding tendencies.

Methods: In total, 31 patients harboring at least one heterozygous variant in *COL1A1*, *COL3A1*, *COL5A1* or *COL5A2* and 20 healthy controls were included. The turnover of collagen type I, III and V was assessed in patients and controls using validated ELISAs based on monoclonal antibodies targeting neo-epitopes specific for collagen formation and degradation. In addition, plasma levels of AA were analyzed in patients using high-performance liquid chromatography. Written informed consent was obtained from patients and controls. The study was approved by regional ethical committees.

TABLE 1 Turnover of collagen type I, III and V. Data are presented as medians (Q1 - Q3). Mann-Whitney tests were used. *, p-value < 0.05

	Patients (*n* = 31)	Controls (*n* = 20)	*p*
PRO-C1 (ng/mL)	63.5 (41.1 - 104)	77.5 (48.8 - 114)	0.48
C1M (ng/mL)	28.7 (21.0 - 36.9)	29.0 (22.5 - 35.9)	0.81
PRO-C1/C1M	2.30 (1.34 - 3.64)	2.43 (1.36 - 4.93)	0.71
PRO-C3 (ng/mL)	9.49 (8.24 - 10.6)	10.5 (8.75 - 12.1)	0.23
C3M (ng/mL)	10.4 (8.95 - 11.6)	10.8 (9.88 - 12.1)	0.29
PRO-C3/C3M	0.91 (0.80 - 1.10)	1.00 (0.83 - 1.07)	0.58
PRO-C5 (ng/mL)	415 (346 - 464)	413 (352 - 472)	0.93
C5M (ng/mL)	3.45 (3.02 - 4.49)	4.44 (3.83 - 5.21)	0.033*
PRO-C5/C5M	114 (67.7 - 155)	89.3 (69.6 - 113)	0.081

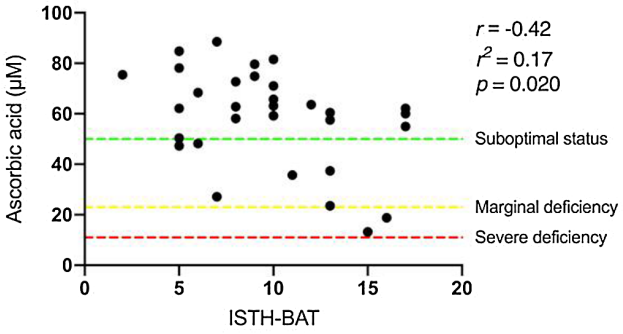

FIGURE 1 Correlation between plasma levels of AA and ISTH-BAT score in the patients (n = 31). A significant negative correlation was found

Results: On a group basis, no significant differences were found between patients and controls regarding collagen turnover, with the exception of lower levels of C5M (degradation of collage type V) in the patients (p = 0.033) (Table 1). A negative correlation between bleeding, assessed by the ISTH-BAT score, and plasma AA levels was shown in the patients (r = -0.42; r^2 = 0.17; p = 0.020) (Figure 1).

Conclusions: Functional investigations of the turnover of collagen type I, III and V were not able to confirm the variants in *COL1A1*, *COL3A1*, *COL5A1* and *COL5A2* as causative of increased bleeding. However, the correlation between bleeding and plasma AA levels warrants further investigations.

PB1183 | Uncharacterised bleeding Disorders Are Associated with Reduced Platelet and Microparticle Thrombin Generation and Decreased Procoagulant Oxidised Phosphatidylcholine

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Background: Patients with a significant bleeding history and normal routine laboratory tests are labelled as having unclassified bleeding disorder (UBD). We have shown previously that enzymatically oxidised phosphatidylcholine
(12-HETE-PC), produced rapidly on platelet activation, supports thrombin generation whereas native PC does not.
Aims: We investigated whether the bleeding phenotype could be explained by the ability of platelets or microparticles (MP) to support thrombin generation (TG) mediated by phospholipids (PL) in the platelet membrane.
Methods: Washed platelets (WP) from healthy controls (HC) (n=27) and UBD patients (n=20) were isolated and activated with thrombin. TG on platelets and MPs, resuspended in standardised platelet poor plasma were activated using liposomes containing tissue factor (10pM) and phospholipid (0.5μM). 12-HETE-PL and externalised phosphatidylserine (PS) were measured using mass spectrometry. In 3 UBD patients, samples were collected pre and post DDAVP.
Results: Peak TG supported by washed platelets and MPs was reduced in UBD patients ($p < 0.05$) (Figure 1). There was no difference in externalised PS in UBD patients and HC. 12-HETE-PC was markedly lower in UBD 9.5 (3.5-18.9) vs 30.7 (13.8-41.8) ng/2x10^7 platelets, p=0.01 (Figure 2). In 3 UBD patients treated with DDAVP for invasive procedures there was increased externalisation of all species of PS ($p< 0.04$) and a trend towards increased peak TG on washed platelets.
Conclusions: In UBDs, a reduced ability of platelets and MPs to support TG may, in part, explain the bleeding phenotype. The reduction in TG was associated with a decreased ability of activated platelets to make procoagulant 12-HETE-PC. This suggests the PL composition of the platelet membrane may be implicated in UBD. DDAVP is effective in UBD and we suggest that this may be because it increases externalised PS and better supports TG. Further studies in independent cohorts of patients with UBD are required to confirm these findings.

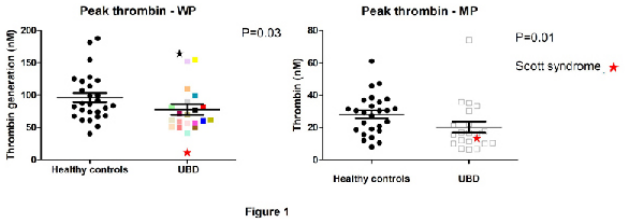

FIGURE 1 Peak thrombin generation in platelets and microparticles of healthy controls and UBD patients

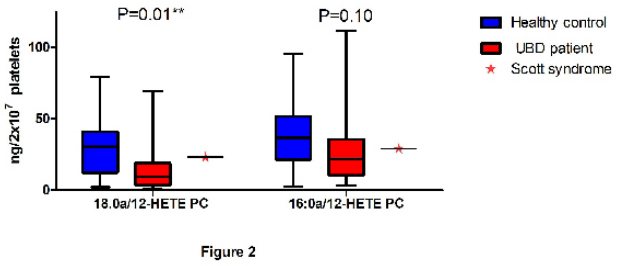

FIGURE 2 Generation of 12-HETE-PC species by activated platelets in healthy controls and UBD patients

PB1184 | Single Centre Experience of Rare Bleeding Disorders

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Background: Rare bleeding disorders (RBDs) are a heterogeneous group of coagulation factor deficiencies, mostly inherited autosomal recessively pattern. This represent approximately 3.5% of all inherited factor deficiencies. Therapeutic options depend on the deficiency and range from plasma-derived (eg, fresh frozen plasma, prothrombin complex concentrates, factor X concentrate) to highly purified and recombinant single factor concentrates.
Aims: To describe the patients with RBD diagnosis, clinical manifestation and severity of bleeding in our centre since 2010.
Methods: Retrospective observational study registered in the last 10 years, since 2010. For this work, retrieved data included: age at diagnosis, type of coagulation factor deficiency, bleeding type, treatment strategies and complications.
Results: The median age at diagnosis was 5 years (range, 6 months to 12 y). 3 females and 2 males. Family background was found in 3 patients. The bleeding symptoms at diagnosis were: post-traumatic bleeding in 2 pts (mouth and fracture arm) menorrhagia, convulsions, and pre-surgical study. The diagnostic were: factor VII deficiency in 2 pts, factor V deficit in 1 patient, factor XIII deficit in one and combined factor V-VIII deficit in 1. The factor VII deficiencies were treated with rFVIIa. In the case of Factor V deficit, the factor dosage was 3% and the patients had 2 haemarthrosis episodes and mucocutaneous bleeding. The episodes were treated with fresh frozen plasma, after the last treatment the child present sudden development of dyspnea, severe hypoxemia, and hypotension with Chest Rx bilateral infiltrates. Transfusion related acute lung injury was diagnosed and required oxygen supplementation and corticosteroids.
Conclusions: RBD are rare, but we must keep their diagnosis in mind, specific factor assay are necessary to identify the deficiencies. Sometimes, the treatment complications can be severe, particularly in case that fresh frozen plasma or cryoprecipitate. When available, specific purified plasma-derived or recombinant factor concentrates are the recommendations.

PB1185 | FXI Gene Mations in Northern Region of Turkey

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Background: Inherited deficiency of clotting factor XI (FXI) is a rare autosomal disorder caused by defects in the F11 gene. The F11 gene is located on the long arm of chromosome 4 (4q35), and consists of 15 exons. Up to now, over 220 mutations in F11 gene have been described in patients with FXI deficiency.

Aims: The aim of this study is to present the genetic analysis results of patients who were diagnosed as severe or mild Factor XI deficiency in northern region of Turkey.

Methods: Factor VIII, IX, XII and VWF deficiency were investigated in patients who were examined for prolonged APTT. Factor XI deficiency has been demonstrated. Families were informed about the genetic transition and screened. Approval was obtained from the ethics committee of our hospital. Genomic DNA was isolated from peripheral blood core cells. Targeted gene region was amplified by PCR. Libraries covering genes were prepared according to the Nextera XT DNA Library Prep protocol and sequenced in Illumina MiSeQ system.

Results: Mutation has been detected in all patients. Five patients were homozygous, one patient was compound heterozygous, and 23 patients were heterozygous. Thirty-five mutant alleles were detected in a total of 29 patients. Sixteen of the patients were male and thirteen were female. p.Ala109Thr was found in 71% (25/35) of the mutated alleles. p.Ala43Thr was found in 17% (6/35) of the mutated alleles. p.Glu135Ter was found in 6% (2/35) of the mutated alleles. c.325+1G>A was found in 6% (2/35) of the mutated alleles.

Conclusions: We report here four different mutations in 10 families with FXI deficiency. The few paper reported previously from Turkey, indicate the genetic heterogeneity of FXI deficiency in the Turkish population. But in this study, p.Ala109Thr was found with high frequency in northern region of Turkey.

PB1186 | A Bypass Surgery with Congenital Factor 5 and Factor 8 Deficiency

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Background: Patients with congenital combined factor 5 and factor 8 deficiency have mild and moderate bleeding than those with isolated factor deficiency. Bleeding is usually seen after surgery and traumas. It is recommended to give fresh frozen plasma and platelet apheresis after bleeding. (1,2,3,4).

Aims: We wanted to present a case of Factor 5 and Factor 8 deficiency with a cardiac bypass.

Methods: A 68 years old male patient with known diabetes, hypertension, benign prostatic hyperplasia, coronary artery disease, and congenital Factor 5 and Factor 8 deficiency with history of tuberculosis treatment. By-pass surgery was made with faktör 8, fresh frozen plasma and platelet apheresis replacement.

Results: Factor 5 is also present on platelet surface, and fresh frozen plasma and platelet apheresis are recommended when bleeding occurs.

Conclusions: Our case is the only one case in the literature that with congenital faktör 5 and 8 deficiency with coroner by-pass surgery.

PB1187 | Molecular Diagnosis of Quantitative and Qualitative Fibrinogen Deficiencies: A Portuguese Centre Experience

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Background: Congenital fibrinogen deficiencies are rare disorders, classified as quantitative (hypofibrinogenemia/afibrinogenemia, with partial/complete absence of fibrinogen) or qualitative (dysfibrinogenemia and hypodysfibrinogenemia, with normal or reduced fibrinogen levels and/or abnormal functional activity). Clinically, quantitative deficiencies are associated with hemorrhagic events, while dysfibrinogenemias are mainly asymptomatic, or can present with bleeding, thrombosis or both. Fibrinogen is a hexameric protein consisting of three pairs of polypeptide chains (Aa, Bb and g), encoded by *FGA*, *FGB* and *FGG* genes, respectively. Variants in these genes have been reported, both in homo- or heterozygosity.

Aims: Our aim was to identify the molecular diagnosis of patients with fibrinogen anomalies and/or hemorrhagic diathesis of unknown cause by next generation sequencing (NGS).

Methods: Seventeen unrelated patients and 5 relatives were analyzed. The molecular diagnosis was done using a custom panel for NGS (43 genes). Library preparation and sequencing was done using IonS5 (Thermo Fisher Scientific) protocol.

Results: We have identified 13 different variants (pathogenic and potentially pathogenic) in *FGA* (5), *FGB* (2) and *FGG* (6) genes, including five new variants in *FGB* (p.Glu240Lys) and *FGG* (p.Tyr27Cys, p.Asp63Val, p.Trp360* and p.Glu422del). The patients were diagnosed as hypofibrinogenemia (n=7), hypodisfibrinogenemia (n=4) and dysfibrinogenemia (n=4). The patients diagnosed with afibrinogenemia (n=2) were homozygous for 2 variants in *FGA* (complete deletion and p.Arg181*) and had severe hemorrhagic manifestations.

TABLE 1 Fibrinogen genetic variants identified in 22 patients with the Thrombosis&Hemostasis (TH)*

Gene	Patients/ Families	Fibrinogen (mg/dL)/ (min-max)	Nucleotide	Amino acid**	Genotype	*In silico*	New Yes/No	Phenotype	Clinical data
FGA	1/1	< 30	Complete deletion		HMZ	P	No	Afibrinogenemia	Spontaneous hemorrhage, intracranial hemorrhage (BS=9) - on prophylaxis
	1/1	< 30	c.541C>T	p.Arg181*	HMZ	P	No		Spontaneous hemorrhage (BS=11) - on prophylaxis
	6/2	(17 - 65)	c.103C>T	p.Arg35Cys	HTZ	P	No (recurrent)	Hypofibrinogenemia	Asymptomatic (BS=0)
	4/3	(<50 - 87)	c.191G>A	p.Cys64Tyr	HTZ	P	No (recurrent)		Bleeding/ Asymptomatic/ Thrombosis (BS=0;2;6;6)
	2/2	84; 70	c.1543G>A	p.Asp515Asn	HTZ	P	No		Bleeding/Asymptomatic (BS=0; 4)
FGB	1/1	625	c.586C>T	p.Arg196Cys	HTZ	P	No	Dysfibrinogenemia	Thrombosis
	1/1	128	c.718G>A	p.Glu240Lys	HTZ	PP	Yes	Hypodysfibrinogenemia	Bleeding (BS=5)

TABLE 2 Fibrinogen genetic variants identified in 22 patients with the Thrombosis&Hemostasis (TH)*

Gene	Patients/ Families	Fibrinogen (mg/dL)/ (min-max)	Nucleotide	Amino acid**	Genotype	*In silico*	New Yes/No	Phenotype	Clinical data
FGG	1/1	256	c.80A>G	p.Tyr27Cys	HTZ	PP	Yes	Dysfibrinogenemia	Thrombosis
	1/1	200	c.140C>T	p.Thr47Ile	HTZ	P	No	Dysfibrinogenemia	Bleeding (BS=5)
	1/1	?	c.188A>T	p.Asp63Val	HTZ	PP	Yes	Hypo-dysfibrinogenemia	Bleeding (BS=5)
	1/1	44	c.901C>T	p.Arg301Cys	HTZ	P	No	Hypo-dysfibrinogenemia	Asymptomatic/ Thrombosis/ Spontaneous miscarriage (BS=0)
	1/1	105	c.1080C>A	p.Trp360*	HTZ	P	Yes	Hypo-dysfibrinogenemia	Asymptomatic, 4 years old (BS=0)
	1/1	471	c.1265_1267delAAG	p.Glu422del	HTZ	PP	Yes	Dysfibrinogenemia	Thrombosis

Fibrinogen (mg/dL) - Reference values: 150-400mg/dL; ** Nomenclature HGVS; P - pathogenic; PP - potentially pathogenic; BS - Bleeding Score. ***Panel TH (43 genes)** - Associated with coagulation factors anomalies: *F2*, *F3*, *F5*, *F7*, *F8*, *F9*, *F10*, *F11*, *F13A1*, *F13B*, *LMAN1*, *MCFD2*, *FGA*, *FGB*, *FGG*, *GGCX*, *VKORC1*, *VWF*; associated with thrombosis: *PROC*, *PROS1*, *SERPINC1*, *SERPINE1*, *SERPINF2*, *THBD*, *PLAT*, *PLG*, *ANXA5*, *F12*; associated with platelet disorders: *GP1BB*, *GP1BA*, *GP9*, *ITGA2B*, *ITGB3*, *NBEAL2*, *NBEA*, *GP6*, *TBXA2R*, *P2RY12*, *ANKRD26*, *CYCS*, *PLA2G4A*, *TUBB1*, *PLAU*.

Five presented with thrombosis: one was carrier for *FGA* p.Cys64Tyr, associated with both thrombosis and bleeding. In total, 11 patients were asymptomatic (Table 1 and 2).

Conclusions: We have identified 5 new variants in the coding region of the fibrinogen cluster, together with high phenotypic variability. A phenotype-genotype correlation was observed in the quantitative deficiencies. In dysfibrinogenemias, it is still a challenge. We add another example of the valuable tool that NGS represents to clinical practice, allowing for a faster and broader diagnosis of fibrinogen hereditary anomalies.

PB1188 | The F7 p.Val22Ile Missense Mutation Affects Splicing and Can Be Counteracted by a Compensatory U1snRNA

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Background: Aberrant splicing is a frequent but still underestimated pathogenic mechanism. We studied the *F7* c.64G→A mutation, associated with factor VII (FVII) deficiency (activity 6-8%). It occurs at -1 position of the 5' splice site (5'ss) of exon 1a and predicts the V22I

change in the FVII pre-peptide, which guides nascent FVII into the endoplasmic reticulum (ER).

Aims: To elucidate the molecular mechanisms leading to FVII deficiency.

Methods: Expression of i) FVII cDNA alone or ii) fused with green fluorescent protein (FVII-GFP) and iii) of the splicing-competent FVII cDNA in Baby Hamster Kidney (BHK) cells. Evaluation of splicing patterns (RT-PCR), intracellular trafficking (fluorescent microscopy) and secreted FVII protein (Western blot, ELISA) and activity (FXa generation assays).

Results: Expression of the $FVII^{22I}$ variant resulted in secreted FVII protein/activity levels comparable with $FVII^{wt}$. Consistently, the $FVII^{22I}$-GFP variant showed an unaltered intracellular trafficking to ER.

To evaluate splicing all introns were, partially or integrally, included into the FVII cDNA (splicing-competent, $SC\text{-}F7^{wt}$). $SC\text{-}F7^{wt}$ expression in BHK cells showed a *F7* splicing pattern that resembled that in human hepatoma cells (HepG2), with the presence of the alternative exon 1b in ~11% of transcripts. Consistently, functional FVII was clearly detectable in medium.

The 64G→A change ($SC\text{-}F7^{64A}$) remarkably reduced the proportion of correctly spliced transcripts (8.0±1.3%) as compared with the $SC\text{-}F7^{wt}$ (43.7±3.5%), and levels of secreted protein (2.1±2.1%). Moreover, a spliceosomal U1snRNA variant with improved complementarity for the mutated 5'ss rescued splicing (up to 33.7±7.2% of correct transcripts) and, consistently, remarkable increased secreted FVII protein (57.0±20.6%) and activity (69.8±22.7%) levels.

Conclusions: Data demonstrate that the c.64G→A mutation affects *F7* pre-mRNA splicing by impairing 5'ss-U1snRNA interaction. The entire splicing-competent FVII construct offers a novel tool to evaluate putative *F7* splicing mutations, both for diagnostic and therapeutic (RNA-based) purposes.

PB1189 | Modulation of Alternative Splicing of the F5 Gene Using Morpholino Antisense Oligonucleotides

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Background: Coagulation factor V (FV) is a liver-derived protein encoded by the *F5* gene. Recently, a low-abundance splicing variant of FV lacking 702 amino acids (FV-short) has been identified. Both activated FV and FV-short express procoagulant activity as cofactors of factor Xa (FXa) in prothrombin activation. However, FV-short also binds tissue factor pathway inhibitor (TFPIα) with high affinity, stabilizing it in the circulation and potently enhancing its anti-FXa activity. Accordingly, genetic mutations that up-regulate FV-short expression have been associated with markedly elevated TFPIα levels and a bleeding tendency (East Texas and Amsterdam bleeding disorders). These disorders may be amenable to splicing modulation therapy using specific antisense oligonucleotides that decrease the relative expression of FV-short.

Aims: To design and test specific morpholino antisense oligonucleotides (MAOs) able to decrease the relative expression of FV-short, as a potential "molecular therapy" for the East Texas and Amsterdam bleeding disorders.

Methods: MAOs targeting the donor and acceptor splice sites of the FV-short-specific intron were designed and tested on a liver cell line (HepG2) that naturally expresses FV and FV-short. After treating the cells with 0-20 μM MAOs, total RNA was isolated and reverse-transcribed. FV and FV-short mRNAs were amplified using transcript-specific primers and quantified by real-time qPCR.

Results: Untreated HepG2 cells expressed 2-3 orders of magnitude more FV than FV-short mRNA. Cell treatment with both MAOs (alone or in combination) decreased FV-short mRNA expression in a dose-dependent manner down to 30-40% of the level in untreated cells ($p < 0.01$, n=3 biological replicates). Differently, treatment with a control MAO did not decrease the FV-short expression level.

Conclusions: We have designed MAOs that decrease the relative expression of FV-short mRNA in HepG2 cells. Once fully validated, these molecules may form the basis for a "molecular therapy" for the East Texas and Amsterdam bleeding disorders, and possibly also haemophilia.

PB1190 | Congenital Combined Bleeding Disorders, a Study on a Large Number of Iranian Patients

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Background: Congenital combined bleeding disorders are extremely rare disorders that mostly presented in a region with a high rate of parental consanguinity.

Aims: This study aimed to report the frequency of these disorders in Iran.

Methods: This study is conducted on 450 patients with combined bleeding disorders referred to Iranian comprehensive hemophilia care center (ICHCC) from 2012 to 2019. Patients were evaluated according to clinical manifestation, family history, and routine and specific laboratory assays.

Results: Out of 450 patients, 43 cases (9.5%) have congenital combined bleeding disorders other than combined factor (F) V and FVIII or combined vitamin K-dependent coagulation factors deficiency (Table 1). Twenty five (58%) were male and 18 (42%) were female. The mean age and mean age at diagnosis were 25.1 and 16.4 years,

TABLE 1 The prevalance of congenital combined bleeding disorders in Iranian population

Type of disorder	Number (%) (each disorder)	Type of disorder	Number (%) (each disorder)
FV-FVII deficiency	8 (61)	FI-VII deficiency FVIII-FIX deficiency	3(6.2)
FXI deficiency-vWD type I	7(10.4)	FVII-XI deficiency FV-vWD type I deficiency	2(4.1)
FVII-FX deficiency FVIII-FXI deficiency	4(8.3)	FVII-FIX deficiency FII-FX deficiency FI deficiency-BSS FXI deficiency-vWD type II M FV-FIX deficiency FVII deficiency-vWD type FVII-FXIII deficiency FVII-FVIII-FIX deficiency FV-FVIII-FIX-FI deficiency FV-FXI deficiency	1 (2.)

respectively. Among all patients, two cases have multiple coagulation factor deficiency including FVII-FVIII-FIX deficiency and FI-FV-FVIII-FIX deficiency. The most common clinical manifestation was mucocutaneous bleeding which results in diagnosis of 40%. Seven patients (16.2%) were diagnosed due to life-threatening bleedings including intracranial hemorrhage (ICH), umbilical cord bleeding, and gastrointestinal bleeding. Severe deficiency was observed in 9 cases that 5 of them associated with FVII level < 1%. All patients received on demand therapy except two with FX-FVII deficiency (FVII level < 1%). The first one was a 4 years old boy who experienced recurrent ICH and received prophylaxis regimen with AryoSeven™ (*AryoGen*, Tehran, *Iran*) in a dose of 30 μg/kg/a day. He developed an inhibitor against FVII and did not respond to high dose of AryoSeven™ (270 μg/kg). He died due to an extensive ICH. Another patient has also experienced ICH and she received a prophylaxis dose of 30 μg/kg/3 times a week now she has no bleeding events.

Conclusions: The coinheritence of bleeding disorders in Iran is surprisingly higher than expected.

PB1191 | Combined Factor Deficiencies Rare among the Rare Bleeding Disorders among Pediatric Population, Seven Years Experience in a Tertiary Care Center of Lahore, Pakistan

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Background: Combined factor deficiencies are very rare among the rare autosomal recessive bleeding disorder with an incidence of 1/1000000. These include Combined factor V & VIII and Vitamin K dependent clotting factors deficiency (VKCFD). Clinical presentation ranges from mild to severe as compared to single factor deficiency.

Aims: The objective of study is to determine the frequency of combined factor deficiencies in paediatric population and their phenotypic presentation.

Methods: It was a cross sectional study carried out in the Coagulation division of Haematology & Transfusion Medicine at the Children's Hospital Lahore from Jan 2013 to Dec 2019. All patients suspected of clotting factor deficiencies aged 0 to 18 years were included. After the initial coagulation screening, specific Factor assays were carried out by using Stago (France) factor deficient plasmas. Other rare bleeding disorders and vWD Patients were excluded from further study on the basis factor assays results.Cases with Combined factor deficiencies were further investigated. Data analysis was done on software Statistical package for Social science (SPSS) version 26. Continuous variable like age were presented as mean ± standard deviations.

Results: Out of total 350 patients of suspected clotting factor deficiencies Rare bleeding disorders cases were 42 (12%). Combined factors deficiency cases among Rare bleeding disorders were 6 (14.2%). Combined factor V & VIII cases were 2 (33.3%) and VKCFD cases were 4 (66.6%). Males to female ratio was 5:1. All six patients were less than 4 years of age. Most common symptom was bruising present in all 6 (100%) cases, CNS bleed was in 2 (33.3%) cases, Umbilical stump bleed in 2 (33.3%), post-circumcision bleed was in 3 (50%), hemarthrosis was present in 2 (33.3%) .

Conclusions: Combined factor deficiencies are rare and improved diagnostic facilities can help in finding more cases and their timely management.

PB1192 | Inhibitor Development in a Patient with Severe Congenital Factor V Deficiency

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Background: A 21-year-old female diagnosed with severe factor V (FV) deficiency (0.01 IU/mL) in childhood presented with sudden abdominal pain related to a grade V atraumatic splenic rupture. EBV IgG was positive. She was successfully treated with embolization and FFP. One week later she started a Cephalosporin for a UTI. She subsequently developed severe epistaxis, treated with FFP and platelets, and then a tension hemothorax. A FV inhibitor was identified (table 1). Bleeding was controlled with recombinant activated factor VII (rFVIIa), platelets and tranexamic acid. The inhibitor was treated with immunosuppression (steroids, IVIg, Rituximab). Course was complicated by a hamstring hematoma related to minor trauma.

TABLE 1 Events, lab values and treatment

	Event	INR	PTT (s)	FV (IU/mL)	Inhibitor (BU)	Hemostatic treatment	Immunosuppression
Baseline		4.78	>169	0.01			
Admission	Splenic rupture	4.80	>169			FFP, pRBC, TEA, embolization	
PAD1-4		1.51-1.89	35.5-48.6	0.17-0.08		FFP daily, pRBC	
PAD5-10	Cephalexin (UTI) (PAD8)	1.70	43.3			Nil	
PAD11-17	Oozing IV site Epistaxis (PAD11) Tension hemothorax (PAD13)	8.40-7.07	>169	0.00	166.4	FFP, plts, pRBC, TEA, rFVIIa	Dexamethasone daily (starting PAD13) Rituximab (weekly x 4) (starting PAD14)
PAD18-31	Epistaxis/ hemoptysis (PAD18) Bleeding at arterial line site (PAD23)	6.38-5.97	>169	0.00	60.8	rFVIIa, plts, pRBC, TEA	Switched to Prednisone PAD18 (continued daily to D/C)
PAD32-47	Right hamstring hematoma	5.58-5.26	>169	0.00	46.4	rFVIIa, plts, TEA	IVIg (PAD36, 37, 40, 41)
PAD48	D/C home	4.73	>169	0.00	24.0		

PAD = post admission day; FFP = fresh frozen plasma; u = units; plts = platelets; TEA = transexamic acid; pRBC = packed red blood cells; rFVIIa = recombinant activated factor VII; D/C = discharge; IVIg = intravenous immunoglobulin

She was ultimately discharged stable on prophylactic rFVIIa with no further bleeding. Although the inhibitor titre decreased, it was still present at follow-up.

Aims: N/A

Methods: N/A

Results: Inhibitors have been rarely reported in congenital FV deficiency, as well as de novo acquired such as in the setting of antibiotic use. In our case, congenital FV deficiency, tissue inflammation, possible EBV infection and aggressive exogenous FV exposure raise concern for an alloantibody, while the Cephalosporin provides argument for an autoantibody. Regardless, the inhibitor further impaired pre-existing abnormal hemostasis - leading to two life-threatening bleeds and necessitating attempt at rapid inhibitor control. While ITI is an untenable eradication strategy given the excessive doses of FFP required, immunosuppression was seen as a viable alternative. Immunosuppression and avoidance of exogenous FV led to a reduction in inhibitor titre. rFVIIa was used to treat bleeds and as prophylaxis.

Conclusions: This case highlights the complexity involved in treating inhibitors arising in the setting of rare factor deficiencies, and the need for better treatment options.

PB1193 | Perioperative Management of Patients with Rare Bleeding Disorders in a Hemophilia Comprehensive Care Centre

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Background: Perioperative management of people with rare bleeding disorders (RBD) continues being a therapeutic challenge for the hematologists.

Aims: Report our experience in perioperative management of people with RBD in our Hemophilia Comprehensive Care Centre (HCCC).

Methods: We reviewed charts of patients with RBD who underwent at least 1 procedure/surgery (excluding exodontics) in our HCCC from September-18 to December-19. Patients with incomplete information, multiple coagulation factor deficits, vitamin K deficiency, autoimmune or liver disease were excluded. Clinical characteristics, type of surgery, perioperative hemostatic treatment and surgical outcome were collected.

Results: We analyzed 62 surgery procedures underwent on 55 patients (16 surgeries in 11 patients with moderate-severe deficiency).

TABLE 1 General and clinical characteristics

	All	Fibrinogen	FV	FVII	FX	FXI
Patients (%)	55	1 (1,8)	2 (3,6)	39 (70,9)	2 (3,6)	11 (20)
Men (%) / Women (%)	30 (54,5) / 25 (45,5)	1	1 / 1	21 / 18	1 / 1	6 / 5
Age Median (Range)	33 (3-88)	71	37 (5-68)	15 (3-88)	55 (37-74)	56 (33-79)
Mild n° / Moderate n° / Severe n°	44 / 7 / 4	0 / 0 / 1	2 / 0 / 0	34 / 5 / 0	1 / 1 / 0	7 / 1 / 3
Above hemostatic level (%)	49 (89)	0 (0)	2 (100)	37 (94,9)	2 (100)	8 (72,7)
Affected relatives Known / Unknown	2 3	1 0	1 0	7 7	0 0	1 3
Surgical History (patients) At least 1 procedure / ≥ 2 procedures / Bleeding	21 /12 / 1	1 /0 / 0	0 /0 / 0	13 / 7 / 0	1 / 1 / 1	6 / 4 / 0

TABLE 2 Surgical characteristics and treatment / **FR (Factor replacement), TXA (Tranexamic Acid), FFP (Fresh frozen plasma)

	Minor procedure		Major procedure	
	Mild Deficiency	Moderate-Severe Deficiency	Mild Deficiency	Moderate-Severe Deficiency
Fibrinogen				1 (FR)
FV	1 (TXA)		1 (TXA)	
FVII	25 (TXA) / 1 (FR) / 2 (FR + TXA) / 1 (None)	1 (TXA) / 1(FR) / 4 (FR + TXA)	4 (TXA) / 1 (FR + TXA)	1 (TXA + FR)
FX	1 (None)			1 (FR + TXA) / 1 (FFP + TXA)
FXI	1 (FFP) / 5 (TXA)	1 (FR) / 1 (FFP + TXA)	3 (FFP + TXA)	4 (FFP + TXA)

Most common deficit was FVII (70.9%). Clinical characteristics of the patients are summarized in Table 1.

Twenty-one patients had surgeries before diagnosis of RBD with only one bleeding complication reported.

We included 15 major and 47 minor surgeries. Three cardiovascular surgeries were undergone in 2 patients with moderate-severe factor deficit. Management of surgeries is shown in Table 2.

Two minor surgeries required no treatment. Replacement therapy without antifibrinolytic agents was prescribed in 4 procedures. Most of the surgeries (90.3%) received tranexamic acid as single agent or associated with factor replacement. Four hemorrhagic complications were reported (3 major procedures in patients with severe deficit). A patient died 5 months after the procedure.

Conclusions: Our perioperative strategy in patients with RBDs achieved good hemostatic results. Adapted use of tranexamic acid to the thrombotic risk in the perisurgical management of patients with no severe factor deficit has been effective and safe. On the other hand, management of patients with severe factor deficiency with replacement therapy +/- tranexamic acid achieved good hemostasis. Three patients had bleeding complications after surgeries requiring factor coagulation products and/or fresh frozen plasma eventually to stop bleeding.

PB1194 | Use of the ISTH Bleeding Assessement Tool in Diagnosis of Bleeding Disorders in West Algeria

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Background: The clinical assessment of the presence and severity of bleeding symptoms is a fundamental step in the evaluation of patients referred for a probable bleedings disorders. The ISTH bleeding assessement tool (ISTH-BAT) was proposed, to standardised a collection of the bleeding history, because the reporting and interpretation of bleeding symptoms is subjective.

Aims: We repport our experience to use ISTH-BAT in diagnosis of bleeding disorders.

Methods: The bleeding symptoms of 1058 patients referred for a probable bleeding disorders and 612 healthy volenteers were assessed retrospectively using ISTH-BAT. Only symptoms and treatment before and at diagnoses are considered. The bleeding score ranged from a minimum of 0 (no symptoms) to a maximum of 56 (blood transfusion or replacement therapy).

Results: In healthy volenteers, sex ratio was 1.25 the most frequents symptoms is menoragia and epistaxis, mean bleeding score was 0 (range 0 to 6).

The use of bleeding score greater than 3 as a screening test for all laboratory phenotype of bleeding disorders has a sensitivity of 57.6% and specificity of 98%; if we use them only for the diagnosis of severe and moderate phenotype, sensitivity become 86% and the specificity 97%. The use of bleeding score as a screening test for Von Willebrand disease has a sensitivity of 97.89% and specificity of 95.73%.

Conclusions: The value of bleeding score ≥ 3 as is a good screening test of bleeding disorders. This bleeding score is an useful clinical tool for the identification of a possible severe or moderate laboratory phenotype, but this score can't differentiating between mild bleeding disorders and normal subject.

PB1195 | Characterization and Management of Patients with Mild or Moderate Hereditary Factor X Deficiency: A Retrospective Chart Review

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Background: Hereditary factor X (FX) deficiency (FXD) is a rare autosomal recessive bleeding disorder resulting in reduced plasma FX coagulant activity (FX:C). FXD patients with FX:C < 1% generally have severe bleeding symptoms, but patients with higher FX:C may also exhibit serious bleeding. Mild/moderate FXD is presumed to be less severe, but the heterogeneity of clinical features may result in delayed recognition and management. There are few reports and no evidence-based guidelines for management of these patients.

Aims: To characterize diagnostic features, evaluate bleeding patterns, and assess current management strategies for patients with mild/moderate FXD.

Methods: This retrospective chart review included patients with FX:C ≥ 1% managed at 2 hemophilia treatment centers (HTCs) in the United States. Expansion to other HTCs is planned.

Results: Five patients were included: 2 siblings aged < 1 year and 3 years, and 3 unrelated patients aged 16, 17, and 20 years at the time of analyses (Table). Three of the five patients were identified due to a hemorrhage; two were identified based on family history of FXD. Two patients with heavy menstrual bleeding (HMB) received antifibrinolytics, iron replacement and hormonal therapy for HMB management. An intracranial hemorrhage was treated with prothrombin complex concentrates until bleed resolution (30 doses), and 3 bleeds (2 external ear trauma, 1 gum biopsy) in one patient were treated with plasma-derived FX concentrate (1 dose per bleed). All bleeds were deemed to have a fair to good response with first-line therapy. One patient did not experience any bleeds requiring treatment (FX:C 52%).

Conclusions: Hereditary FXD is a heterogenous disorder with variable bleeding phenotype. The association between the bleeding phenotype and laboratory results is essential for establishing the appropriate diagnosis. Evaluation of additional patients with mild/moderate FXD will provide information about the bleeding manifestations and response to therapy, allowing formulation of clinical management guidelines.

TABLE 1 Patient Characteristics and Diagnostic Laboratory Results

			PT, sec		PTT, sec		FX:C, %		
Patient no.	Age at analysis, y	Presenting complaint	Test result	Normal range	Test result	Normal range	Test result	Normal range	FX antigen, %
1	<1	Family history	21.3	9.8 - 13.1	118.2	27.0 - 36.3	7.4	50 - 150	89
2	3	Intracranial hemorrhage after a fall	26.3	9.8 - 13.1	99.5	27.5 - 37.4	8	50 - 150	82
3	16	HMB	13.5	9.4 - 11.7	29.0	23.1 - 33.1	36	64 - 144	Pending
4	17	Epistaxis	13.3	9.4 - 11.7	39.0	23.1 - 33.1	48	64 - 144	78
5	20	Family history	12.0	9.4 - 11.7	30.4	23.1 - 33.1	52	64 - 144	43

PT, prothrombin time; PTT, partial thromboplastin time.

PB1196 | A Digestive Hemorrhage Like No Other

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Background: Scurvy is the clinical manifestation of vitamin C deficiency. It is historically linked to the era of large shipping expeditions. Today, it affects the patients with malnutrition. Its clinical presentations are multiple and sometimes misleading.

Aims: To present an unusual hemorrhage aetiology.

Methods: A new case report is reported.

Results: A 69-year-old patient, followed for primary cutaneous vasculitis treated with long-term corticosteroid therapy, was hospitalized for febrile impairment of the general condition.

Clinical examination showed a fever with extracellular dehydration, a BMI at 18 kg/m^2, alopecia and edentulousness. The patient had diffuse bruising of different age and skin vasculitis. The abdomen was tender but there was no rectal hemorrhage.

In biology, the patient had hyponatremia without dyskaliemia, a hypocholesterolemia, a hypocalcemia and an inflammatory biological syndrome. The hemoglobin was at 11 g/dl, there was a lymphopenia and a thrombocytopenia at 119000 el/ mm^3. The AAN, the anti-ENA, ANCA and the antiphospholipid antibodies were negative. The myelogram was normal.

Initially, infectious endocarditis was evocated due to the presence of vegetation on the small mitral valve, however, during his stay at the hospital, the patient developed a digestive hemorrhage of a great abundance.

The oeso-gastroduodenal endoscopy, colonoscopy and entero-CT scan did not show any abnormality that could explain the hemorrhage.

The diagnosis of scurvy was suspected regarding hair loss, malnutrition, teeth loss, bruising and thrombocytopenia. It was confirmed by a low rate of vitamin C (2.1 mg/l). The evolution with 1,5 g/day of Vitamin C was favorable. The melena disappeared and the thrombocytopenia and hemoglobin stabilized.

Conclusions: Scurvy is a potentially serious historical disease that has not completely disappeared despite an improvement in living standards. Its treatment is simple and inexpensive if we think about it.

PB1197 | Inherited Factor VII Deficiency in Women: Low Rate of Bleeding in Non-severe Disease

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Background: Factor VII (FVII) deficiency is a rare inherited bleeding disorder. There is poor correlation between FVII level and bleeding phenotype. There is little published information about the relationship with bleeding in women.

Aims: To describe diagnosis, type of bleeding, treatment and outcome of women with FVII deficiency.

Methods: Retrospective cohort study. Consecutive women from a university hospital, age > 18y.o. with FVII deficiency (2016-2019) were included (FVII < 50%). Prothrombin Time (PT) was performed by a rabbit brain thromboplastin (PTFibrinogenHS+) and FVII dosage by one stage clotting assay with human recombinant thromboplastin (RecombiPlasTin2G) at three dilutions in a ACL TOP. Inhibitor presence was ruled out by PT and FVII mixing studies.

Reasons for determining FVII were evaluated. Spontaneous, peri-surgical bleeding and bleeding related to the peri-partum were recorded. Bleeding: classified according to ISTH guidelines. Statistic: basic descriptive analysis in Stata13.

Results: Twenty-eight women with FVII deficiency were included. Basic characteristics of the population in table 1. In 54% the diagnosis was suspected by a prolongation (frequently slight) of PT in patients without a history of bleeding.No FVII deficiency was acquired. Only 17% were diagnosed by bleeding.

Most patients (62%) had a PT greater than 60%. No patient had FVII < 10% at diagnosis and only 32% FVII < 30%.

Eight (29%) of the patients had a history of abnormal uterine bleeding, but none from menarche. Two patients had an alternative gynecological cause of bleeding.

Of the total population, only two patients had a history of major bleeding.

Table 2 shows the results of patients facing hemostatic challenges.

Conclusions: In our cohort of mild FVII deficiency we observed a low rate of bleeding complications. Most of the patients had no history of menorrhagia and no complications related to delivers were observed. We did not have patients with severe deficiency, being this a study limitation.

TABLE 1 Baseline characteristics of the population

Patients included, n	28
Age in years, median (IQR)	35 (25-45)
Factor VII in U/dL, median (IQR)	37 (28.5-44.5)
PT activity in %, median (IQR)	64 (57.5-69)
Reason for determination of FVII	
PT alteration in routine controls without bleeding, n(%)	15 (54)
Pre-surgery study, n (%)	7 (25)
Bleeding, n (%)	5 (18)
Family history of FVII deficiency, n (%)	1 (3)

TABLE 2 Hemostatic Challenges

Major surgery, events (patients)	13 (12)	Minor surgery, events (patients)	12 (12)	Deliveries, events (patients)	11 (10)
Preventive treatment, n (%)	8 (61.5)	Preventive treatment, n (%)	2 (17)	Preventive treatment, n (%)	0
Preventive treatment: Recombinant factor VII / fresh frozen plasma, n / n	4/4	Preventive treatment: Recombinant factor VII / fresh frozen plasma, n / n	1/1	Caesarean section, n (%)	8 (73)
Bleeding, n	0	Bleeding, n	1	Bleeding, n	2 (18)

PB1198 | Congenital Afibrinogenemia. Report of a Case in the Children's Hospital of Mexico Federico Gomez

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Background: Congenital afibrinogenemia is an extremely rare disorder (1 / 1,000,000) that generally occurs as an autosomal recessive condition in which the affected individuals are homozygous or compound heterozygous for truncated mutations in the gene encoding the fibrinogen α chain (FGA).
Aims: Report the case of a mexican patient with congenital afibrinogenemia.
Methods: Review of a clinical case and comparison with medical literature.
Results: An 11-month-old female patient reported significant diameter ecchymosis at the vaccine application site; 45 days prior to arrival at our hospital, she falls from its support plane with subsequent neurological deterioration, CT scan was performed finding epidural hematoma that required surgical drainage; she is sent to a second level hospital where it is received with data of active bleeding in puncture sites and neurological deterioration data, it was attempted to place central venous access presenting hemothorax that drained 200 ml of fresh blood, presented coagulation times that do not coagulate and improved with the administration of fresh frozen plasma, so they send with reference to our institution for suspected coagulopathy, TP and TTPa that do not coagulate, fibrinogen not detectable, are confirmed, so that fibrinogen quantification is requested which reports < 10 mg / dL and a sample is sent for DNA sequencing, congenital afibrinogenemia is integrated; currently receiving treatment with plasma fibrinogen.
Conclusions: Congenital afibrinogenemia is a rare pathology, which is undoubtedly a diagnostic challenge. Early diagnosis is very important for prevent serious or fatal bleeding. Traditional treatment includes cryoprecipitate transfusion, and is currently treated with prophylactic replacement therapy with plasmatic fibrinogen. The present case is relevant since it is a rare pathology but it should be suspected before a patient with clinical data of coagulopathy with TP and TTPa that do not coagulate.

PB1199 | Afibrinogenemia: Identification of a New Mutation in *FGB*

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Background: Afibrinogenemia is a rare inherited autosomal recessive trait characterized by bleeding events due to the absolute lack of fibrinogen. To date, genetic defects causing afibrinogenaemia are mostly identified in *FGA* and *FGG*, but less frequently in *FGB*.
Aims: We describe a case of afibrinogenemia with a new mutation in *FGB*.
Methods: A Pakistan 4-years-old girl and her parents were referred to our Center for a likely diagnosis of congenital fibrinogen deficiency. The proband showed severe bleeding from the umbilical cord after spontaneous delivery and oral mucosa bleeding with melena at the age of 6 months. At the age of 2 years, she showed a post-trauma subdural hematoma. Plasma fibrinogen was functionally assayed by Clauss clotting method and quantitatively evaluated using the Partigen Fibrinogen kit. Amplifications of *FGA*, *FGB*, *FGG* coding regions were subjected to a direct cycle sequencing. In silico analysis of potential splicing mutation was performed by using Human Splicing Finder.
Results: In Pakistan the patien was treated with prophylactic plasma infusion (200 ml once a month). At the age of 4 years, she moved to Italy where the diagnosis of afibrinogenemia was confirmed (undetectable functional and immune-reactive fibrinogen). Therefore, she started prophylaxis with 55 mg/kg of lyophilised human fibrinogen every 4 days to maintain fibrinogen activity higher than 0.5 g/L. Sequencing showed a homozygous mutation in *FGB* intron 4 (c.718 + 1G>A). Her parents are first-degree cousins, so far asymptomatic for bleeding events and have fibrinogen within normal ranges. Both carry the c.718 + 1G>A in heterozygous (Table). In silico analysis revealed that mutation is likely to affect splicing of exon 4.
Conclusions: We report a novel splicing site mutation in *FGB*. The c.718 + 1G>A is likely to lead to exon 4 skipping or shortening or inclusion of intronic material, as expected for a null allele variant, with the impair fibrinogen assembly and secretion, resulting in afibrinogenemia.

TABLE 1 Genotype-Phenotype relationship

Patient reference	Gender	Age (year)	Fibrinogen functional (g/l) Reference range 1.50-4.00 g/l	Fibrinogen antigen (g/l) Reference range 1.82-3.39 g/l	Genotype	Gene region	Nucleotide	Clinical features
Proband	Female	4	Undetectable	Undetectable	Homozygous	FGB Intron 4	c.718 + 1G>A	Umbilical cord bleeding. Oral mucosa bleeding with melena. Post-trauma subdural hematoma.
Mother	Female	28	2.46	1.82	Heterozygous	FGB Intron 4	c.718 + 1G>A	Asymptomatic
Father	Male	32	2.50	1.40	Heterozygous	FGB Intron 4	c.718 + 1G>A	Asymptomatic

PB1201 | Menorrhagia in Egyptian Adolescents with Inherited Bleeding Disorders: A Single Centre Study

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Background: Menorrhagia, Heavy menstrual bleeding (HMB) is a common gynecologic problem among adolescent females with inherited bleeding disorders(IBD) which are more prevalent in Egypt due to consanguineous marriage. HMB in IBD is an overlooked problem inspite of its negative impact on the quality of life(QOL) of those patients.

Aims: To assess the severity of HMB,its management and iimpact on the QOL of our studied cohort.

Methods: Retrospective review of the medical records of 31 adolescent girls with a diagnosed IBD in Cairo University Pediatric Hematology clinic and attending the multidisciplinary clinic for menorrhagia. Assessment included a detailed menstrual history and bleeding history using the ISTH BAT.Menorrhagia was evaluated using the pictorial blood assessment chart (PBAC) and the patients' QOL using a questionnaire designed by Kadir et al, 1998.

Results: 32/63 (50.8%) teenagers diagnosed with IBD had menorrhagia. They included 30.7% (4/13) diagnosed with platelet function disorders, 66.7%(12/18) with Von Willebrand disease(vWD) and 50%(16/32) with rare coagulation disorders.They were all nulliparous except 2 (1FV deficiency and 1 vWD) and 2 afibrinogenemic patients had repeated abortions.The mean age at menarche was 12 years and 81.3% had a positive family history of a similar condition. 56.2% of our studied cohort with menorrhagia required hospital admission because of acute menorrhagia and severe anaemia and received blood transfusion. 68.7% were on tranexamic acid, 40.6% on oral contraceptive pills and 53.1% received replacement therapy. 3 patients had rupture ovarian cyst(1 died, 1 living, 1 did oophrectomy), 1 had a bilateral oophrectomy. and 1 had orchidopexy after torsion. ISTH -BAT was severe in all patients.The PBAC and QOL showed improvement after treatment and/or control of menorrhagia.

Conclusions: Menorrhagia is a common symptom in Egyptian adolescents with IBD and is usually severe affecting their QOL adversely which can show improvement on treatment.

PB1202 | Important Sex Differences in Patients with Autosomal Inherited Bleeding Disorders

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Background: Sex differences may influence bleeding phenotype, diagnosis and management of patients with autosomal inherited bleeding disorders.

Aims: Therefore, we investigated sex differences in a large cohort of well-defined patients with autosomal inherited bleeding disorders (i.e. von Willebrand disease (VWD) and rare bleeding disorders (RBDs)).

Methods: We included all patients with VWD and RBDs in the Netherlands from two nationwide cross-sectional studies on VWD (WiN study) and RBDs (RBiN study). In both studies, the bleeding score(BS) was obtained, patients filled in an extensive questionnaire and blood was drawn for central measurement of coagulation factor levels.

Results: We included 1030 patients (834 VWD; 196 RBD patients), the baseline characteristics are shown in Table 1. The mean age of diagnosis was 15.7 ± (SD: 20.3) years in men, and 21.1 ± 20.9 years in women ($p < 0.001$). Women were more often referred for bleeding than men: 276 (46.1%) vs 133 (34.3%) ($p < 0.001$, Figure 1). In patients referred for bleeding, mean age of first bleeding was not different between men (9.1 ± 13.0 years) and women (10.8 ± 11.1 years, $p = 0.229$). However, mean age of diagnosis was much lower in men referred for bleeding than in women: 18.0 ± 19.5 years vs 25.4 ± 19.3 years ($p < 0.001$). Thus, the diagnostic delay after the first bleeding episode was much longer in women (14.0 ± 16.6 years) than in men (8.1 ± 17.0 years, $p < 0.001$). Total BS was lower in men than in women (9.7 ± 6.9 vs 11.6 ± 7.3, $p < 0.001$). However, after deduction of sex specific bleeding, BS seemed higher in men (9.7 ± 6.9) compared to women (8.8 ± 6.1, $p = 0.053$). In women aging 12 years or older, 63.9% had undergone treatment because of menorrhagia or postpartum hemorrhage.

Conclusions: In this large combined nationwide study we found important differences in bleeding phenotype, diagnostic delay, and management of males and females with autosomal inherited bleeding disorders.

TABLE 1 Patient characteristics

	Type 1 VWD		Type 2 VWD		Type 3 VWD		RBDs[1]	
	Malen = 173 (35%)	Femalen = 321 (65%)	Malen = 148 (47%)	Femalen = 164 (53%)	Malen = 14 (50%)	Femalen = 14 (50%)	Malen = 70 (36%)	Femalen = 126 (64%)
Age inclusion	33.9 ± 20.9*	43.1 ± 17.9*	36.4 ± 21.6	38.9 ± 20.9	30.7 ± 23.0	28.7 ± 21.2		
VWF antigen	0.35 ± 0.24	0.40 ± 0.22	0.27 ± 0.16	0.27 ± 0.15	0.01 ± 0.01	0.01 ± 0.01		
VWF activity[2]	0.44 ± 0.34	0.49 ± 0.32	0.14 ± 0.24	0.12 ± 0.12	0.00 ± 0.01	0.00 ± 0.00		
Coagulation factor activity[3]							0.23 ± 0.28	0.24 ± 0.30
Fibrinogen activity[4]							0.46 ± 0.26	0.70 ± 0.40
Euglobulin clot lysis time ratio[5]							11.0 ± 2.4	8.1 ± 2.8
Bleeding score	8.1 ± 6.3*	10.7 ± 7.0*	10.7 ± 6.5*	13.0 ± 7.7*	18.9 ± 6.0	19.9 ± 8.4	9.9 ± 7.9	11.4 ± 6.7

Data presented as mean ± standard deviation or frequency (percentage). *$p < 0.05$ between men and women.[1]152 patients with rare coagulation factor deficiencies and 44 with disorders of fibrinolytic system. [2]Measured with the monoclonal antibody assay.[3]Measured in patients with a rare coagulation factor deficiency.[4]Measured in patients with a fibrinogen deficiency.[5]Measured in patients with PAI-1 deficiency and hyperfibrinolysis

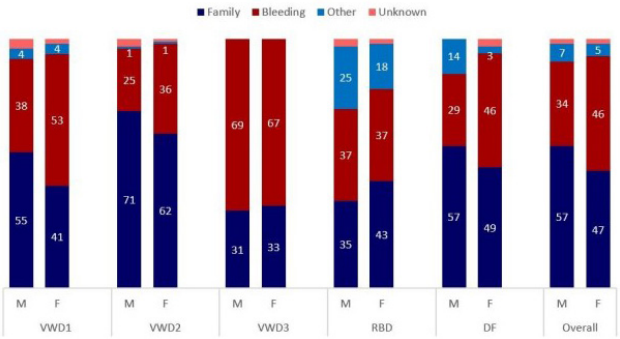

FIGURE 1 Reason for referral in patients with autosomal inherited bleeding disorders

PB1203 | Fibrinolytic Alterations in a Thrombomodulin-sociated Coagulopathy Are Diminished by Coinheritance of a TAFI Mutation

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Background: Thrombomodulin (TM) regulates coagulation and fibrinolysis by interaction with thrombin promoting activation of Protein C and thrombin activatable fibrinolysis inhibitor (TAFI, also denoted procarboxypeptidase U). Familial cases of TM-associated coagulopathy have been described displaying augmented plasma TM levels due to a premature stop codon in THBD (p.Cys537stop).

Aims: To investigate the impact of a novel THBD variant (*THBD: c.1487delC*, p.Pro496Argfs*10) on coagulation and fibrinolysis parameters in a family with 4 affected individuals.

Methods: The index case presented with 'unexplained bleeding' and abnormal prothrombin consumption index. Family members, recruited to NIHR BioResource, were analysed by ThromboGenomics (panel v2.8) and Sanger sequencing. Plasma levels of TM, TAFI and endogenous thrombin potential were determined by ELISA, activity assay and calibrated automated thrombography respectively. Plasma clot lysis rates were determined by turbidity assay ± carboxypeptidase inhibitor (CPI).

Results: THBD p.Pro496Argfs*10 cases reported excessive bleeding (median ISTH BAT score 6). TM plasma levels were significantly elevated (median 556 ng/ml, normal 2.9-7.6 ng/ml). The presence of the *THBD* p.Pro496Argfs*10 attenuated the endogenous thrombin potential and delayed clot lysis. Inhibition of TAFIa by CPI reduced the prolongation of lysis. Two affected individuals and one additional family member were found to harbour a monoallelic high impact variant in the TAFI gene (*CPB2* p.Arg114*). Individuals with the *CPB2* p.Arg114* variant exhibited low plasma TAFI levels (median 542 U/L, normal 677-1309 U/L). Coinheritance of *CPB2* p.Arg114*with THBD p.Pro496Argfs*10 diminished the impact of the TM mutation on lysis. In contrast, the individual with only the *CPB2* p.Arg114* variant showed enhanced lysis compared to control.

Conclusions: The novel THBD variant, THBD p.Pro496Argfs*10 gives rise to marked elevation of plasma TM levels. The functional consequences are enhanced potential for thrombin generation and TM-mediated TAFI activation. Coinheritance of *CPB2* p.Arg114* results in an intermediate fibrinolytic profile highlighting the complexities of independently inherited variants on pathogenesis of mild coagulopathies.

PB1204 | Missense Mutations and Predicted Effects on Splicing in Factor XI Deficiency

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Background: Congenital Factor XI (FXI) deficiency is a rare bleeding disorder caused by mutations in the F11 gene, with highly heterogeneous bleeding tendency, poorly correlated with plasma FXI activity. Among different reported mutations, the majority are missense, but most were not analyzed at the mRNA level or expressed in cells. Recent studies suggest unexpected deleterious effects of nucleotide substitutions located in specific exonic cis-acting elements (ESEs), important for correct splice-site identification and distinct from the classic splicing signals.

Aims: To investigate possible effects on splicing and functional consequences of missense mutations in F11 and implications on genotype-phenotype relationships.

Methods: In 37 subjects diagnosed with FXI deficiency (FXI:C < 0.6 IU/mL) missense mutations were identified and analyzed with the ESEfinder tool (a web resource that applies defined scoring matrices to identify possible ESEs in DNA sequences) to check the effect of selected nucleotidic substitutions on binding of proteins involved in the splicing pathway (Silico Splicing prediction).

Results: Among 21 different mutations identified in our cohort, 11 were missense variants. Ten missense variants were found in heterozygosity in 27 patients, whereas compound heterozygosity was shown in 2 patients (2 missense variants and missense/intronic mutations, respectively). Through Silico Splicing prediction 6 missense mutations were found as "aberrant splicing", i.e. introducing or deleting splicing sites (NM_000128.3: c.1252G>A; c.1693G>A; c.1721A>G; c.1786G>T; c.1822T>C; c.943G>A); 3 as "affecting splicing" i.e. affecting the score of a splicing site (c.1199C>T; c.183C>A; c.578G>A) and 2 as "no effect on splicing" (c.1052C>A; c.168T>G).

Conclusions: According to this prediction, most missense mutations can significantly and variably affect the splicing pathway. These predicted effects should be evaluated by in vitro splicing analysis to

assess possible consequences on pre-mRNA processing. A correct classification of mutations is essential for understanding structure-function relationships in the corresponding protein and searching for correlations with clinical and laboratory phenotype.

PB1205 | Genotype and Phenotype Characterization in RBDs: A Spanish Pilot Study

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Background: Rare bleeding disorders (RBD) are an inherited heterogeneous group of lower prevalence diseases, classified as fibrinogen disorders and several factor deficiencies (FD): FIID, FVD, FVIID, combined FV/FVIIID, FXD, FXID and FXIIID, which cause bleeding diathesis. The molecular characterization is only partial and genotype-phenotype correlation is not well known, mainly in FVIID.
Aims: To identify the pathogenic variants of RBD and the genotype-phenotype relationship in FVIID.
Methods: We have designed a prospective multicentre pilot Spanish study including 126 patients. Molecular characterization was performed in 82 patients by 23-gene panel using High-throughput sequencing (HTS), previously published. Bleeding score (BS) was evaluated by ISTH-BAT. Genotype-phenotype correlation was evaluated using the software package SPSS (v.25).

TABLE 1 Baseline characteristics

	n = 126
Age (years)	30 (4-85)
Sex	Female: 53%, Male:45%
Severity	Mild: 67%, Moderate:6%, Severe: 11%, No data: 16%
RBD	FII: 1%(1), FV: 6%(8), FVII: 58%(73), FX: 5%(6), FXI: 11%(14), FXII: 2%(3), FXIII: 1%(1), FV+FVII: 1%(1), FV+FVIII: 1%(1), FV+FXI: 2%(2), Prekallikrein: 2%(2), Fibrinogen: 9%(11)
Pathogenic variants	Missense: 77%(63/82), Deletion: 10%(8/82), Nonsense: 7%(6/82), others: 6%(5/82)

Results: Median age was 30 (range, 4-85); 53% was female; The most frequent RBD was FVIID (58%) and FXID 11%. Deficiencies were classified in mild (67%), moderate (6%), severe (11%). The median BS was 1 (0-22); the most frequent bleeding symptoms were mucocutaneous bleeding and epistaxis. 82 pathogenic variants were identified: 77% missense, 10% deletion, 7% nonsense, 6% others. Twenty-seven variants were novel. Forty-four patients are being sequenced to update the data for the congress. In the cohort of FVIID (n = 73), most of them was classified in mild (73%), moderate (8%), severe (11%). Most of genetic variants affected to catalytic FVII domain (83%). Until, all FVII patients included, were sequenced, in the uni and multivariate analysis we found any correlation between genotype, BS and FVII levels ($p > 0.05$).
Conclusions: We performed a pilot study to characterize the molecular-clinical-laboratory phenotype for RBDs in Spain. Molecular test was 100% successful and 27 novel variants were found. To date, we don't find relationship phenotype and genotype in FVIID.
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PB1206 | An Italian Survey on the Real-world Use of rFXIII (Catridecacog) in Patients with Factor XIII Deficiency

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Background: FXIII deficiency is a rare coagulation disorder. Bleeds are especially mucocutaneous, but life-threatening intracranial haemorrhages (ICHs) can occur in case of patients with severe disease. These subjects should therefore be treated in prophylaxis from childhood to prevent bleeding.
However, in the literature the data relating to the use of rFXIII in real life are insufficient.
Aims: To evaluate the characteristics of patients with FXIIII deficiency treated with rFXIII, the real dosage and frequency of the catridecacog administration used, and the clinical outcomes.

Methods: All patients with FXIII deficiency treated with catridecacog at nine Italian Haemophilia Centres were collected in this Survey. All data were subsequently statistically evaluated.

Results: Overall 17 patients with FXIII deficiency were collected, 75.0% presenting severe disorder. 8/15 were females. Mean age at diagnosis was 11 years (range birth-32 years). Two-thirds were a family disorder. In the 13/17 of cases the diagnosis was performed after major bleeding, only one of them was an ICH. These acute events were treated respectively with plasma (eight cases), pdFXIII (five cases), embolization (one case), rFXIII (one case), not reported (two cases). 76.5% of patients were subsequently put on prophylaxis with catridecacog at mean dosage of 33.1 IU/kg (range 25.0-80.0 IU/kg), on average every 4.0 weeks (range 3.0-8.0 weeks). Only two of them had muscular haematomas during a mean follow-up of 31 months, quickly resolved. Pharmacokinetics assessment was performed in the 88.2% of cases before starting prophylaxis. The other four patients, ¾ with severe FXIII, remained on-demand treatment. One of them experienced a subsequent severe ICH.

Conclusions: This is a first survey on real-world use of catridecacog performed in patients with FXIII deficiency. The efficacy and safety of rFXIII, used in most cases at the recommended dosage and frequency, was proven in all treated patients. The role of prophylaxis to prevent recurrences was also highlighted.

PB1207 | Severe Factor V Deficiency Caused by Two Novel Pathological *F5* Variants - First Insights into their Effect on Factor V Function

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Background: Autosomal recessively inherited FV deficiency is associated with a bleeding tendency of variable severity, but phenotype determinants are largely unknown. Recently, a 2.5-year-old girl presenting with recurrent mild bleedings (e.g. oral, already before dentition, easy bruising, and delayed wound healing) was referred for evaluation. Coagulation tests identified a severe FV deficiency explaining the mild bleeding disorder.

Aims: Our aim was to identify the patient's F5 variants and evaluate their impact on coagulation factor V synthesis and function.

Methods: The patient's *FV* gene was sequenced on an Ion Torrent S5. We confirmed found F5 variants by Sanger sequencing in the patient and her parents. We performed thromboelastometry, and assessed thrombin generation (TG) in platelet poor plasma (PPP) trigged by~ 5pM tissue factor (TF) with/without thrombomodulin or by~ 20pM TF by the automated STGenesia.

Results: The patient was found to be compound heterozygous for two novel *F5* variants, a missense variant p.Pro2098Arg (annotated according the LOVD FV database) inherited form her father, and a premature stop, p.Gln1598* on the maternal allele. Coagulation tests showed a prolonged PT of 59s and an aPTT of 116s, in the presence of FV:C < 5%, while all other factors, TT, and fibrinogen were normal. A partial FV deficiency was found in the both parents in line with their heterozygous carrier status. The patient's whole blood clot formation assed by thromboelastometry was prolonged. In patient's PPP, thrombin generation in the presence of low TF (~ 5pM) was absent, and reduced with high TF (~ 20pM; ETP 18% Lag Time ratio: 6.3 and PH: 7.0%). Both parents had normal TG with either TF concentration, however, thrombomodulin resistance (ETP inhibition 23%) was noted in the father's PPP.

Conclusions: The demonstration of the pathogenic role of the novel compound heterozygous variants requires further experimental validation.

PB1208 | Comparative Analysis of Three Custom Gene Panels Based on Next Generation Sequencing for Diagnostic of Inherited Coagulation Bleeding Disorders

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Background: Gene panels based on next generation sequencing (NGS) are a valuable diagnostic tool for congenital coagulopathies. Nowadays, there are several alternatives for panel design. Since 2014, our laboratory has designed and tested three different custom gene panels for diagnosis of bleeding disorders.

Aims: Compare the performance of three NGS gene panels in routine diagnostic activity.

Methods: All gene panels tested were amplicon-based: GeneRead, 4 tube multiplex-PCR; LP 48.48, 48 multiplex-PCR in an integrated

TABLE 1 Characteristics of the three custom gene panels designed for molecular diagnosis of congenital coagulopathies

Gene Panel	Year of implementation	Workflow Time (total time)	€ Library prep/sample	# Samples processed	DNA concentration (ng/µL)	# Genes	Pools/sample	# Amplicons	Coverage
GeneRead panel (QIAGEN)	2014	10 hours	50	>500	>4	23	4 multiplex PCR	1,285	98.7%
LP 48.48 integrated fluidic circuit (IFC) (Fluidigm Corporation)	2017	9.5 hours	11	>300	50-100	30	48 pools in IFC	697	99.3%
CleanPlex Custom NGS Panels (Paragon Genomics)_A+B	2019	3 hours	64	>150	10	16	2 multiplex PCR	479	99.3%
CleanPlex Custom NGS Panels (Paragon Genomics)_ Subset A						4	2 multiplex PCR	195	99.2%
CleanPlex Custom NGS Panels (Paragon Genomics)_ Subset B						12	2 multiplex PCR	284	99.4%

fluidic circuit; CleanPlex, 2 tube multiplex-PCR (Table 1). Sequencing was performed in an Illumina MiSeq instrument.

Results: GeneRead requires to process ≥ 24 samples simultaneously to be cost-effective as it has a 2 days' workflow but is affordable in basic laboratories. LP needs samples at higher concentrations and 48 are required to fill the fluidic circuit. This is a very cheap protocol but specific equipment is necessary. CleanPlex is the most flexible, as few samples can be processed and the panel can be divided into two subsets of genes depending on the clinical orientation. Moreover, it is the fastest protocol with only 75 minutes of hands-on time. A minimum coverage of 10X was established to be considered acceptable in variant calling analysis. Under these settings the number of failed exons (in > 5% of samples) were: 19/332 in GeneRead, 16/400 in LP, and 11/239 in CleanPlex.

Conclusions: All tested panels were useful for an accurate molecular diagnosis of congenital coagulopathies. GeneRead and LP are the most cost-effective for large number of samples, while CleanPlex is the most versatile regarding the number of samples and genes analyzed simultaneously. To sum up, several parameters have to be considered to select the panel that best fits our requirements: number of samples, available facilities, number and size of selected genes, turnaround time and determination price.

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PB1209 | Unclassified Bleeding Disorder (UBD)-Bleeding Phenotype and Management

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Background: Patients with UBD experience spectrum of bleeding symptoms and there is no standardised management.

Aims: Review bleeding patterns and outcomes in patients with UBD.

Methods: Retrospective cohort analysis of patients with UBD at Katharine Dormandy Haemophilia and Thrombosis Centre, Royal Free Hospital, London and Coagulation Centre Rhine-Ruhr, Duisburg, Germany.

Results: Out of 4969 patşents referred to both clinics over 10-year period, 162 patients with UBD were identified (3.2% incidence).

All patients underwent full haemostatic assessment with normal results.

109 (68%) were female and 52 (32%) male. Mean age at diagnosis was 25 years (range 2 months-84 years). There was only one case of consanguinity.

Leading presenting symptoms in women were heavy-menstrual bleeding (HMB) (32%), haematoma/easy bruising (26%), post-operative bleeding (16%) and epistaxis (15%). Most frequent symptoms leading to diagnosis in men were epistaxis (71%), haematoma/easy bruising, post-operative bleeding and bleeding after tooth-extraction were all equally prevalent (6%).

19% incidence of anaemia (range 8.3-11.9 g/dL), all received oral iron.

Mean BAT score was 3.56 (range 1-17) and in women mean PBAC score was 266 (range 68-1085).

24 patients experienced acute bleeding episode requiring A&E attendance, with female:male ratio of 9:1. Postpartum haemorrhage (63%) and HMB (25%) were the commonest emergencies. Blood transfusion was required in 10 patients.

33 patients underwent 59 major/minor surgeries. Haemostatic cover was administered in 38 cases (65%); Tranexamix acid (TXA) (n = 27), DDAVP (n = 8), platelet transfusion (n = 3). There were five cases of post-operative bleeding requiring blood transfusion.

Of women with HMB, 39% suffered from menarche. 30% received TXA, 25% combined hormonal-contraception and 14% combination therapy. Six women required hysterectomy.

Conclusions: Patients with UBD suffer from a variety of bleeding symptoms and are at risk of acute bleeding. Women are more likely to suffer with bleeding symptoms leading to diagnosis, with HMB being their commonest symptom.

PB1210 | Whole Exome Sequencing Approach to Address the Genetic Heterogeneity of Inherited Platelet Disorders

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Background: Inherited platelet disorders (IPDs) have variable clinical presentations and their genetic basis is highly heterogeneous. Mostly, the molecular characterization of these patients is currently not feasible in routine clinical diagnosis through Sanger sequencing. However, Next Generation Sequencing (NGS) offers new diagnostic and research possibilities as it allows the simultaneous study of larger numbers of genes at a low cost.

Aims: To address the genetic analyses of IPDs through a Whole Exome Sequencing (WES) strategy with differential analysis based on the clinical and laboratory phenotype.

Methods: Twenty-three patients with IPDs diagnosis were recruited. The WES was performed using the *Nextera Flex for Enrichment* kit which ran on a *NextSeq 500* system (Illumina). BWA Enrichment and Variant Interpreter tools were used for the analysis and filtering of genetic variants based on population frequency, quality, consequence and candidate genes related with IPDs.

Results: Before filtering, an average of 41,169 variants per patient were detected. In 12 patients, 15 heterozygous variants were prioritized in candidate genes. Of them, 9 variants were predicted to be pathogenic or likely pathogenic and/or were previously described (Table 1) and 6 variants were of uncertain significance. In the remaining 11 patients, the variants identified did not fully correlated with clinical data, but the possibility to extend the study to new genes and re-analyse the results may help to identify new variants. Furthermore, the identification of 10 variants in coagulation genes could contribute to explain the presence of bleeding.

Conclusions: The possibility to enlarge the panel for new candidate genes makes WES ideal for research and diagnosis of genetically heterogeneous diseases. The application of this strategy will contribute to elucidate the role of the different actors in IPDs improving the quality of care of these patients.

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TABLE 1 Patients with the 9 candidate genetic variants related to inherited platelet disorders predicted to be pathogenic or previously described

ID	Suspected disease	Gene	Gene function	Inheritance	Nucleotide change	Aminoacid change	dbSNP	InterVar prediction	References
RBDS0001	Thrombocytopathy/ thrombocytopenia	*ACTN1*	Cytoskeletal protein	AD	c.137G>A	p.(Arg46Gln)	rs387907348	Likely Pathogenic	Kunishima S *et al.*
RBDS0002	May-Hegglin anomaly	*MYH9*	Cytoskeletal protein	AD	c.5797C>T	p.(Arg1933Ter)	rs80338835	Likely Pathogenic	Kelley MJ *et al.*
RBDS0003	Thrombocytopathy	*ITGA2BTUBB1*	Agonist platelet receptorCytoskeletal protein	AD/ARAD	c.998 + 1G>Ac.35delG	-p.(Cys12LeufsTer12)	-rs773248042	PathogenicUncertain Significance	-Bastida JM *et al.*
RBDS0008	Familial macrothrombocytopenia	*GP9*	Agonist platelet receptor	AR	c.395T>G	p.(Leu132Arg)	-	Likely Pathogenic	-
RBDS0010	Familial thrombocytopenia	*ACTN1*	Cytoskeletal protein	AD	c.673G>A	p.(Glu225Lys)	rs387907350	Likely Pathogenic	Kunishima S *et al.*
RBDS0012	Familial thrombocytopenia	*CYCS*	Transcription Factor	AD	c.193C>G	p.(Leu65Val)	-	Likely Pathogenic	-
RBDS0023	Familial thrombocytopenia	*ACTN1*	Cytoskeletal protein	AD	c.2255G>A	p.(Arg752Gln)	rs387907347	Likely Pathogenic	Kunishima S *et al.*
RBDS0026	Familial thrombocytopenia, abnormal platelet function	*GFI1B*	Transcription Factor	AD/AR	c.814 + 1G>A	-	-	Pathogenic	-

PB1211 | Safety of Antithrombotic Therapy in Subjects with Hereditary Hemorrhagic Telangiectasia

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Background: Medical conditions requiring antithrombotic therapy (AT) may develop in subjects with hereditary hemorrhagic telangiectasia (HHT). However, safety of AT in HHT is uncertain and the only data currently available derive from retrospective analysis.
Aims: The aim of our study is to objectively determine number and severity of bleeding complications in patients with HHT treated with AT.
Methods: We performed a retrospective analysis of the database of our HHT Center and identified 26 patients who had received AT, for a total of 30 courses. Number and severity of hemorrhagic events were determined by medical record review and dedicated interviews. We also performed a prospective study, by enrolling 15 HHT subjects treated with AT and registering hemorrhagic events during follow-up.
Results: The retrospective analysis of the 30 AT courses (mean treatment period 18.5 ± 22.6 months) revealed three major bleedings (two gastrointestinal bleedings, one hematuria), no clinically relevant non-major (CRNM) bleedings, and no minor bleedings, except for epistaxis. Worsening of epistaxis, assessed with the Epistaxis Severity Score (ESS), was reported during 9/30 AT courses. There were no significant changes between the mean ESS reported before and during AT. Likewise, there were no significant changes in the mean hemoglobin levels before and after initation of AT (Table 1).

TABLE 1 Characteristics of the population and bleeding complications in the retrospective study

Number of AT courses / Number of patients	30/26
Gender (male/female ratio)	14/12
Mean age of patients (years±SD)	59.3 ± 17.0
Mean treatment period (months)	18.5 ± 22.6
Worsening of Epistaxis (n/total of AT courses)	9/30
Mean ESS±SD during AT versus prior to AT	4.1 ± 2.6 vs 3.8 ± 2.2 (95% CI -0.5682-1.155, p = 0.4918)
Major bleedings (n/total of AT courses)	3/30
CRNM bleedings (n/total of AT courses)	0/30
Minor bleedings different from epistaxis (n/total of AT courses)	0/30
Hb levels (gr/dl±SD) during AT versus prior to AT	10.8 ± 2.2 vs 11.1 ± 2.5 (95% CI -0.9035-0.3102, p = 0.3256)

TABLE 2 Characteristics of the population and bleeding complications in the prospective study

Number of AT courses / Number of patients	15/15
Gender (male/female ratio)	8/7
Mean age of patients (years±SD)	67.5 ± 13.4
Mean follow-up (months)	8.9 ± 2.4
Worsening of Epistaxis (n/total of AT courses)	1/15
Mean ESS±SD during AT versus prior to AT	3.2 ± 2.6 versus 3.9 ± 2.8 (95% IC -3.2-1.5, p = 0.2988)
Major bleedings (n/total of AT courses)	0/15
CRNM bleedings (n/total of AT courses)	1/15
Minor bleedings different from epistaxis (n/total of AT courses)	0/15
Mean Hb levels (gr/dl±SD) during AT versus prior to AT	11.5 ± 2.5 versus 11.4 ± 2.9 (95% CI -1.5-1.5, p = 0.9609)

In the prospective study (mean follow-up 8.9 ± 2.4 months), we reported one CRNM bleeding (hematuria). No major and minor bleedings, except for epistaxis, were registered. Worsening of epistaxis, upon initiation of AT, was documented only in one patient. There were no significant changes in the mean ESS and in the mean hemoglobin levels measured before and during AT (Table 2).
Conclusions: Our study suggests that HHT patients may tolerate AT and that antithrombotic agents may be used in a sufficiently safe manner in these patients, when required and when prescribed by experienced physicians in a multidisciplinary setting.

PB1212 | Inherited Factor VII Deficiency Associated with Thrombo-hemorrhagic Phenotype: A Case Report

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Background: Factor VII deficiency is a rare autosomal recessive bleeding disorder characterized by a deficiency or reduced activity of clotting factor VII (FVII). Bleeding in severe FVII deficiency can sometime result in life-threatening complications. However, also a seemingly paradoxical increased incidence of clotting (thrombosis) has been noted in FVII deficiency in recent years.
Aims: We want to point out the coincidence of serious hemorrhagic as well as thrombotic complications in the same individual with severe inherited FVII deficiency.
Methods: Description of a lifelong clinical course in 70-year-old female with severe inherited FVII deficiency and repeated thrombo-hemorhagic complications.

Results: A woman born in 1950 with severe inherited FVII deficiency (FVII: 1%). Clinically, she suffered by reccurent menorrhagia and hypermenorrhea in the adolescent age associated with anemia (anemia for most of her life), gingivorrhagia, later also GM epilepsy. In 1983-1985 patient had repeated radiotherapy targeted to the uterus due to major hypermenorrhea. Surprisingly, in 1990 she had to be anticoagulated with LMWH because of iliofemoral thrombosis and diabetes mellitus typ 2 (DM2) was discovered (from 1992 treated with insulin). In 1991 hysterectomy was performed due to reccurent menorrhagia. Later she suffered by attacks of erosive esophagitis, gastritis, duodenitis and reccurent epistaxis. Then in 2004, the thrombosis of a. and v.centralis retinae was unexpectedly diagnosed and treated. In 2005 reccurent hematuria and hemophtalmus developed. In 2018 patient started her hemodialysis sessions due to the end stage kidney disease (ESKD) caused by diabetic nephropathy and one year later the serious cerebral hemorrhage led to death of patient.

Conclusions: Despite of the fact that bleeding complications are the most common clinical manifestation also the serious thrombotic symptoms can simultaneously develop in patients with inherited FVII deficiency as depicted in our case report.

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PB1213 | Evaluation of the Skeletal Lesions in the Lebanese Patients with Afibrinogenemia

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Background: Afibrinogenemia is a rare hereditary bleeding disorder associated with severe bleeding phenotype and thromboembolic event. It is characterized by absence of fibrinogen with a complex physiopathology. Bones pain related to bones cysts have been described as rare complication

Aims: We report the clinical characteristic of bone pain in the Lebanese afibrinogenemic patient.

Methods: It is a retrospective descriptive study in a well-defined cohort during a follow up of 16 years.

Results: 20 patient complain of spontaneous recurrent skeletal pain with no obvious trigger factor. There was 7/29 women and 13/39 men. Pain was described from age 5 till 25 with a maximum of crisis at the adolescence period. There is no specific rhythmicity. Some patients do not complain anymore. Pain was localized in the femur, tibia, humerus and spine. It was described as intermittent low intensity crises at the beginnings with periods of rest the first 24 h. It increases over time to became continuous intolerable preventing sleep and activity after 48 h. Pain resolved after fibrinogen substitution. A substitution with a target level of 1 g prevent crisis 15 to 21 day. There was no correlation with spleen rupture or thromboembolic event. No fracture was observed in the cohort.

MRI of femur, tibia, humerus and spine was performed in 10 patients on several time during follow up. Several lesions were observed: abnormal intra-medullary fluid signal with heterogeneous T2 and T1 hypersignal and intermediate signal T1 in connection with subacute hemorrhagic suffusions, diffuse central medullary bone edema of all the diaphysis and metaphysis, circumferential signal anomalies type hyper T2 around the cortex of the diaphysis in connection with a probable periostitis and lack of fluid in the joints. the study of the MRI lesions promotes hemorrhagic etiology.

Conclusions: Bone pain is a frequent complication that could be prevented by prophylaxis. Studies are needed for a better understanding.

PB1214 | Thrombotic Events in the Patients with Haemorrhagic Disorders of Haemostasis

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Background: In spite of the natural bleeding pattern of the inherited haemorrhagic disorders of haemostasis, these diseases do not protect particular patients from the development of the thromboembolic event. Dominant risk factors for the thrombosis in these individuals are the traditional risky features, such as arterial hypertension, smoking, hyperlipidaemia, immobilization, advanced age, pregnancy, surgery and also the coincidence of the thrombophilic state, substitution therapy and the use of by-passing agents.

Aims: To present the topic of the coincidence of bleeding and thrombotic episode.

Methods: The authors describe the case reports of the individuals with bleeding disorder of haemostasis who developed thromboembolic episode and report on the successful treatment of their complicated clinical states.

Results: In all cases, the antithrombotic therapy was used without any complications and repetition of the thrombotic events.

Conclusions: Older patients with haemorrhagic disorders of haemostasis have the prothrombotic risk features, that are comparable with the risky factors of general population. In the case of the coexistence of more than one such prothrombotic risk factor, the risk of the development of thrombosis increases. Globally, large studies providing the universal guidelines for such challenging situations are missing. Thus, the combination of the substitution and antithrombotic therapy ought to be individualized.

Keywords: Bleeding, thromboembolic episode, antithrombotic treatment

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Informed consent of the patients was obtained and the study was approved by a medical Ethics committee of the Jessenius Faculty of Medicine in Martin.

NURSES AND ALLIED HEALTH

PB1216 | Warfarin - A Real Threat in Evaluation of Acute Poisoning in the Emergency Department (ED) of a Tertiary Care Hospital - A Base Line Study to Make a Strategy for Better Patient Outcome

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Background: Warfarin toxicity is an increasing concern globally. Accidental and intentional poisonings is a significant source of morbidity, mortality, and health care expenditure around the world. Unfortunately, limited literature on toxicological data in Pakistan available. Information about acute poisoning is essential for the prevention and management.

Aims: Evaluation of Acute Poisoning cases in the emergency department (ED) of a tertiary care hospital in Karachi, Pakistan.

Methods: Retrospectively medical records were reviewed of patients presenting with acute poisoning in ED between 2011 to 2016. Total 892 cases of various acute poisoning due to drugs and chemicals in people of all ages were reviewed. Data regarding age, sex, name of poisonous substances, and outcome were reviewed. Poisons involved were classified into 3 categories: pharmaceuticals agents, pesticides (organophosphorus agents) and also non medicinal substances (food poisoning).

Results: Based on data review, total 892 patients presented with acute poisoning in which 512(57.39%) patients were female and adults were 614 (68.83%). A majority of the poisoning cases were due to miscellaneous drug overdose. Total drug overdose patients were 405 in which adults were 363(89.62%). Warfarin toxicity cases presented in total 217 patients in which geriatrics were 131(60.36%). Organophosphorus poisoning cases were 26 in which adult were 25(96.15%). 53 cases presented with Food poisoning in which 34 (64.15%) were peads.

Conclusions: These up-to-date data provide us important information which serve as a baseline to make a guidelines to improve activities such as professional trainings, preventive measures, community education and new research across Pakistan. Warfarin finding is very significant and it gives us clue to work on this important toxicity which is a neglected area of concern in developing country.

Keyword: Warfarin, a Real threat, Acute poisoning, Emergency deartment (ED), Tertiary care hospital, Patient outcome.

PB1217 | Implementation of Prefilled Doses- A Novel Approach to Decrease Medication Time to Administration and Errors In Critically Ill Pediatric Patients of Emergency Department (ED)

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Background: Pediatrics are a vulnerable group for dosing and dispensing errors .Most of the drugs used are available in dosages and units ready for dispensing in adults. This needs a lot of calculation and has a potential for errors. Further, ED has a high volume and acuity of work adds to errors.

Aims: The aim of these initiatives was to enhance medication safety in pediatric Emergency through implementing pre-filled syringes of selected IV medications.

Methods: The aim of these initiatives was to enhance medication safety in pediatric Emergency through implementing pre-filled syringes of selected IV medications.

Results: We retrieved data from January - December 2017 to see the impact of the project. Increased utilization of all IV pre-filled doses were analyzed (total magnesium sulphate 97, 3% HTS = 271, paracetamol = 1591 consumed during mentioned period) than calculated the cost saving on each dose .Total cost saving of all 3 selected pre-filled syringes were found to be Rs 151905.

Conclusions: Results showed that the use of pre-filled doses not only saved the cost and time per administration but also enhance patient safety by decreasing the chance of medication error. This novel dosing approach may impact on standard resuscitation practices in this unique patient population. In future we will try to incorporate more pre-filled doses. This project is continuing successfully and in coming days, we are planning to incorporate more pre filled doses for ED admitted peads.

Keywords: Prefilled Doses, Critically Ill Pediatric Patients, Emergency Department (ED), Emergency Department (ED).

PB1218 | Socio-Demographic Data and some Haematological Parameters of Nigerian Children Living on the Street: A Comparative Study

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Background: Homelessness is a problem in urban communities worldwide particularly when children fend for themselves on the street. Haematology is greatly influenced by proper nourishment and care in growing children.

Aims: The study's aim was to assess the socio-demographic profile and some haematological parameters of children living on the street in Calabar, Nigeria.

Methods: A total of 180 male and female children aged between 6 and 16 years, were enrolled, comprising ninety children living on the street at Mary Slessor Avenue and Lemna Road, Calabar. With

TABLE 1 Socio-Demographic data of children living on the street versus children living in homes

Parameter		Children living on the street n = 90	Children at home n = 90
Mean Age (years)		11.91 ± 2.19	12.91 ± 2.61
Gender	Males n(%) Females n(%)	48 (53.3) 42 (46.7)	32 (35.6) 58 (64.4)
Education	Primary n(%) Junior Secondary n(%) None n(%)	10 (11.1) 6 (6.7) 74 (82.2)	60 (66.7) 30 (33.3) 0 (0.0)
Survival strategies (street children)	Street dependence n (%)	90 (100)	-
	Support group n (%)	56 (62.2)	-
	Odd jobs n (%)	54 (60)	-

TABLE 2 Body mass index and some haematological parameters of children living on the street versus control

Parameter		Children on the street n = 90	Children at home n = 90	P-value
Body mass index	(Kg/m2)	18.21 ± 3.33*	21.06 ± 3.68	0.001
Packed cell volume	(L/L)	0.37 ± 0.08	0.39 ± 0.06	0.590
Haemoglobin	(g/L)	120..10 ± 20.5	124.70 ± 18.8	0.270
Total white cell count	(x109/L)	7.87 ± 4.18*	5.90 ± 1.94	0.005
Platelet count	(x109/L)	280.69 ± 198.31	216.31 ± 120.79	0.066
Serum iron	(μmol/L)	34.10 ± 7.81	33.35 ± 9.61	0.683
Total iron binding capacity	(μmol/L)	51.54 ± 9.89	52.82 ± 11.65	0.577
Transferrin	(%)	35.24 ± 8.39	37.93 ± 9.25	0.152
Serum ferritin	(ng/ml)	100.67 ± 24.18	111.00 ± 31.57	0.085

parental consent, ninety healthy, age and gender- matched children who live in homes were selected from Lourdes Academy, a private school in Calabar, as control. Socio-Demographic data was compiled by interview. Weight and height were measured and body mass index (BMI) calculated. The packed cell volume, haemoglobin, total white cell and platelet counts were determined by standard manual techniques. Serum iron and total iron binding capacity were determined by colorimetry, serum ferritin by enzyme linked immunosorbent assay while transferrin saturation was calculated. Data were analyzed using student t-test on statistical package for social sciences version 21. Significance was set at $P \leq 0.05$.

Results: Results showed that more males were found on the street (53.3%) with the majority (82.2%) having no formal education. All children living on the street depend fully on the street, 62.2% benefits from support groups with 60% engaged in odd jobs. The BMI of children on the street was significantly lower while total white cell count was significantly higher ($P < 0.05$) versus those at home. Other measured parameters were comparable ($P > 0.05$).

Conclusions: The lower BMI of children living on the street implies slower growth compared to control while higher white cell count suggests a response to exposure to infectious agents and environmental hazards.

PB1219 | Knowledge and Attitude towards Safe use of Direct Oral Anticoagulants (DOACs) among Doctors, Nurses and Pharmacist in a Developing Country, Karachi, Pakistan

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Background: Direct oral anticoagulants are high alert medications as per ISMP. It is imperative for healthcare professionals to have adequate knowledge for their safe handling.

Aims: This study aimed at evaluating knowledge and attitude of various health professionals towards safe use of direct anticoagulants (DOACs) in two hospitals setting.

Methods: This study was conducted at two large academic care centers including one private (Aga Khan University hospital) and one public sector hospital (National institute of cardiovascular institutes) during January 2019. Study was ethically approved by ERC of both hospitals

(ERC2019-0763-1198, ERC 05/2019). A survey questionnaire was developed to evaluate critical knowledge related to dosing, indications, laboratory monitoring and antidotes of DOACs. SPSS version 21 was used for assessment of cognitive scores and stratified for age, gender, profession and as per experience for using DOACs. Attitude and behavior were recorded using a 5-point Likert's scale and analyzed through SPSS AMOS for regression analysis.

Results: 445 of 550 participants responded while 15 forms were rejected. Final response rate was 78%. There were 234 female (54.4%) and 196 (45.6%) male participants and included 63.3% nurses, 8% pharmacists and 28% residents & 8% consultant doctors. There were 15% participants who had never used DOACS .Overall better scores were observed for pharmacokinetics (61%), bridging (62%) and patient safety (60%) while borderline scores were obtained for reversal (56%), indication usage (50%)and laboratory monitoring (48%). No difference in knowledge with respect to age group and gender was observed .Pharmacists were more knowledgeable for pharmacokinetics and antidote compared to other groups. Value of R^2 was 0.623 indicating moderate correlation between independent and dependent variable for estimation of behavior with Cronbach's Alpha of 0.712

Conclusions: The study identified knowledge gaps for DOACS. Seminars and discussion need to be organized for health professionals to improve safe use of DOACS.

PB1220 | A Nurse Led Service Providing Safe and Effective Peri-Operative Anticoagulation

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Background: Withdrawal of anticoagulation around surgery and medical procedures presents risks of bleeding and thrombosis. There is also the risk of cancellation of vital procures due to anticoagulation not being withdrawn appropriately or inadequate coordination and communication between the anticoagulation team and operating teams.

Aims: An established service to ensure appropriate peri-operative anticoagulation and follow up was audited to establish rates of cancellation, numbers of peri operative thrombotic events and post procedure bleeding events.

Methods: Referrals are made electronically by the pre-operative assessment nurses for all anticoagulated patients requiring surgery in the hospital. A peri-operative anticoagulation plan is created by the anticoagulation specialist nurses on a database. The plans are approved by the lead specialist nurse or the lead consultant haematologist. The plan is uploaded to electronic records for review by surgical teams.

The patient is phoned by the anticoagulation nurses 1-2 weeks pre procedure and informed of the date they are to stop their oral anticoagulation. All patients taking Warfarin are given a 2-3 day pre-operative appointment where the INR is checked and bridging commenced with injectable anticoagulants if indicated. Those requiring bridging are taught self-injection or community nursing support is arranged. Oral vitamin K is administered to those who have an INR > 2.0 at the appointment.

Post-operative follow up is arranged.

The patients were phoned by the anticoagulation nurses post procedure to audit the following:

- Procedure cancellation rates.
- Adherence to peri-operative plan.
- Peri-operative thrombosis rates
- Post-operative bleeding rates

Results: Patients adhered to recommended plans well

Cancellation of procedure was almost always not related to anticoagulation but other reasons.

Thrombosis rates were negligible.

Severity and character of bleeding was noted.

Conclusions:

- Patient peri-operative adherence was good
- Cancellation of procedures due to anticoagulation was prevented
- Peri-operative thrombosis was not a problem
- Post-operative bleeding occurred only among those bridged with low molecular weight heparin.

PB1221 | Effect of Foot Bath on the Plasma Concentration of Plasminogen Activator Inhibitor-1 Produced by Endothelial Cells in Healthy Subjects

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Background: Endothelial cells express various fibrinolytic factors such as tissue plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1), which play an important role in adequate blood clot formation. Foot bath is a common nursing technique and previously it has been reported that foot bath has various effects such as a sedative effect on the autonomic nervous system and a preventive effect against diabetic feet. However, preventive effect of foot bath against thrombosis has not been reported.

Aims: In the present study, to evaluate the effect of foot bath on prevention of thrombosis, changes of plasma concentrations of PAI-1, which are produced by endothelial cells and function as a physiological inhibitor to t-PA, were measured during and after foot bath using water of various temperatures.

Methods: We divided healthy volunteers into 37°C, 40°C and 43°C foot bath groups, and obtained plasma samples from the subjects of each group before foot bath, immediately after 10 and 20 min foot bath, and also 10 min and 20 min after 20 min foot bath. Plasma PAI-1 concentration was measured using commercially available ELISA kit.

Results: Plasma PAI-1 concentration at each time point of 37°C and 43°C foot bath was not significantly changed as compared with that before the foot bath. However, plasma PAI-1 concentration in "10 min foot bath" and in "20 min after 20 min foot bath" using 40°C water was significantly decreased as compared with before foot bath. In addition, minimum PAI-1 concentration for each subject in every temperature of foot bath during and after foot bath shows a

significant overall decrease as compared with that in the same subject before foot bath.

Conclusions: These results suggest that foot bath reduced PAI-1 expression from endothelial cells and/or secretion of PAI-1 from endothelial cells, and foot bath using 40°C water has a preventive effect against thrombosis.

PB1222 | Elderly Patients with Atrial Fibrillation Have Higher Risk of Geriatric Syndromes: Results of the Nursing Study

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Background: The prevalence of atrial fibrillation (AF) and geriatric syndromes (GS) increases with age, however the associations between these conditions have not yet been studied.

Aims: To evaluate the associations between AF and GS in elderly patients.

Methods: Four hundred and seventy-two in-patients (18% male) aged 75-100 (mean 81 ± 4) years were included in the nursing study. Patients were examined by nurses on a specially designed nursing assessment algorithm that included Mini-Cog test, Morse Fall scale, Norton scale, Barthel Activities of daily living Index, Short Physical Performance Battery (SPPB) tests, and a number of other questionnaires.

Results: AF was observed in 104 patients (22%). CHA_2DS_2-VASc score was 5,6 ± 1,4 points. HAS-BLED score was 2,9 ± 0,7 points. Patients with and without AF did not differ by age and gender. AF patients had lower points on Norton scale (17,4 ± 2,1 vs 18,2 ± 1,7; p < 0,001), Barthel Index (89,3 ± 16,7 vs 95,1 ± 8,7; p < 0,001), SPPB (4,9 ± 3,2 vs 6,0 ± 3,3; p = 0,002), and higher points on Morse Fall scale (46,3 ± 19,5 vs 39,8 ± 20,9; p = 0,004) compared non-AF patients. AF patients had higher frequency of pressure ulcers high risk (4,8% vs 0,8%; p = 0,015), frailty (79% vs 66%; p = 0,015), and functional dependence (Barthel Index ≤ 60 points) (5,8% vs 1,4%; p = 0,018). CHA_2DS_2-VASc score was correlated with Norton scale (r = -0,29; p = 0,003), Barthel Index (r = -0,24; p = 0,015), and SPPB (r = -0,32; p = 0,001). Univariate regression analysis showed that presence of AF increases the frailty risk in 1,9 times (OR 1,89; 95% CI 1,13-3,18; p = 0,016), functional dependence risk - in 4,5 times (OR 4,45; 95% CI 1,33-14,87; p = 0,015), and pressure ulcers high risk - in 6,2 times (OR 6,15; 95% CI 1,44-26,15; p = 0,014). Also in AF patients, increasing CHA_2DS_2-VASc score by 1 point increases the frailty risk on 79% (OR 1,79; 95% CI 1,18-2,73; p = 0,007).

Conclusions: Elderly AF patients have higher risk of GS such as frailty, pressure ulcers high risk, functional dependence in daily living.

PB1223 | Monitoring of Adverse Event of INR > 6 Using IHI Global Trigger Tool in Cardiology Hospital, Pakistan

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Background: The IHI Global Trigger Tool for Measuring Adverse Events provides an easy-to-use method for accurately identifying adverse events (harm) and measuring the rate of adverse events over time. It is also strongly recommended by the Food and Drug Administration (FDA), the Joint Commission on Accreditation that Hospitals must have active surveillance system for safe use of medications and detection of any Adverse event in system.

Aims: Tabba heart institute provides specialty base cardiac services. All hospitalized patients went through high risk procedures and they treated with high risk medications. Aim of the study is to closely monitored the adverse event of patients on warfarin whose INR goes > 6 during hospitalization and checked for evidence of bleeding. Warfarin is an important drug required more vigilance for patient safety.

Methods: Retrospective data of one year is evaluated from a key performance indicator to monitor the INR > 6 of all hospitalized patients. The data is measured through = INR > 6 of hospitalized patients/ total number of patients on warfarin *100.

Results: Software driven data for INR > 6 was evaluated from the period of January 2019 to December 2019. The mean percentage of INR resulting > 6 is 12.6%, which is a trigger. Highest percentage of INR > 6 reported in month of August 2019 (26.67%). Reasons for INR > 6 include poor patient compliance, lack of focused guidance to the patients and drug-drug interactions.

Conclusions: Our study results strongly recommends pharmacist supervised Warfarin therapy counseling should be initiated for this high risk cardiovascular population. Also, there is an opportunity to evaluate data for bleeding events on INR > 6 that might also result in mortality.

PB1224 | Recommendation on the Treatment of Venous Thromboembolism in Cancer Patients at a Brazilian Public Oncological Institution: Educational Plan

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Background: In October 2018, a recommendation on the treatment of venous thromboembolism in cancer patients was developed at

ICESP-HCFMUSP and included a new anticoagulant. To raise awareness on it an educational plan was organized and a pilot outpatient clinic was carried out to check patients eligibility to switch from enoxaparin to rivaroxaban. 'Alô Enfermeiro' is a 24/7 institutional call center managed by nurses. In order to empower them and ensure they would be able to reply accordingly, their training was prioritized.

Aims: To present results of the first 3 months of the recommendation, focusing on questions about rivaroxaban. To identify knowledge gaps on cancer associated thrombosis.

Methods: Healthcare professionals were trained presentially and with residual materials. Physicians and nurses of the pilot clinic were additionally trained on how to evaluate and educate patients on the new anticoagulant. A folder for patients was developed and locally approved before delivery. Knowledge gaps were identified by records of 'Alô Enfermeiro' and opinion requests for the thrombosis and hemostasis team from October 22nd 2018 to January 31st 2019. Results were described.

Results: From October 2018 to January 2019, 215 physicians, 363 nurses and 48 pharmacists of the institution were trained on the recommendation. All patients who had started rivaroxaban received the folder. During the 3 months of the pilot clinic, 57/358 patients (16%) on rivaroxaban called 'Alô Enfermeiro', 27 (47%) with questions about the drug (management facing invasive procedures, lab tests and chemotherapy, duration of anticoagulation and bleeding). Fourteen inpatients were evaluated by the thrombosis and hemostasis team due to rivaroxaban (eligibility to switch from enoxaparin, perioperative management, bleeding episodes and thrombosis recurrence). All requests could have been independently solved if physicians were more familiar to the recommendation.

Conclusions: Education was crucial to ensure a broad use of the recommendation. Knowledge gaps inspired a continuous educational program.

PB1225 | The Role of the Nurse in the Therapeutic Education of Hemophiliac Patients in Preparation for Physiotherapy

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Background: the care of the patient with hemophilia can only be multidisciplinary. Physiotherapy is as important and as effective a therapeutic means as substitution for deficient factor VIII or IX. Like all chronic diseases, patients with hemophilia must benefit from therapeutic education. TE plays a key role in the acceptance, compliance, regularity and success of physiotherapy sessions.

Aims: The interest of physiotherapy to preserve joint capital.

Methods: Patient training for physiotherapy and self-treatment began in January 2016. 21 patients underwent TE for their care in Physical Medicine and Rehabilitation at the University HOSPITAL in Western of Algeria Oran. 17 H.A severe and 0 4 H.B severe Educational objective: -The interest of physiotherapy to preserve joint capital, Hand washing, dosing calculation and product preparation, practical courses on peripheral venous approaches on artificial arms, Individually or in groups of 3 patients at the rate of one to two sessions per week. Hemophilia and waste management. -All procedures performed at home must be recorded in the hemophiliac's diary.

Results: The healthcare team should monitor the use of FAH and assess the effectiveness of the Physiotherapy and Self-treatment program. In 05 university students and 07 high school students, there has been a reduction in school absenteeism. Among the 09 salaried hemophiliacs, 06 report a significant improvement in the quality of life, with more autonomy and taking responsibility and less absenteeism at work. Self-treatment and physiotherapy allowed better preservation of joint capital and improved quality of life for hemophiliac patients.

Conclusions: The quality and safety of care are conditioned by a commitment on the part of the patients and carers while ensuring the application of safety measures as well as the quality of self-treatment and ensuring the continuity of physiotherapy sessions. to preserve joint capital and good muscle condition and introduce hemophilia patients to self-rehabilitation.

PB1226 | Effectiveness of a Self-management Program in the Treatment Control of Oral Anticoagulants Antivitamin K

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Background: To compare the efficacy of a self-monitoring programme vs. the conventional method used before the intervention in maintaining the international normalised ratio (INR) in the therapeutic range in patients receiving oral anticoagulants, as well as complications, quality of life and the time invested in the tests.

Aims: To compare the efficacy of a self-monitoring programme vs. the conventional method used in patients receiving oral anticoagulants, as well as complications, quality of life and the time invested in the tests.

Methods: Pre-pospilot and feasibility study. The study included 52 patients over the age of 18 years who had been attending the monitoring programme for more than 6 months. In the pre phase, patients performed the tests and follow-up in the outpatient clinic. After conducting an individual training session with each patient to teach them how to perform venipuncture, use the coagulometer, manage dosing tables and subsequent follow-up from the virtual clinic, we compared the percentage of in-range INR tests, complications, quality of life, and the time invested in performing the tests pre- (conventional) and post-intervention (intervention for self-monitoring).

Results: The percentage of INR tests in the therapeutic range was significantly higher in the post-phase than in the pre-phase (70% vs. 55%, $p < .001$). Likewise, the incidence of both minor and serious

complications decreased in the post-phase (20% vs. 0%, and 6.7% vs. 0%, respectively). Finally, all 5dimensions of the quality of life questionnaire improved significantly, while the time invested decreased.
Conclusions: In our experience, OAT self-monitoring is associated with a significant improvement in patient management, a reduction in the rate of complications, improved quality of life and timesaving.

PB1227 | Successful Development and Implementation of Pharmacist-led VTE Stewardship. An Australian Tertiary Hospital Perspective

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Background: Venous thromboembolism (VTE) is a leading cause of preventable death in hospital. Pharmacist-led interventions can improve prescribing of VTE prophylaxis, however the effectiveness of a dedicated VTE stewardship pharmacist to provide hospital-wide oversight has not been explored.
Aims: To determine if a VTE stewardship pharmacist for a 750-bed Australian tertiary hospital can increase:
(1) the prevalence of appropriate VTE prophylaxis prescription,
(2) VTE risk assessment using an electronic clinical decision support (eCDS) tool,
(3) any form of documented risk assessment and
(4) risk assessment within 24 hours of admission. Single-site data will form part of a planned multi-site study of four network hospitals.
Methods: Barriers to eCDS tool were identified. Stewardship interventions were implemented in two stages:
(1) multi-disciplinary education, daily medication chart auditing and weekly clinician performance feedback within 3 months, and
(2) health promotion and performance feedback gamification within 6 months. A cross-sectional audit of random electronic patient medical records (n = 150) was conducted at baseline, 3 and 6 months.
Results: Single site analysis found improvement in VTE risk assessment using the eCDS tool at baseline, 3 and 6 months (21%, 50% and 87%, respectively, $p < 0.05$) and any documented risk assessment (75%, 78% and 96%, respectively, $p < 0.05$). There was no improvement in prevalence of risk assessments made within 24 hours of admission. There was no difference in the prescription of appropriate prophylaxis (79%, 83% and 83%, respectively, $p = 0.63$). However, pooled results indicate using the eCDS tool was associated with appropriate VTE prophylaxis prescription (appropriate prophylaxis with eCDS 87%, without eCDS 76% $p = 0.004$). Multi-site analysis is currently in progress.
Conclusions: VTE stewardship pharmacists are a useful addition to the Australian hospital setting and can increase VTE risk assessment, particularly through encouraging use of eCDS tools. Planned multi-site analysis could highlight the full impact of VTE stewardship on prescribing.

PB1228 | Psychosocial Burden of Hemophilia in a Canadian Cohort

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Background: Patients with chronic diseases have increased levels of health-related (HR) anxiety, depression and stress, negatively affecting quality of life (QoL). In hemophilia, improvements in QoL have been associated with prophylaxis and access to safe factor concentrates. Literature discussing specific aspects of the psychosocial burden of hemophilia is lacking.
Aims: 1) Assess prevalence of HR depression, anxiety and stress
2) Evaluate health-related quality of life (HRQoL)

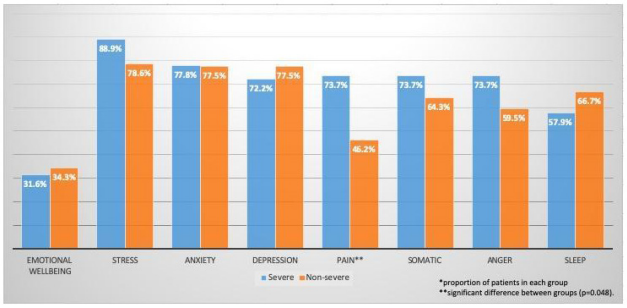

FIGURE 1 Trends for the Hemophilia Cohort in Psychosocial and HRQoL Scores

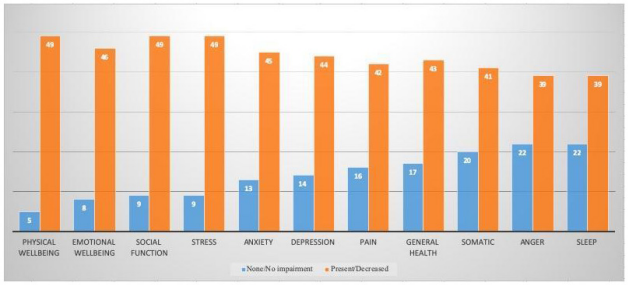

FIGURE 2 Trends in Psychosocial and HRQoL Scores by Severity*

Methods: 61 participants (>18 years) with hemophilia A or B completed the SF-36v2, HEM-QoL-A, DASS & DSM-5 cross cutting measures (designed to differentiate between HR depression, stress, anxiety and related symptoms). Results were analyzed using T-test/chi-square test as appropriate and grouped by disease severity. Informed consent was obtained. The study was approved by the University of Calgary Research Ethics Board.

Results: Most respondents reported greater than average amounts of HR stress (84.4%, 49/58), anxiety (77.6% 45/58), and depression (75.9%, 44/58) secondary to their disease. 64% (39/61) of participants reported disturbed sleep and increased anger concerning their disease. Somatic symptoms, related to outside perceptions of their illness, were reported by 65.6% (40/61) of participants (Figure 1). Most participants reported lower functioning across all SF-36v2 domains, however, only pain (p=< 0.001) and general health (p = 0.008) were significantly lower than Canadian normative data. Participants with severe hemophilia had significantly more pain (SF-36v2; t(1) = 3.9; p = 0.048), and reported more HR stress, anger and somatic symptoms than those with non-severe disease (Figure 2).

Conclusions: This study demonstrates that hemophilia negatively influences specific aspects of HRQoL for all patients, irrespective of severity. Participants reported significantly more pain and poorer overall wellbeing than the general population. The majority of respondents in this cohort reported increased anger and somatic symptoms; the relationship between these domains and hemophilia are unclear and require further study. Understanding the components of HRQoL is necessary for provision of holistic, multidisciplinary hemophilia care.

PB1229 | A Multi Centre Non-randomised Clinical Trial to Establish the Efficacy of Peer Reviewed Physiotherapy Treatment Protocols among PWH in India

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Background: This was done as part of a bigger service implementation project sponsored by NNHF to improve the physiotherapy resource for pwh in India.

Aims: Test efficacy of peer reviewed physiotherapy protocols.

Methods: Necessary protocols regarding data security, ethical approval for study, Pwh consent to participate was fulfilled. 3 peer reviewed physiotherapy protocols were developed as interventions. 5 institutes that participated in protocol development were intervention centres. 9 similar capacity centres for physiotherapy facilities who consented were included as Controls. We trained physiotherapists from intervention centres who provided treatment to eligible PWH as per protocols; PWH in control centres underwent routine physiotherapy.

Inclusion: Hemophilia A: 0 - 17 years

Exclusion: Children suffering from other bleeding disorders, muscle bleeds, entrapment neuropathy, associated HIV, Hepatitis-B, Hepatitis-C, on inhibitors, and amputees.

Outcomes: Standard Physical Evaluation, HJHS, FISH and monitoring Bleed episodes.

Data: The physiotherapists providing care recorded data on a web portal. PwH were measured at baseline, 6, 12 weeks.

Analysis: Repeated measures analysis of score for within the group trends and between group comparisons.

Results: Characteristics: 106 and 167 PWH were recruited into the study in intervention and control groups respectively. Both intervention and control groups had similar mean age (10 years), mean weight (30 kgs).

Outcomes: *FISH* - The scores in each of the components and the total significantly improved within both the intervention and control groups over time. When comparing the two groups, scores improved significantly in the Locomotion component of the intervention group.

HJHS - Improvement noted in HJHS knee scores within both groups, in left ankle scores in intervention group.

Bleed Episodes-Frequency of bleeding was significantly lower in intervention group as compared to control group from baseline to endline.

Conclusions: Intervention group did better for locomotion and ankle joints. Continued treatment with longer follow up will provide evidence for sustained effect.

PB1231 | Patient Compliance with Aspirin and Rivaroxaban Post Elective Joint Replacement Surgery: A Quality Assurance Initiative

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Background: Aspirin is a common, over the counter (OTC) medication. In conjunction with a five-day lead-in of Rivaroxaban, it is effective VTE prophylaxis for total knee & hip arthroplasty (TKA & THA) (EPCAT II study).

Aspirin may be thought of as a benign medication by patients given its non-prescriptive nature. Considering its role in VTE prophylaxis, it is important to ensure patient adherence to the full treatment regime [5 days Rivaroxaban then Aspirin 81 mg x 9 days (TKA) or x 30 days (THA)]. This retrospective quality assurance (QA) study analyzed compliance rates with Rivaroxaban and Aspirin in an elective TKA/THA population.

Aims: The main goal was to measure patient adherence with Aspirin post TKA & THA surgery. Patients were also assessed for GI symptoms with Aspirin use. Compliance with the initial five-day run-in Rivaroxaban was also evaluated.

Methods: Patients undergoing elective TKA or THA on or after December 1, 2019 were eligible. REB was notified; given the QA

nature of the study, formal REB approval was not required. Patients were called once VTE prophylaxis was completed so as not to influence compliance. To minimize recall bias, phone calls were completed within 60 days.

Results: 100 consecutive patients with TKA or THA surgery on or after December 1, 2019 were followed. Preliminary results on the first 50 patients show 96% compliance with each of Rivaroxaban and Aspirin. Of patients who were non-adherent, 25% reported no concern for VTE risk after surgery versus 48% of adherent patients. 98% of patients reported receiving adequate VTE education; some reported this as a contributor to compliance. 2% reported stomach upset. Complete analysis pending.

Conclusions: Interim results reveal no difference in adherence with prescription Rivaroxaban versus OTC Aspirin. Overall results show excellent compliance rates. Education on VTE appears to positively influence adherence. Aspirin was not associated with GI discomfort.

PB1232 | Canadian Guidelines Regarding Safe Blood-draw Volumes for Research in Pediatric Participants

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Background: Pharmaceutical research in pediatrics has increased in recent years. As children are exceptionally vulnerable, care must be taken in the design of research studies to ensure minimal risk. One essential contributor of risk is blood sampling. EU (European Union) and Food and Drug Administration (FDA) guidelines confirm it is the responsibility of the research ethics board (REB) and the investigator to ensure safe volumes are drawn. The guidance from the literature is highly variable (table 1) and is dependent on the age of the participant. The EU guidance is the most conservative at 1% of blood volume in 24 hours, maximum 3% over 4 weeks. Consideration of patient health and need for clinical testing must also be taken in account.

Aims: We performed a literature search to identify Canadian Guidelines for research blood sampling volumes in pediatric patients.

Methods: An OVID/Pubmed search was performed using the following search terms: pediatrics; humans/blood specimen collection/ blood volume draw guidelines; ethics, research/nursing methodology research/ operations research/ research/ clinical nursing research/pharmaceutical research/ nursing research; Canada.mp or Canada. A Google search was performed of guidelines for blood collection volume for pediatric research in Canada.

Results: No results were returned from the OVID/pubmed search. No guidance from Health Canada was found regarding the safe volume of blood collection from pediatric patients in research. No evidence-based Canadian studies were located. Guidelines found from Canadian REBs were identified from the Hospital for Sick Children, Children's Hospital of Eastern Ontario, University of British Columbia and Alberta Health Services (table 2). These guidelines were variable and referenced the World Health Organization and US-based guidelines and research.

Conclusions: There is a need to develop evidence-based guidance for Canadian sites regarding blood draw maximums for research involving pediatric patients to ensure minimal risk for participation.

TABLE 1 Blood Volume Draw Guidelines for Research in Pediatric Participants

Reference Institution	Single draw percent of Total Blood Volume (TBV)	Single draw in mL/kg	Total allowable cumulative draw volume
USC/LA Children's Hospital	2.5-2.7% (within 24 hours)	2 mL/kg (within 24 hours)	4 ml/kg within 30 days
Wayne State University	1%	0.8 mL/kg	10% TBV or 8 mL/kg within 8 weeks
Partners Human Research Committee	3.6-3.9%	<3 mL/kg	<3 mL/kg within 8 weeks
University of California Davis	2.5%	2 mL/kg	5% TBV within 30 days
KEMRI-Wellcome Trust Research Programme, Kilifi	1.9-2.3%	1.7-2.4 mL/kg	2008 guideline 1.3%TBV for research purposes in addition to volume needed for routine care
Gambia Government - MRC Joint Ethics Committee	0.3 to 2.4%	2 mL up to max 30 mL depending on age of child	Within 3 months same as for single draw maximum
EU Directive 2001/20/EC (2008)	1%	0.8 mL/kg	3% TBV during period of 4 weeks
Children's Hospital of Pennsylvania	6%	5 mL/kg	9.5 ml/kg maximum over any 8 week period

TABLE 2 Canadian Research Ethics Board Guidelines for Allowable Blood Draw Volume for Research in Pediatric Participants

Reference Institution	Single draw % of Total Blood Volume (TBV)	Single draw in ml/kg	Total allowable cumulative draw volume	References
Hospital for Sick Children REB	5% (divided portions)	3.75-4 mL/kg (divided portions)	5% TBV within 3 months	Pearson HA "Blood and Blood-forming Tissues" in Rudolph's Pediatrics, McGraw-Hill, 21st Edition 2003 pg 1521
Children's Hospital of Eastern Ontario	2.5%	2 mL/kg	5% TBV monthly or 4 ml/kg (should not occur more than 3 months in a row)	CMRC IRB, Chicago IL. (2006) Howie (2011) Bull World Health Organ 89: 46-53
University of British Columbia	2.5%	2 mL/kg	5% TBV over 30 period (must occur no more than 3 consecutive months)	CMRC IRB, Chicago IL. (2006) Howie (2011) Bull World Health Organ 89: 46-53
Alberta Health Services	1%	1 mL/kg	1% or 1 ml/kg maximum volume in 24 hours	Ernst, D.J. Applied Phlebotomy. Lippincott Williams & Wilkins (2005)

PEDIATRICS

BLEEDING IN NEONATES AND CHILDREN

PB1233 | Single Center Analysis of the Phenotype of Children Immune Thrombocytopenia with Mutation Related to Common Variable Immunodeficiency

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Background: Common variable immunodeficiency (CVID) is the most common primary immune deficiency diseases(PID). Nearly 20-25% of its first manifestation is autoimmunity. Some patients are characterized by cytopenia represented by immune thrombocytopenia (ITP),which is easily confused with primary ITP. Most ITP caused by CVID usually developed into chronic/refractory ITP(C/RITP) with poor prognosis. Early diagnosis is essential in adequate management of the disease.

Aims: We aim to detect children CVID combined with ITP by high-throughput, next-generation sequencing (NGS) from children with apparent C/RITP and analyze their characteristics, providing evidence for the early identification of the disease.

Methods: Though performing NGS for CVID related genetic variation on C/RITP admitted to Beijing Children's Hospital from April 2016 to May 2019. We scored and analyzed the clinical and laboratory data of CVID with ITP confirmed by their phenotype, genetic rule and serum immunoglobulin (Ig) level.

Results: Among 176 children C/RITP, 19 (10.8%) were diagnosed with CVID related genetic variation, 8 (4.5%) were confirmed with CVID, 5 with TNFRSF13B, 1 with LRBA, 1 with NFKB2 and 1 with CARD11 mutation. Six boys and 2 girls with a median onset age of 5.00 (0.37-11.32) years were included. Seven presented with mild bleeding and 1showed profound bleeding. Repeated infection existed in all. Two were associated with hypersensitivity. Positive indexes of autoimmunity were noted in 5. Five (62.5%) had a definite positive family history. The median of serum IgA concentration was 0.3875 (0.078-0.792) g/L, IgG concentration was 6.14 (4.1-13.4) g/L and IgM concentration was 0.522 (0.216-0.667) g/L.

Conclusions: In this study, diagnosis of CVID with ITP is likely to be delayed due to the onset manifestation as ITP, atypical immunodeficiency symptoms, intravenous immunoglobulin (IVIG) intervention and absence of family history. NGS performing and thorough review of the medical history may beneficial to identification and management of CVID.

PB1234 | High-throughput Sequencing in the Diagnosis of Inherited Immune-Thrombocytopenia from among Children with Apparent Chronic/Refractory ITP

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Background: Inherited thrombocytopenia (IT) is a group of hereditary diseases characterized by thrombocytopenia and bleeding, which is hard to distinguish from primary ITP with similar manifestations and response to treatment. Nevertheless, management and follow-up in life-long are different. ITs are frequently incorrectly diagnosed and thus with poor clinical prognosis and evolving into chronic and refractory ITP (C/RITP). Currently, limited data about the detection rate of ITs in Chinese children C/RITP leads to further investigations.
Aims: To detect immune IT by high-throughput next-generation sequencing (NGS) from children with apparent C/RITP and investigate their clinical profile.
Methods: We retrospectively collected 252 children C/RITP with transient response to Intravenous Immunoglobulin (IVIG) /steroid, all of whom underwent NGS targeted for ITs from Apr 2016 to Apr 2019. Clinical data were systematically collected. We diagnosed immune ITs and suspicious ITs depending on genes, phenotype and family history and we analyzed their characteristics.
Results: Forty-nine patients without analyzable information were ruled out. Among the remaining 203 cases, 36(17.8%) was confirmed as IT and 65(32.0%) was suspicious of IT. Twenty-three (63.9%) from confirmed group and 51(76.9%) from suspicious group had immune-related mutations. In analyzing the clinical characteristics, we found more boys (82.6%) in confirmed immune IT group than in the suspected(62.7%) and negative group (53.9%), while age of onset is younger in the confirmed(5.47 ± 3.78 years) and older in the suspected(7.30 ± 3.94 years). Buchanan scores showed more patients of level-3 bleeding in the confirmed(30.4%), while more of level-2(58.8%) in the suspected and more of level-1(46.6%) in the negative.
Conclusions: In total, 35.9% of children C/RITP were found with mutations of ITs. For the confirmed cases, younger boys with more severe the degree of disease. As for the suspected cases, their onset age is older and manifests obvious immunological abnormalities and more severe disease state. In summary, early identification is essential for children ITs.

PB1236 | Chronic Refractory Immune Thrombocytopenia is Associated with a High Frequency of Potentially Damaging Variants in Immune Genes

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Background: Chronic refractory autoimmune thrombocytopenia(C/RITP) comprises complex and heterogeneous pathogenesis.
Aims: We sought to the incidence, clinical and genetic characteristics of chronic/refractory immune thrombocytopenia children with a high frequency of potentially damaging variants in immune genes.
Methods: Whole Exon Sequencing (WES) was performed by Next Generation Sequencing (NGS) to summarize and analyze the clinical characteristics, and genetic characteristics of cases with a high frequency of potentially damaging variants in immune genes that could not be clearly diagnosed as hereditary immune thrombocytopenia by NGS.
Results: In a national, retrospective analysis of 252 patients with chronic refractory autoimmune thrombocytopenia initiated in 2016, 51 children with a high frequency of potentially damaging variants in immune genes(M+ group) and 103 children with no genetic abnormalities (M- group). Compared with M- group, children in M+ group were more likely to show skin and mucous membrane bleeding, and the proportion of bleeding degree was higher than that in the M- group. Meanwhile, we found that M+ group with a higher incidence of abnormal immunological indexes, more abnormal immunological indexes. And the peripheral T lymphocytes and B lymphocytes in the children with a high frequency of potentially damaging variants in immune genes were significantly increased. Nine patients(5.84%) had probable pathogenic variants in 1 of 4 genes known to be involved in primary immunodeficiencies (TNFRSF13B, CARD11, CBLand RAG2), whereas 42 patients (27.27%) carried uncertain mutations in 23 genes in the context of autoimmune disease. Lastly, no genetic abnormalities were found in the remaining 102 patients (66.88%, the M- group). The M+ group displayed more severe bleeding than the M- group, with more abnormal immunological indexes.
Conclusions: Wide-ranging genetic screening should be offered in chronic refractory immune thrombocytopenia patients associated with long history, easy recurrence, severe bleeding, positive indicators of autoimmune diseases and abnormal immune function tests.

PB1237 | Retrospective Analysis of Characteristics and Outcomes of 340 Pediatric ITP Patients in One Center

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Background: Immune thrombocytopenia (ITP) is a bleeding disorder characterized by increased platelet destruction and suboptimal

TABLE 1 Demographic,clinical,and laboratory data of different types of pediatric ITP

Characteristics	Newly diagnosed ITP	Persistent ITP	Chronic ITP	P values	Sustained response group	Chronic group	P values
Girls	47.8%	53.3%	47.5%	0.627	42.7%	57.4%	0.042
Age at diagnosis(mean ± standard deviation)	8.93 ± 4.57	9.45 ± 4.06	7.60 ± 4.21	0.007	7.89 ± 4.19	10.06 ± 4.28	0.000
platelet count at diagnosis, ×109/L (median, range)	7.5(1~68)	10.5(0~91)	15(1~87)	0.001	10(0~68)	9(1~80)	0.785
preceding infection	54.9%	42.5%	40.2%	0.050	54.9%	47.8%	0.328
insidious onset	25.4%	61%	74%	0.000	22%	60.5%	0.000
platelet antibody	41.7%	23.5%	26.1%	0.007	44.4%	26.7%	0.015
ANA	55.1%	43%	46.9%	0.164	47.4%	48.7%	0.860
Mucosal bleeding	56.7%	54.2%	64.6%	0.284	51.2%	54.8%	0.621

TABLE 2 Prognostic factors for chronic ITP prognostic factors for chronic ITP

Characteristics	Sustained response group	Chronic group	P value	Odds ratio	CI
girls	42.7%	57.4%	0.398	0.744	(0.375-1.477)
age	7.89 ± 4.19	10.06 ± 4.28	0.045	1.085	(1.002-1.175)
insidious onset	22%	60.5%	0.000	4.348	(2.154-8.777)
platelet antibody	44.4%	26.7%	0.049	0.491	(0.242-0.997)

platelet production resulting in thrombocytopenia. Childhood ITP usually resolves with or without intervention for most affected children while a certain number of children may progress to chronic illness.

Aims: To identify clinical characteristics of childhood ITP and risk factors for developing chronic disease and outcomes in children with ITP.

Methods: 340 children with ITP hospitalized in our institute between January 2014 and October 2018 were retrospectively analyzed.

Results: ITP occurred with equal frequency in boys(50.6%) and girls(49.4%).134(39.4%), 107(31.5%) and 99(29.1%) children were classified as newly diagnosed, persistent and chronic ITP respectively.The mean age at presentation was 8.70 ± 4.36 years.The most frequent complaint were petechia(87.9%), ecchymosis(56.2%) and epistaxis(46.5%). One child died of fatal bleeding, the mortality rate in the cohort was 0.29%. Of the 198 newly diagnosed/persistent ITP patients for whom follow-up data were more than 12 months, 115(58.1%) progressed to chronic type. The remaining 83 obtained sustained response including one persistent ITP child underwent splenectomy 8 months after initial diagnosis. The mean age at diagnosis was significantly higher in chronic group(p = 0.000). Spontaneous remission was achieved by 41.4% of non-splenectomized children with newly diagnosed/persistent ITP within 12 months from initiation of disease and by 30.4% of chronic patients at the last follow-up(October 2019). Females developed chronic ITP significantly more often (p = 0.034). In multivariable regression analysis, the risk of chronicity was associated with age (OR = 1.085, CI = 1.002-1.175) and insidious onset of symptoms (OR = 4.348, CI = 2.154-8.777), inversely associated with platelet antibody (OR = 0.491, CI = 0.242-0.997), gender was not a risk factor (OR = 0.744, CI = 0.375-1.477).

Conclusions: Childhood ITP is a benign condition in most cases, with rare severe hemorrhage, low mortality and relatively high spontaneous remission rate.The relevant risk factors for chronicity included insidious onset of symptoms and age at presentation, platelet antibody might be a favor factor.

PB1239 | Variability in Diagnosis and Management of Immune Thrombocytopenia in Children: Real Life According to Spanish Pediatricians and Hematologists

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Background: Evidence-based guidelines for the management of immune thrombocytopenia (ITP) recommend an observation-based approach for the majority of pediatric patients with newly diagnosed ITP. A variability in adherence to this recommendations has been described. Moreover, there is still a lack of evidence regarding the utility of different management approaches, including general recommendations for sport participation.

Aims: To evaluate the current practice patterns of pediatric ITP treating physicians across Spain.

Methods: A questionnaire regarding management of a newly diagnosed pediatric ITP, studies at ITP onset, treatment, and general recommendations for daily life and sports participation in pediatric ITP patients was designed. A web-based survey tool, *docs.google.com/forms* was used to generate an online version of the questionnaire (table 1). Pediatricians, pediatric hematologists and hematologists treating pediatric ITP in Spain were invited to participate. Responses were collected and formatted using *google forms*.

Results: A total of 81 responses were collected from pediatric hematologists (66,7%), hematologists (23,5%) and pediatricians (9,9%).

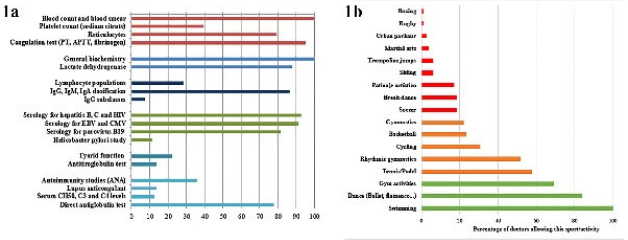

FIGURE 1 a: variability in additional test requested in ITP. 1: recommedations in sport participation

TABLE 1 Summary of responses. *More relevant in those doctors treating less than 10 ITP every year

Clinical situation	Answers
Cases 1-3: Newly diagnosed ITP. Platelets 18.000/mmcc. Blood smears: normal. No hemorrhagic symptoms but skin lesions.	·7-month-old toddler: 55.6% admission and observation and 23.5% home observation ·7-year-old child: 63% home observation; 19.8% admission and observation ·13-year-old teenager: 67.9% home observation and 22%* of admission and observation
Case 4: Newly diagnosed ITP. 5-year-old girl. 6.000/mmcc platelets. Normal blood smears. No hemorrhagic symptoms but skin lesions.	·Admission and observation: 24.7% ·Treatment administration: GC: 29.6% IVIG: 37%
Bone marrow aspiration in newly diagnosed ITP	·No one patient: 49.4% ·No response to GC and IVIG: 34.6%
Case 5: Newly diagnosed ITP. 3-year-old girl. Platelets 2.000/mmcc. Normal blood smears. Large hematoma secondary to traumatism. Positive direct coombs test. Would this change your attitude?	·Similar treatment to other patients: 86.4% ·Long term GC: 8.6%*
Case 6: Persistent ITP. 7-year-old. Bone marrow aspiration compatible with ITP. Transient response to IVIG three times. No response to GC. Epistaxis during the last 48 hours. 9.000/mmcc platelets. Attitude:	·IVIG administration: 77.8%
Case 7: Similar to case 6 but there is no response to IVIG. Attitude:	·TPO analogs: 72.8% ·IV GC bolus: 16%*
Case 8: Chronic ITP. 8-year-old girl. Transient response to IVIG (6 doses), all of them followed by mild headache as secondary effect. No response to GC. Epistaxis and bruises. She begs for participating in rhythmic gymnastics classes. Would you offer other treatment?	·TPO analogs: 92.6% ·Others*
Cases 9 and 10: Minimum level of platelet count to allow a completely normal life in:	·3-year-old child: 19.8% >20.000/mmcc; 34.6% >30.000/mmcc; 22.2% >50.000/mmcc ·10-year-old child: 23.5% > 20.000/mmcc; 30.9% >30.000/mmcc; 17.3% > 50.000/mmcc
Case 11: The patient insists on practicing a sport that you don't recommend (bleeding risk). Would you start treatment to increase his platelet count?	Yes: 85.2%

A 65,4% of respondents had treated pediatric ITP patients for more than 6 years, but only 37% of them treat more than 10 cases per year. Main responses are summarized in table 1. Figure 1a shows the variability in additional test requested in a newly diagnosed ITP. Figure 1b, the percentage of doctors that recommend the participation in sports of a chronic ITP teenager with a platelet count between 15-30.000/mmcc.

Conclusions: Despite of the existence of national and international guidelines, there is some variability in the diagnosis and management of pediatric ITP in our country. Those doctors treating less than 10 ITP per year tend to administrate treatment more frequently.

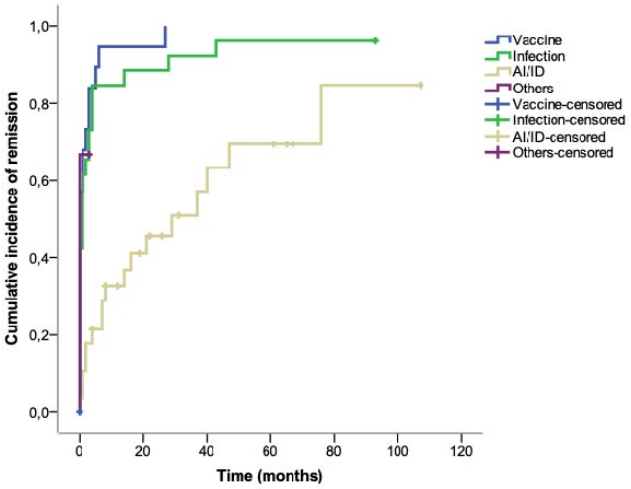

FIGURE 1 Cumulative incidence of remission depending on the underlying diagnosis of secondary ITP

PB1241 | Secondary Immune Thrombocytopenia in Children: Characteristics and Outcome of a Large Cohort from Two Spanish Centers

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Background: Immune thrombocytopenia (ITP) is the most common cause of thrombocytopenia in children and adolescents. Most cases are considered primary, but others are secondary to coexisting conditions. The occurrence of secondary ITP has been estimated based on clinical experience of experts and short series (around 20%), but there is a lack of data regarding its incidence in our country.

Aims: To study the incidence and outcome of secondary ITP in a large cohort of pediatric Spanish patients.

Methods: A retrospective observational study was conducted in two pediatric University hospitals in Spain between 2009 and 2019, which included children from 4 months to 18-year-old

TABLE 1 Secondary conditions of ITP, clinical characteristics and treatment administered

Coexisting condition	n	Age ITP onset. Mean (SD)	Gender	Age at diagnosis of coexisting condition. Mean (SD)	Clinical characteristics, observations and treatment administered
Vaccines	21	3.8 (4.3)	F: 12 M: 9	At onset	-Most of them received IVIG -Two cases of persistent ITP -Two cases of chronic ITP (suspicion of CVID in one case)
Viral infection	26	6.7 (5.0)	F: 15 M: 11	At onset	-VEB: 12; CMV: 7; Parvovirus: 1; Others: 6 -Most of them (n = 15) received IVIG -Persistent ITP: 3 cases. Chronic ITP: 4 cases.
Immunodeficiency: CVID	7	10.8 (3.8)	F: 5 M: 2	13.7 (3.7)	-All cases of CVID are in remission after receiving substitutive IVIG. Four of them also received immunosuppressive treatment (MMF and sirolimus).
Immunodeficiency associated to other syndromes	6	9 (5.7)	F: 3 M: 3	At onset	-DiGeorge and one Kabuki sd patients received TPO analogs. -Down sd. patients are in remission.
EVANS	4	9 (5.4)	F: 3 M: 1	10.6 (3.3)	-Three patients received immunosuppressive treatment (MMF). -Underlying ALPS in one case.
Autoinmunity	11	10 (3.06)	F: 9 M: 2	12.2 (3.0)	-Five patients in complete remission after receiving specific treatment: hydroxychloroquine +/- glucosteroids. -Four patients are under treatment with TPO analogs and specific treatment -One patient in complete remission after receiving rituximab (refractory to other treatments, corticodependent)
Others	3	13 (5.0)	F: 1 M: 2	At onset	-Chemotherapy (2) and cotrimoxazole (1) -All in CR after avoiding the cause.

diagnosed with ITP. Data were recorded from clinical charts: gender, age at diagnosis, coexisting condition and associated characteristics (at diagnosis or during the follow-up), outcome and treatment.

Results: A total of 442 patients were diagnosed with ITP during the period study. Mean age was 5.4 (SD 4.4); a 49.1% were boys. Most of them (n = 284; 64.3%) were catalogued as newly diagnosed ITP, 55 (12.4%) as persistent ITP, and 102 (23.1%) as chronic ITP.

Secondary ITP was diagnosed in 78 cases (17.4%). Clinical characteristics and outcome depending on coexisting conditions are shown in table 1 and figure 1. Apart from them, there are 10 chronic ITP patients under suspicion of an underlying autoimmune disease or immunodeficiency.

Conclusions: Characteristics of our cohort are comparable to that describe in the literature. Secondary ITP identification is important as it predicts outcome. Whereas most of secondary ITP are diagnosed at onset (especially those secondary to vaccines or infections), others like autoimmune diseases or immunodeficiencies should be rule out periodically as they are usually diagnosed later, even years after or later of ITP onset. An early diagnosis of the underlying disease could have an impact on choosing a tailored treatment.

PB1242 | Impact of a Standardized, Moderate-Intensity Aerobic Exercise Regimen on Hemostasis in Adolescent Hemophilia A Carriers: A Pilot Investigation

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Background: In a previous study (Kumar et al. Thromb Haemost; 2016), we documented a 2.3 fold increase in FVIII activity (FVIII:C) in adolescent males with mild hemophilia A (n = 8) performing moderate-intensity aerobic-exercise
(MIA-exercise).

Aims: In this pilot-study, we investigated the impact of MIA-exercise on hemostasis in adolescent, hemophilia A carriers.

Methods: The study (clinicaltrials.gov: NCT03379974) was approved by the research ethics committee. 5 females who met eligibility criteria were invited to participate. Written consent/assent was obtained from parent/participant. After baseline (T0) blood work was drawn, participants were instructed to exercise on a cycle-ergometer using the previously-validated, progressively incremental Godfrey protocol, until they completed 3-minutes of cycling at 85% of their predicted maximum heart-rate. Blood work was repeated at 30 (T1), 75 (T3) and 135 (T4) minutes from the start of exercise. Specimens were assessed for multiple coagulation labs including global hemostatic assays.

Results: Baseline demographic data are summarized in Table 1. All participants successfully completed the study protocol. Median (range) duration of exercise was 11 (10-15) minutes. MIA-exercise was associated with a 1.06
(1.0-1.9) fold change in FVIII:C (T1 compared to baseline), 0.94 (0.8-1.4) fold change in von Willebrand antigen (VWF:Ag), 1.03 (0.8-1.6) fold change in VWF activity, 1 (0.8-1.8) fold change in platelet count and 1.06 (1.01-1.14) fold change in endogenous-thrombin-potential. We additionally compared the FVIII:C and VWF:Ag changes associated with MIA-exercise to the participant's historic desmopressin-challenge (Figure 1). FVIII:C and VWF:Ag changes associated with exercise were observed to be inferior to desmopressin.

Conclusions: This is the first study to investigate the impact of aerobic-exercise on hemostasis in carriers of hemophilia A. Despite exercising for a similar duration and intensity as males with mild hemophilia A, females did not show a significant increase in FVIII:C, VWF:Ag or platelet counts. Future directions include confirmation of this gender-based discrepancy and further investigation of its etiology.

TABLE 1 Baseline characteristics of study cohort

Characteristics	Participant 1	Participant 2	Participant 3	Participant 4	Participant 5
Age (years)	16	13	15	15	16
Body mass index (kg/m2)	18.7	26.7	20.7	23.5	19.2
Baseline FVIII:C (%)	57	24	54	60	52
F8 variant identified	c.3808insA	c.1910A>G	c.1034T>C	c.1034T>C	c.6445G>T
Severity of hemophilia reported with F8 mutation	Severe	Mild/Severe	Moderate	Moderate	Mild
Historic desmopressin response	Responder	Responder	Responder	Responder	Responder
Baseline Physical Activity (measured using IPAQ)	High	High	High	High	Moderate
Duration of exercise (min)	15	12	10	11	11

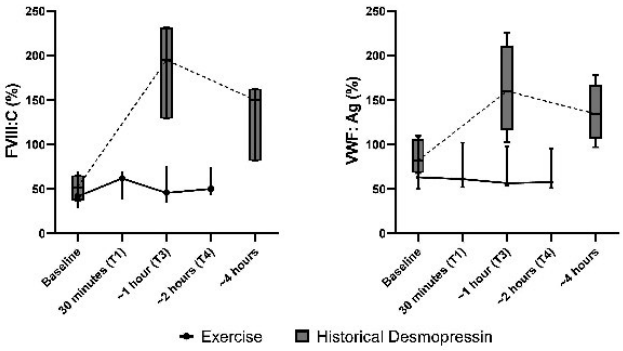

FIGURE 1 Comparison of FVIII:C and VWF:Ag change with exercise and desmopressin

PB1243 | Moderate Intensity Aerobic Exercise and Intranasal Desmopressin Additively Increase Factor VIII and von Willebrand Factor (VWF) Levels in Adolescent Males with Mild Hemophilia A - Principal Findings from a Randomized Trial

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Background: Persons with mild-hemophilia A (MHA) may use intranasal desmopressin prior to sports. In a previous study we documented a 2.3-fold increase in FVIII activity (FVIII:C) in adolescents with MHA after moderate-intensity aerobic exercise (MIA-exercise).

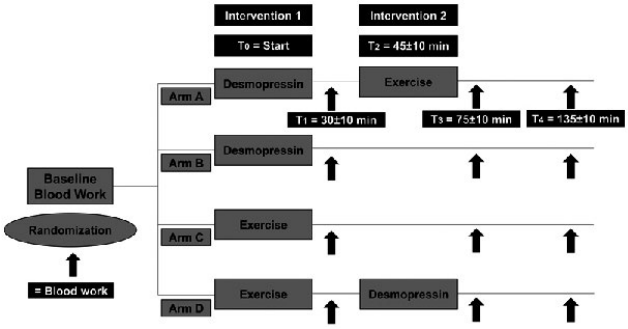

FIGURE 1 Study design

Aims: We performed a randomized-trial of MIA-exercise versus intranasal desmopressin in adolescents with MHA. Our primary-objective was to compare the increase in FVIII:C with MIA-exercise versus desmopressin. We also examined changes in hemostatic parameters with the sequential administration of these interventions.

Methods: The study (clinicaltrials.gov: NCT03379974/NCT03136003) was conducted simultaneously at SickKids and Nationwide Children's Hospital with research ethics approval. Written consent was obtained from 32-participants meeting eligibility criteria. Participants were randomized to one of four study arms (Figure 1). Blood work was obtained at baseline and three subsequent time-points. Participants randomized to exercise, cycled on an ergometer till they completed 3-minutes of cycling at 85% of their predicted maximum heart-rate. Standard weight-based dosing of desmopressin was used. Primary analysis was performed using a non-inferiority t-test. Secondary analyses were done using t-test and linear mixed-models.

Results: Mean age (±SD) at participation was 16.1 (±2.6) years, and mean baseline FVIII:C was 27.9 (±18.4)%. FVIII:C increased by 1.73 fold with MIA-exercise versus 2.24 fold with desmopressin (non-inferiority p = 0.04) (Figure 2A). The change in VWF antigen, activity (Figure 2B) and endogenous thrombin potential were similar with both interventions. MIA-exercise, but not desmopressin was associated with a significant increase in platelet count. Sequential administration of MIA-exercise and desmopressin was associated with an additive increase in FVIII:C and VWF activity (Figure 2C,2D), though the impact of exercise alone was short-lived.

Conclusions: While MIA-exercise (compared to desmopressin) was associated with a similar increase in FVIII:C and VWF levels, its impact on hemostasis was brief. Combination of MIA-exercise and desmopressin resulted in an additive increase in multiple hemostatic parameters. These findings have significant implications for persons with MHA.

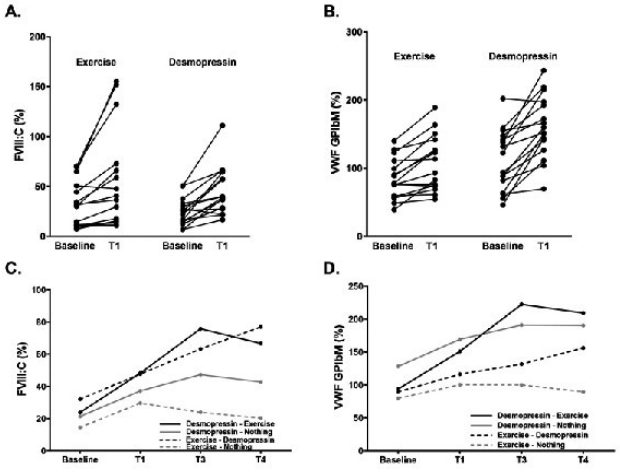

FIGURE 2 Change in FVIII:C (2A) and VWF:GPIbM (2B) from baseline to T1. Change in FVIII:C (2C) and VWF:GPIbM (2D) across the 4 study arms

PB1244 | Haemostatic Disorders and their Correlation with Hepatic Fibrosis Markers in Case of Hepatic Cirrhosis in Children

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Background: Currently data is increasingly accumulated on the correlation of hepatic diseases and coagulation disorders and on the role of haemostatic proteins in the hepatic fibrosis pathogenesis.

Aims: To assess haemostatic disorders and their correlation with hepatic fibrosis markers in children suffering from hepatic cirrhosis.

Methods: The study group consisted of 31 children with hepatic cirrhosis aged between 3 and 17 years old. To assess haemostatic disorders, there were determined the platelet count, the activated partial thromboplastin time (APTT), thrombin time, the Quick's

TABLE 1 Changes in hemostasiologic indicators and fibrosis markers (Me (Q1;Q3)) (Note: n is the number of observations, p is the significance level)

Indicator	The study group n = 31	The control group n = 15	p
Thrombocytes, (109/l)	150(117; 212)	311.0(266.0;349.0)	p=0.000
Endothelin-1, (fmol/ml)	1.80(0.82; 5.05)	0.55(0.22; 0.63)	p=0.000
VWF, (%)	167.5(149.0; 267.0)	76.6 (58.85;105.4)	p=0.000
APTT, (s)	40.4(34.2; 42.4)	30.53(28.9; 33.2)	p=0.000
PT (%)	81.0(70.0; 88.5)	102.5(89.0; 124.0)	p=0.000
C-protein, (%)	69.4(59.0; 86.3)	99.5(88.1; 113.1)	p=0.002
α2-AP, (%)	81.3(63.0; 95.8)	115.8(102.1; 124.6)	p=0.000
Collagen IV, ng/ml	50.55(29.67; 74.20)	18.48(14.12;21.31)	p=0.000
PDGF, pg/ml	4137.5(2812.5; 5677.9)	2862.6(2462.4; 3437.6)	p=0.045

TABLE 2 Correlation relationships of haemostatic indicators and fibrosis markers (Spearman's method) (Note: p is the reliability of correlation relat)

Fibrosis markers	Haemostatic indicators	Spearman's rank correlation coefficient (r)	p
Collagen IV	VWF	r = 0.519	p=0.019
Collagen IV	Endothelin - 1	r = 0.527	p=0.012
Collagen IV	Quick's PT	r = - 0.453	p=0.023
Collagen IV	C-protein	r = - 0.482	p=0.031
PDGF	VWF	r = 0.661	p=0.002
PDGF	Endothelin - 1	r = 0.600	p=0.005
PDGF	Quick's PT	r = - 0.459	p=0.027

prothrombin activity (PT), antithrombin activityIII, C-protein, von-Willebrand factor (VWF), plasminogen, alpha-2-ntiplasmin (α-2AP), XIIa-dependent fibrinolysis, fibrinogen content, SFC (soluble fibrin complexes), D-dimers, endothelin-1. The concentration of the following hepatic fibrosis markers was also determined: collagen IV, the platelet-derived growth factor (PDGF). The control group (n = 15) consisted of children included in health groups I-II.

Statistic methods: Mann-Whitney U-test, Spearman's rank correlation coefficient. Quantitative data are herein presented as Me (Q1;Q3).

The study was approved by the Local Ethics Committee (Protocol No.16, December 5, 2017). All the study participants signed their informed consent.

Results: The clinical evidence of haemorrhagic events (ecchymoses, petechiae, frequent nosebleeds) was recorded in each second patient. Changes in the haemostatic system were characterized by liability to thrombocytopenia, endothelium hyperthrombogenicity and moderate decrease in the coagulation, anticoagulant and fibrinolytic activity (Table 1). All patients had higher concentrations of hepatic fibrosis markers, which were directly correlated with indicators of coagulation hemostasis, anticoagulant system and endothelium dysfunction markers (Table2).

Conclusions: Haemostatic disorders in case of cirrhosis are directly correlated with fibrosis markers. So, it proves their role in the fibrogenesis development and determines the need to monitor all links of the haemostatic system in order to comprehensively assess the disease severity and to prevent the further progression of grave complications. Financing: the research was completed under the Governmental task for 2017-2020.

PB1245 | The Relationship between Platelet Phenotype and Function with Clinical Outcomes in 57 Paediatric Veno-Arterial ECMO Patients

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Background: Extracorporeal membrane oxygenation (ECMO) as a form of life support and the underlying diseases are associated with platelet dysfunction and coagulopathy. Maintaining haemostasis in ECMO patients is challenging. Understanding haemostasis and platelet function in the context of ECMO, particularly their association with patient outcomes, is essential in improving outcomes for children requiring this support.

TABLE 1 VA-ECMO patient demographics

Total Number of Patients	n = 57	
Age	Neonates (0 - 30 days)	24 (42%)
	Infants (31 days - 1 year)	11 (19%)
	Children (1 - 18 years)	22 (39%)
Gender	Male	29 (51%)
	Female	28 (49%)
Cannulation site	Central	40 (70%)
	Peripheral	17 (30%)
At least one major bleed	>4 ml/kg/hr for > 4 hrs Intracranial or pulmonary haemorrhage Gastrointestinal bleed requiring endoscope or surgical intervention Surgical site bleed requiring re-operation	25 (44%)
At least one thrombosis	Thrombosis (venous or arterial) requiring acute intervention or formal anticoagulation beyond ECMO Radiologically proven CNS embolic stroke ECMO circuit thrombosis requiring circuit change	8 (14%)

TABLE 2 Relationship between platelet phenotype and function and major bleeding. (MFI = Median Fluorescence Intensity; AUC = Area Under the Curve

Major Bleeding	Odds Ratio	Standard Error	p-value	[95% Confidence Interval]	
CD61 MFI	1.000	0.0001	0.0905	1.000	1.000
CD42b MFI	1.000	0.0001	0.3251	1.000	1.000
GPVI MFI	1.000	0.0002	0.8195	1.000	1.000
Basal CD62P %	0.877	0.1902	0.5320	0.573	1.341
Basal PAC1 %	1.041	0.0279	0.1093	0.988	1.097
Basal CD63%	**1.258**	**0.1091**	**0.0033**	**1.062**	**1.491**
Activated CD62P (AUC)	1.017	0.0138	0.2006	0.990	1.045
Activated PAC1 (AUC)	1.020	0.0106	0.0519	0.999	1.041
Activated CD63 (AUC)	**1.048**	**0.023**	**0.0192**	**1.003**	**1.094**

Aims: This study aimed to determine the association between platelet phenotype and function, and bleeding and clotting outcomes in children on ECMO.

Methods: This study was approved by the Royal Children's Hospital (RCH) Ethics in Human Research Committee and written informed consent was obtained from parents. ECMO patients were recruited from the RCH Paediatric Intensive Care Unit (PICU) from March 2017 to December 2019. Samples were collected from the arterial line of 57 neonates and children placed on veno-arterial ECMO (VA-ECMO) within 24 hours of ECMO initiation. Whole blood flow cytometry was used to measure markers of platelet phenotype and function (response to thrombin receptor activated peptide-6 (TRAP-6)). Clinical data was recorded and logistic regression was used to determine associations between platelet phenotype and function, and bleeding and thrombosis.

Results: 57 patients (Table 1) were recruited between the age of 1 day and 18 years (median age of 2.2 months). The median duration of VA-ECMO was 5 days (range 1 to 112 days). A significant association between both increased basal platelet activation and response to TRAP-6 with major bleeding was found using CD63, a lysosome release indicator (p = 0.0033 and p = 0.0192 respectively, Table 2).

Conclusions: An association between increased basal activation state and the ability to further activate platelets with bleeding in paediatric VA-ECMO patients was identified. CD63 could potentially be used as an early biomarker of bleeding events in ECMO patients. Analysing platelets by flow cytometry could benefit ECMO patients by facilitating early intervention to prevent bleeding and clotting events.

PB1246 | Isolated Thrombocytopenia in Children Is Not Always an Immune-Associated Disorder

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Background: Immune thrombocytopenia (ITP) remains the most frequent diagnosis in isolated thrombocytopenia in children, however other causes must always be investigated before reaching diagnosis.

Aims: To present 3 cases of thrombocytopenia initially considered as ITP that proved to be attributed to other causative mechanisms.

Methods: Retrospective review of patient files.

Results: The first case concerns a 14-year old girl presenting to the ER with mild intermittent abdominal pain. Isolated thrombocytopenia with a platelet number of 60.000/μl was found at work up and the child was admitted with a diagnosis of ITP. Abdomen ultrasound routinely performed in such cases revealed abnormal position of the spleen with fluid presence underneath. CT scan established the diagnosis of a "wandering spleen". The patient underwent splenectomy and spleen autotransplantation, with platelets returning to normal following surgery.

The second case concerns a 17-year old girl diagnosed with mild chronic ITP before referral. She reported a mild bleeding phenotype and a platelet count of approximately ~70.000/μl. Platelet morphology was normal but testing of other family members revealed thrombocytopenia in two of her five adult siblings. Hereditary thrombocytopenia was suspected and genetic testing followed.

The third case concerns a 5-year old boy referred with a diagnosis of ITP from infancy. A mild bleeding phenotype was reported along with persistent eczema and occasional infections. Wiscott-Aldrich Syndrome was suspected and then diagnosed by genetic testing.

Conclusions: Although ITP remains the main diagnosis in isolated childhood thrombocytopenia, low platelet count may arise from decreased production or mechanical destruction and should always be excluded before setting diagnosis.

PB1247 | Thrombin Generation Measurement Using ST-Genesia System in a Cohort of Healthy Children Including Neonates: Normal Values and Variability

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Background: The evaluation of coagulation in children remains challenging particularly in newborns. Thrombin generation assay measurement is an integrative functional method that may be more relevant than current global assays (APTT, PT). ST-Genesia ™is a dedicated instrument providing standardization with the use of reference plasma allowing expressing each parameter as normalized values. Full automation facilitates its use in practice.

Aims: Evaluate TGA using ST Genesia™ in a paediatric healthy population including neonates.

Methods: Two hundred and sixty healthy children and thirty nine adults were consecutively recruited. TGA was analysed in citrated platelet poor plasma on a ST-Genesia™, using STG-Bleedscreen ™to trigger thrombin generation. Activated Partial Thromboplastin Time assay (APTT), Factor V and Factor VII were determined.

Results: The precision of the ST Genesia ™was excellent. TGA parameters gradually change according to the age of the children (3 days, 1-3 months, 3-6 months, 6-12 months, 1-2 years, 2-6 years, 6-12 years, 12-15 years, and 15-18 years). However, TGA values were in a comparable range to adults (median age: 31.1 years) whatever age group or TGA parameter. No strong correlation was found between TGA parameters and FV or FVII levels.

Conclusions: ST Genesia can be used in children including neonates with good performance. This integrative method indicates that thrombin generation gradually changes with age but remains in comparable range than in adults in a healthy population.

PB1248 | U.S. Cohort Study of Previously Untreated Patients with Congenital Hemophilia (ATHN 8: PUPS Matter Study) Interim Analysis of the 2010-2019 Birth Cohort

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Background: The American Thrombosis and Hemostasis Network's (ATHN) ATHN 8: PUPs Matter Study is the first U.S. cohort study of previously untreated patients (PUPs) with congenital hemophilia. Children with baseline factor VIII or IX levels ≤ 5% born after January 1, 2010 that are followed at ATHN-affiliated hemophilia treatment centers (HTCs) are enrolled in this multi-center study. The primary objective is to determine the percentage of PUPs with confirmed inhibitors within the first 50 exposure days (ED).

Aims: To provide an interim analysis of the ATHN 8: PUPs Matter Study 2010-2019 birth cohorts.

Methods: The study was approved by each HTC's institutional review board and informed consent was obtained prior to implementing study procedures. Demographic, laboratory, and clinical data are being collected. All participants are being followed until 50 exposure days or until inhibitor development. Descriptive statistics are used to analyze participant characteristics for an interim analysis through August 31, 2019.

Results: Nineteen HTCs have enrolled 79 male participants thus far. The majority have severe hemophilia A. [Table 1] Family history for hemophilia at diagnosis was present in 52%. Age at first product exposure is known for 56 (71%). Forty-nine (88%) had their first treatment within the first year of life, with 20 (25%) receiving treatment within the first month. Most participants (59%) received recombinant factor at first exposure. [Table 2] One or more inhibitor titer test is documented for 59 (75%), and, to date, 14 (18%) have met the primary endpoint for inhibitor development.

Conclusions: The interim analysis provides clinical information for PUPs born between 2010-2019. Planned enrollment is 250 participants. This study will provide important information regarding inhibitor development and the changing treatment landscape.

TABLE 1 Demographic and Diagnostic Characteristics

N = 79	Birth Cohort 2010-2019
Age, years (median, range)	4.2 (0.3-9.1)
Birth year, N (%) 2010-2012, 2013-2015, 2016-2018, 2019-	15 (19), 29 (37), 33 (42), 2 (3)
Race, N (%) White, Black/African American, Asian, Mixed Race, Unknown	66 (84), 6 (8), 3 (4), 3 (4) 1 (1)
Ethnicity, N (%) Non-Hispanic	63 (80)
Primary Diagnosis, N (%) Hemophilia A, moderate; Hemophilia A, severe; Hemophilia B, moderate; Hemophilia B, severe	8 (10), 60 (76), 2 (3), 9 (11)
Prenatal Diagnosis, N (%) Yes	3 (4)
Reason for diagnostic testing, N (%) Bleeding symptoms, Mother known carrier, Other family history, Abnormal pre-operative screening, Other Unknown	43 (54), 16 (20), 10 (13), 1 (1), 3 (4), 6 (8)
Gene variant identified, N (%) Hemophilia A (N = 68); Hemophilia B (N = 11)	43 (63), 7 (64)

TABLE 2 Treatment Characteristics

N = 79	
Primary treatment type, N (%) Prophylaxis (continuous), Prophylaxis (event-based), Episodic, None, Unknown	39 (49), 2 (3), 23 (29), 1 (1), 14 (18)
Primary treatment product type, N (%) Recombinant factor, Plasma-derived factor, Bypassing therapy, Non-factor therapy, None Unknown	52 (66), 9 (11), 2 (3), 1 (1), 1 (1), 14 (18)
Home infusion, N (%)	56 (71)
Age at first home infusion (N = 56), <1 y, 1-1.99 y, 2 + y	27 (48), 21 (38), 8 (14)

PB1249 | Inflammation Associated Cell-Free microRNA Levels in Cerebrospinal Fluid of Premature Infants Upon Intraventricular Hemorrhage

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Background: Intraventricular hemorrhage (IVH) is a frequent complication of prematurity that is associated with high neonatal mortality and increased risk of neurodevelopmental impairment. In IVH, extravasation of red blood cells (RBCs) is followed by RBC lysis, oxidation of extracellular hemoglobin (Hb) and local inflammation in the subarachnoid space. We recently described elevated cell-free miRNA levels in cerebrospinal fluid (CSF) of preterm infants with IVH.
Aims: We have further characterized the relationship between oxidized Hb induced inflammatory response and CSF miRNA expression after IVH.
Methods: CSF samples were analyzed in 47 preterm infants at the gestational age of 28.2 ± 3.2 weeks with grade III or IV IVH vs. clinical controls (n = 14). miRNAs were isolated with miRNeasy kit, and miR-223, miR-155, and miR-181b were quantified by RT-qPCR after normalization to "spike-in" cel-miR-39. Oxidized Hb and total heme levels were determined by spectrophotometry. Concentrations of IL-8, VCAM-1, ICAM-1 and E-selectin were measured by ELISA. *In vitro* controlled hemolysis experiment was performed to investigate whether these miRNAs were released from RBCs upon IVH.
Results: Expression of miR-223, miR-155 and miR-181b and the levels of oxidized Hb/total heme with IL-8 were the highest in CSF samples obtained between days 1-14 after the onset of IVH vs. controls and were significantly decreased by post-IVH days 29-49. Significant correlation was observed between these miRNAs, oxidized Hb and soluble adhesion molecules levels. During follow-up, oxidized Hb was eliminated with attenuated miRNAs and VCAM-1/ICAM-1/E-selectin expression in CSF. miR-223 indicated the presence of oxidized Hb in CSF (ROC-AUC value: 0.745 [95% CI 0.597-0.892]; p = 0.007). Importantly, these miRNAs remained unaffected by increasing degree of artificial hemolysis *in vitro* excluding their RBC origin.
Conclusions: Hb oxidation induces CSF miRNA expression during inflammatory response in preterm infants with IVH. These miRNAs may serve as potential CSF biomarkers to monitor clinical conditions after IVH.

PB1250 | Challenges in Diagnosing and Treating Polycythemia Vera with Acquired von Willebrand Disease in a Pediatric Patient

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Background: Myeloproliferative Neoplasms (MPN) are rare in pediatrics and management is complex due to limited literature. Classical MPNs include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis. The most common complications are hemorrhage, thrombosis, and leukemogenic conversion. Management is further complicated in patients with acquired von Willebrand (VW) disease. Treatment options for PV in adults include phlebotomy, hydroxyurea, and pegylated-interferon but treatment in pediatrics is less well-characterized.
Aims: Report diagnosis and management of refractory PV with acquired VW disease in a pediatric patient.
Methods: Literature review and expert consultation for single case report.
Results: Our patient is a 10 year old female who presented with a longstanding history of headaches and intermittent pain, numbness and cyanosis of her extremities and a platelet count of 1,125 X 10^9/L. A diagnosis of ET was made by bone marrow evaluation and presence of JAK2 V617F mutation. Laboratory evaluation confirmed low VW factor and aspirin was discontinued despite symptom improvement. Her diagnosis was changed to PV due to increased hematocrit at 46.6% with low erythropoietin level. The patient was started on hydroxyurea but was switched to pegylated interferon due to severe persistent oral ulcers. Patient's course has been complicated by significant transaminitis and treatment was changed to a combined regimen of low dose hydroxyurea (5 mg/kg) and pegylated-interferon (45mcg every other week). Patient is currently symptom-free on this regimen with platelet counts below 600 X 10^9/L and normal hemoglobin/hematocrit with minimal side effects.
Conclusions: Distinguishing PV vs ET in children is challenging as presentations can be similar and diagnostic criteria are extrapolated from adults. The distinction between PV and ET is vital as PV patients have a poorer prognosis. Management includes preventing thrombohemorrhagic complications, which can be challenging in patients with acquired VW disease. This case demonstrates the complexity of diagnosis and management of MPNs in the pediatric population.

PB1251 | Predictive Factors for Disseminated Intravascular Coagulation in Childhood Acute Lymphoblastic Leukemia

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Background: Recent advance in the treatment of childhood acute lymphoblastic leukemia (ALL) results in a substantially high remission

TABLE 1 Multivariate logistic regression results showing independent predictive factors of DIC in children with ALL

Variables	Adjusted Odds Ratio (95% CI)	P value
Age group: 10-13 years 1-9 years ≥ 14 years	Ref 10.67 (0.41, 280.77) 125.54 (2.16, 7303.95)	0.021
Liver size: <6 cm below costal margin ≥ 6 cm below costal margin	Ref 4.21 (0.6, 29.51)	0.152
Baseline albumin level (g/dL): ≥3.5 < 3.5	Ref 52.76 (4.1, 679.35)	<0.001
Chemotherapy regimen: Standard risk High/very high risks	Ref 10.61 (0.61, 185.21)	0.062

rate. However, the outcomes in developing countries are relatively lower owing to the induction-related deaths secondary to either malignancy or infection-associated disseminated intravascular coagulation (DIC).

Aims: To investigate the risk factors predicting an occurrence of DIC following the induction chemotherapy in children with ALL.

Methods: The hospital records of children aged 1-15 years diagnosed with ALL and received chemotherapy between October 1st, 2014 and September 30th, 2019 were retrospectively reviewed. Patients were classified into two groups based on an occurrence of DIC according to the International Society of Thrombosis and Hemostasis criteria. Variables were compared between two groups using univariate analysis; multivariate logistic regression model were used to identify independent factors for DIC and the prediction capability was evaluated using a receiver operating characteristics (ROC). Ethics approval was obtained and informed consent was waived.

Results: Eighty-one children were included in the study; 12 (14.8%) with DIC and 69 (85.2%) without DIC. Age < 10 or ≥ 14 years, liver size ≥ 6 cm below costal margin, hypoalbuminemia (< 3.5 g/dL) at presentation, and chemotherapy regimen for high/very high risk ALL patients were associated with DIC in the univariate analysis. There was no difference in sex, bleeding at presentation, other presenting features, initial white blood cell count, ALL immunophenotype, or cytogenetics between two groups. Independent predictive factors associated with DIC in the multivariate analysis were age ≥ 14 years (OR 125.54, 95% CI 2.16-7303.95) and hypoalbuminemia at presentation (OR 52.76, 95% CI 4.1-679.35) (Table 1). The discriminative ability of the logistic model was shown by the ROC curve (Figure 1) with an area under the curve of 0.95.

Conclusions: Age ≥ 14 years and hypoalbuminemia at presentation were independently associated with DIC in children with ALL.

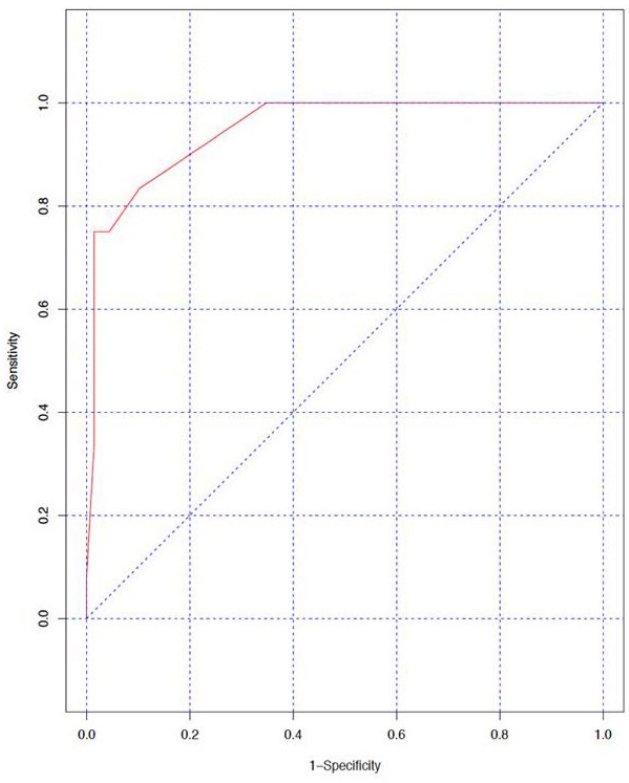

FIGURE 1 ROC curve of logistic regression model for the prediction of DIC in children with ALL

PB1252 | Massive GI-Bleeding Caused by Acquired von Willebrand Syndrome in Pediatric Allogeneic Hematopoetic Stem Cell Transplantation

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Background: Acquired von Willebrand syndrome (avWSII) causing bleeding complications in patients with extra corporal circulation or mechanical heart valve replacement is well known. Reports of avWSII occurring in children after allogeneic hematopoetic stem cell transplantation (HSCT) are rare. Diagnosis is complicated by elevated Willebrand parameters obscuring the relative loss of large vWillebrand factor (vWf) multimers that are most important for coagulation.

Aims: Raise awareness for a differential diagnosis that causes bleeding after HSCT.

Methods: We report four children (aged 4 - 10 years) who suffered recurrent Hb relevant gastrointestinal bleeding episodes after undergoing HSCT due to hematological malignancies or metabolic disorders. One patient suffered from an additional intracranial bleed in context of arterial hypertension and posterior-reversible-encephalopathy-syndrome. No patient had had a bleeding history before HSCT.

Results: Bleeding started abruptly 3 - 9 months after HSCT, during complicated recoveries after high grade graft versus host disease (GvHD). Histologically gastrointestinal GVHD was under control at the time of bleeding. All patients required significantly more blood transfusions than expected. Comprehensive coagulation work up revealed an elevated level of vWf in all patients. Quotient of vWf antigen and vWf activity lay between 1.2 and 1.5; indicating an

inadequate quality of vWf. Diagnosis was later confirmed by multimer analysis. All patients were treated on demand with FVIII & vWf combination-products. Desmopressin was avoided due to expected tachyphylaxis and difficult fluid/ electrolyte management. With treatment, bleeding episodes were significantly reduced. In one patient multimer analysis had normalized 12 months after HSCT.

Conclusions: AvWSII seems to be a relevant, transient factor in bleeding patients with a history of HSCT and GI GvHD, because adequate treatment decreased bleeding. Diagnosis of avWSII is challenging. Prospective research evaluating the incidence of avWSII in patients undergoing HSCT is necessary in order to reduce bleeding complications, blood transfusions and immunosuppression.

PB1253 | Validity and Reliability of Self-Administered Thai Pediatric Bleeding Questionnaire (TPBQ) Application to Predict Risk of Bleeding in Pediatric Bleeding Disorders

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Background: Congenital and acquired bleeding disorders present with various symptoms ranging from mild to severe bleeding. In recent years, there has been attempted to remodel bleeding histories into questionnaire to predict risk of bleeding and describe symptoms severity. Pediatric Bleeding Questionnaire (PBQ) is a pediatric-specific bleeding questionnaire that has been used worldwide. In Thailand, Thai Pediatrics Bleeding Questionnaire (TPBQ) - has been developed but still hasn't been widely used.

Aims:

(a) To test validity and reliability of self-administered TPBQ application compared to original PBQ in Thai children with bleeding disorders.

(b) To predict risk of bleeding by using self-administered TPBQ application.

Methods: This is a descriptive analytic study. Patients who were diagnosed as bleeding disorders (von willebrand disease (VWD), hemophilia, congenital factor deficiencies and immune thrombocytopenia (ITP)) and visited at King Chulalongkorn Memorial Hospital between 2019-2020 were interviewed by a physician using original PBQ then followed by self-administered TPBQ application in the next two weeks. Results were analysed by using mean and test-retest reliability by intraclass correlation (ICC) and internal consistency.

Results: 41 patients were enrolled in this study (17 patients with VWD, 8 patients with severe hemophilia A, 3 patients with congenital factor VII deficiency and 13 patients with ITP). The mean score in VWD by TBPQ and original PBQ are 5.7 and 6.5, severe hemophilia A are 10.8 and 12.5, congenital factor VII deficiency are 21 and 21, and ITP are 5 and 5.2 respectively. The duration of the interviews for self-administered is 6.4 minutes and expert-administered is 3.8 minutes. The reliability proved excellent with intra-class correlation coefficient 0.86 for test-retest. The internal consistency was strong (Cronbach-alpha = 0.92).

Conclusions: Self-administered TPBQ application is comparable to original version of PBQ interviewed by physicians in predicting severity and risk of bleeding in Thai children with bleeding disorders.

PB1254 | Comparison between Bleeding Time and PFA-200 to Evaluate Platelet Function Disorder in Children

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Background: The bleeding time test, one of the tests used in evaluating platelet function, is difficult to perform, standardize and interpret in children.

Aims: To evaluate the sensitivity, specificity and efficiency of bleeding time test and PFA-200 in a series of pediatric cases with platelet dysfunction condition.

Methods: This prospective study was conducted at King Chulalongkorn Memorial Hospital (KCMH) during 2017-2019. Children, age ranging from 6 months to 18 years, were sent to undergo bleeding time together with other routine blood test. The authors used the residual sample, about 1.6 ml, to perform PFA-200. Ninety-seven children (51 of them females, mean age 9.14 ± 4.35 years) were enrolled into this study. The subjects were divided into 2 groups: platelet dysfunction group and normal platelet function group. All subjects were sent to undergo bleeding time and PFA-200. Subjects with congenital platelet disorder, including von Willebrand disease and Glanzmann′s thrombasthenia, and those who are Aspirin users represent the platelet dysfunction group. All of the patients in this group were confirmed to have platelet dysfunction by the gold standard test, platelet aggregation test.

Results: Use of PFA-200 offered 81.3% sensitivity and 95.9% specificity for detection of qualitative platelet abnormalities, whereas bleeding time testing showed 33.3% and 93.9% respectively.

Conclusions: The PFA-200 is a more efficient test; it, therefore, can replace the bleeding time test as a component of the laboratory evaluation of children with platelet dysfunction.

PB1255 | Hemostasis in Parenteral Nutrition Associated Cholestasis in Preterm Infants: An Observational Study

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Background: Parenteral nutrition (PN) associated cholestasis (PNAC) affects one-fifth of neonates receiving PN for more than 2 weeks. Experts suggest vitamin K administration, based on the assumed

TABLE 1 Demographical data of the study population. GA: gestational age; PCA:post-conceptional age; CRIB II: clinical risk index babies score

	1. NO PNAC (n = 60)	2. PNAC (n = 32)	1 vs 2, p-value
GA (median, range); weeks	30 (24-32)	29 (25-32)	ns
PCA at the time of sample (median, range); weeks	34 (28-37)	35 (30-36)	ns
Birthweight (median, range); grams	1275 (700-1495)	1065 (660-1490)	0.001
Gender, male n(%)	24(40%)	16(50%)	ns
Singleton, n(%)	25(41.7%)	17(51.3%)	ns
Cesarean section, n(%)	54(90%)	26 (81.3%)	ns
CRIB II (median, range)	6 (2-16)	8(3-14)	0.018
APGAR 5 minutes (median, range)	8 (6-10)	9 (6-10)	ns
Lenght of stay (median, range); days	54 (27-270)	75 (30-269)	0.005

increased risk of bleeding. However, the hemostatic profile is poorly defined in PNAC.

Aims: To investigate the effect of PNAC on the hemostatic profile of preterm infants.

Methods: This is an ancillary study of a larger prospective observational study aimed at defining hemostasis in preterm infants with gestational age below 32 weeks, in the first month of life. The study was approved by the Ethical Committee and informed consent was obtained from patients' guardians. In this secondary analysis, we included preterm infants exposed to at least 14 days of PN (standard vitamin K content) and in full enteral feeding at the time of sampling. We excluded patients receiving extra doses of vitamin K before blood withdrawal. We compared thromboelastography-TEG, thrombin generation assay-TGA (lag time-LT; endogenous thrombin potential-ETP with/without thrombomodulin), PT, aPTT and blood count between infants who developed PNAC versus those not affected by PNAC (controls), at 30 days of life.

PNAC was defined based on the value of direct bilirubin ≥ 2mg/dl.

Results: We enrolled 92 preterm infants: 32 neonates affected by PNAC and 60 controls (Table 1). As expected, cholestatic patients had a lower birthweight (1065-vs-1275;p = 0.001), longer exposure to PN (28-vs-21 days;p = 0.001) and longer hospitalization (75 vs 54 days;p = 0.005). Infants with PNAC showed longer median PT (12.8 vs 12 sec;p = 0.02), aPTT (39.2 vs 36.5 sec;p = 0.04) and LT (4.9 vs 4.4 min;p = 0.008) than controls. TEG and ETP did not differ between groups (Table 2).

Conclusions: Despite the prolongation of PTs, aPTTs and LT in preterm infants affected by PNAC, both TEG profile and ETP with/without thrombomodulin were similar between the two groups. Further studies are needed to validate the clinical significance of these results.

TABLE 2 Hemostatic profile of the study population. Data are expressed as [number] median (range)

	1. NO PNAC	2. PNAC	1 vs 2, p-value
Reaction time (sec)	[56] 4.85 (02-16.9)	[31] 4.7 (2.1-14.0)	ns
Maximum amplitude (mm)	[56] 71.1 (35.8-79.7)	[31] 71.1 (48.0-77.9)	ns
PT (sec)	[57] 12.0 (9.7-16.6)	[32] 12.8 (10.4-16.3)	0.02
aPTT (sec)	[57] 36.5 (26.1-58.2)	[32] 39.1 (28.3-59.9)	0.041
Fibrinogen (mg/dl)	[60] 229 (103-393)	[32] 222.5 (117-442)	ns
LT_TM (min)	[57] 5.1 (3.1-10.5)	[29] 5.7 (4.6-11.2)	0.022
ETP_TM	[57] 1108 (444-1905)	[29] 1007 (329-1829)	ns
Platelet count (103/µL)	[56] 443 (98-853)	[31] 318 (45-581)	0.001

PB1256 | Assessment of Vitamin K Level in Newborn Presenting with Bleeding in Population of Rural Sindh, Pakistan

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Background: Vitamin K deficiency bleeding(VKDB) has been classified by age of onset into early (< 24 hours), classical (days 1-7) and late (1 week to 6 months), and by etiology into idiopathic and secondary in 1999 by the Pediatric and Perinatal Subcommittee of ISTH. Limited data are available on the relative frequencies of early, classic, and late vitamin k deficiency bleeding and exact level of vitamin K has not been assessed before in the population of Sindh.

Aims::

1- To assess Vitamin K level in newborns presenting with bleeding.

2- To determine demographic profile of VKDB patients.

Methods: This descriptive cross-sectional study was conducted at the department of Pathology and department of Pediatrics LUMHS from August 2018 till December 2019. Patients who met the criteria were selected from Paediatrics department. Detailed bleeding history and clinical examination of the patient was done. All patients underwent primary screening for bleeding including PT and APTT that was performed on Coagulation Analyzer CA- 600. Samples were analyzed using Vitamin K1 (VK1) ELISA Kit by Abbexa(UK). Data was analyzed using SPSS 20.

Results: Sample size was 114, with age ranging from day 01 up to day 180 (up to 6 months) out of which 56 were males and 58 were females. P- value: 0.03. The mean age was 44.01 days. PT, aPTT, were prolonged in almost all of the cases. Mean PT was recorded as

21.14 and mean aPTT was 51.84. Normal VK level found in 63.2%, whereas 36.8% had low Vitamin K level. (< 0.16 ng/ml).
Conclusions: Vitamin K deficiency presents with a variable pattern of bleeding. It is more frequent in male, preterm neonates.

PB1257 | Application of the First Russian Biosimilar of Recombinant Clotting Factor VII in an Infant with Antenatal Cryptogenic Hepatitis and Bleeding Developed After Laparosopic Liver Biopsy

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Background: There is a necessity of effective hemostasis with the possible occurrence of delayed bleeding after laparoscopic liver biopsy in conditions of coexisting prothrombotic and hemorrhagic disorders.
Aims: To analyze hemostasis system disorders and opportunities of their correction during laparoscopic liver biopsy in an infant with antenatal cryptogenic hepatitis.
Methods: An infant, 2 months old, was treated in the perinatal center of Saint Petersburg State Pediatric Medical University clinic with antenatal cryptogenic hepatitis complicated by hepatocellular failure. To assess the severity of damage and fibrosis, laparoscopic liver biopsy was made. The hemostatic system was assessed by platelet count, APTT, PT-INR, fibrinogen level, antithrombin (AT), PrC, PrS activity, thromboelastography data.
Results: There were no clinical manifestations of hemorrhagic syndrome before laparoscopic liver biopsy. Platelet level was 125x10.9/l, APTT - 2,31/68,2s(0,80-1,2/25,1-36,5s); PT - 21,1/44%(10,6-13,7s/80-120%); INR - 1,8(1,0); fibrinogen - 0,8 g/l(2-4 g/l). The results of thromboelastography: R min 5,6; K min 3,9; Angle deg 47,7; MA mm 35,9 (hypocoagulation). AT and PrC activity levels were extremely low (AT < 7,2%; PrC - 0%). Thus, the patient had a potential risk of both hemorrhagic and thrombotic complications.
Conclusions: Hepatocellular failure was key factor in the imbalance of coagulation and anticoagulation systems with the threat of both hemorrhagic and thrombotic complications during laparoscopic liver biopsy. In our opinion, the combination of FFP and the recombinant factor VII effectively stopped intraabdominal bleeding and was safe without possible thrombotic complications at critically low levels of AT and PrC.

PB1258 | Features of Epistaxis Predictive of a Mucocutaneous Bleeding Disorder (MBD) in Children

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Background: Epistaxis is a common childhood bleeding symptom, not only in children with an underlying inherited MBD, i.e., von Willebrand disease (VWD) or a platelet function disorder (PFD), but also in healthy children. An epistaxis-specific bleeding assessment tool (epistaxis-BAT) could be useful in screening for affected children. In addition to an indication of clinical severity, as determined by the Pediatric Bleeding Questionnaire (PBQ) or ISTH-BAT, an epistaxis-BAT should also include features of epistaxis that are predictors of a diagnosis of a MBD.
Aims: To determine which features of epistaxis are predictors of a diagnosis of a MBD in children.
Methods: Univariate and multiple logistic regression analyses were performed on previously collected data from the epistaxis section of the PBQ administered to children diagnosed with VWD or a PFD and healthy controls, ≤ 18 yrs (Stokhuijzen et al, J Pediatr 2018). The epistaxis features analyzed were: duration (>10 min); frequency (>5/yr); spontaneous onset; site, specifically, occurring from both nostrils; and presence of seasonal correlation.
Results: 66 patients with a diagnosis of VWD or a PFD (median age: 12 yrs; 59% male) and 56 controls (median age: 12 yrs; 50% male) were included in the analysis. Using univariate logistic regression, the features of epistaxis that were found to increase the odds of a MBD diagnosis were: duration (odds ratio (OR) 16.7; 95% confidence interval (CI), 6.4-53.2); frequency (OR 8.2; 95% CI, 3.7-19.6); site (OR 7.7; 95% CI, 3.4-18.3); and seasonal correlation (OR 2.9; 95% CI, 1.3-6.6). Subsequent multiple logistic regression analysis showed that duration (OR 10.9; 95% CI, 3.2-46.8) and frequency (OR 4.36; 95% CI, 1.6-12.8) were the major predictors for an underlying MBD.
Conclusions: Duration and frequency of epistaxis are the strongest predictors of a diagnosis of a MBD in children. Thus, these features would be important to include in an epistaxis-BAT.

PB1259 | Quantitation of Bivalirudin Effect Using Microliter Scale Sample Microfluidic Assay in a Pediatric ECMO Population

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Background: Bivalirudin, a direct thrombin inhibitor, is an evolving alternative to unfractionated heparin (UFH) for anticoagulation therapy in the pediatric critical care setting. A functional test for bivalirudin associated anticoagulation status is not available.

TABLE 1 Patient Descriptive Statistics

Age	Indication	Drug	Drug Dose Range	No. Samples	Sample 1 (FIIa CTS)	Sample 2 (FIIa (CTS)	Sample 3 (FIIa CTS)	Sample 4 (FIIa CTS)	% Samples Within Target Range (UFH CTS 6.0-14.5) (Bivalirudin CTS 5.4-15)
5 days	ECMO (VA ECMO)	UFH	24-25 U/kg/hr	2	High (18.0)	Low (4.0)			0%
9 months	ECMO (VA ECMO)	UFH (x2), Bivalirudin (x2)	28-38.5 U/kg/hr; 0.28-0.7 mg/kg/hr	4	Target (8.9)	Low (3.10	Target (11.7)	High (19.9)	50%
11 years	ECMO (VV ECMO)	Bivalirudin	0.65-0.82 mg/kg/hr	2	Low (1.5)	High (17.2)			0%
9 months	ECMO (Berlin)	UFH	16-20 U/kg/hr	3	High (18.5)	Target (13.2)	High (19.9)		33%
11 months	ECMO (Jarvik)	Bivalirudin	0.72-0.82 mg/kg/hr	3	High (14.6)	Target (14.1)	High (15.0)		33%
17 years	Sub Massive Pulmonary Embolism	UFH	10-18 U/kg/hr	3	Low (5.5)	High (24.6)	Low (-3.1)		33%
18 years	Ventricular Assist Device (LVAD)	Bivalirudin	0.22-0.3 mg/kg/hr	3	High (21.3)	High (21.6)	Target (13.4)		33%

Aims: Demonstrate the ability of a microliter volume capillary assay to detect the effect of bivalirudin and UFH and quantify the level of anticoagulation using a functional, clot time end point in pediatric critical care patients.

Methods: Under IRB approval, a retrospective analysis of 20 citrated, frozen, bio-banked plasma specimens from 7 anticoagulated pediatric patients was performed. Five patients were on extracorporeal membrane oxygenation, one patient had a sub massive pulmonary embolism and one patient was on a ventricular assist device, providing 11 bivalirudin and 9 UFH samples. (Table 1). 100 µL of each sample was re-calcified in the presence of Coagulo's reagents (combinations of Factor IIa/Xa), the time to clot at 37°C was measured and the Clotting Time Score (CTS) was calculated using optimized algorithms. Reference clotting time curves for each drug were made using spiked healthy adult samples. The target CTS range was determined by comparison to clinical target drug levels: Bivalirudin (0.7-2.0 ug/mL), UFH (0.2-0.4 IU/mL) (Figure 1A-B).

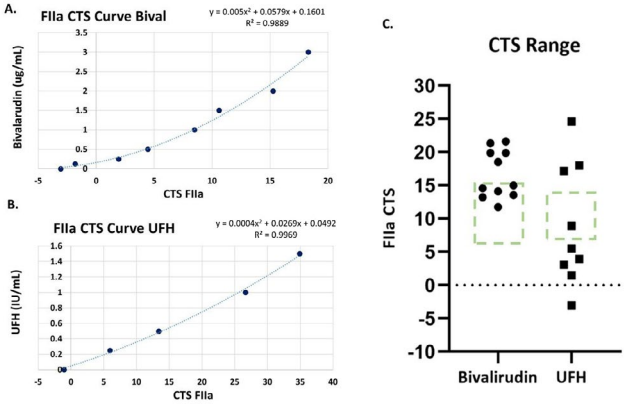

FIGURE 1 Factor IIa inhibition related Clotting Time Score (CTS) analysis of Bivalirudin and UFH Patient samples

Results: The CTS range corresponding to clinical target drug levels was 6.0-14.5 (Bivalirudin) and 5.4-15.0 (UFH), providing an assessment of below-, on- or above- target drug range (Figure 1C). Using the CTS, only 25% of samples were within the target range. Bivalirudin samples were on (36%) or above (64%) target while UFH samples were below (56%), on (11%) and above (33%) target.

Conclusions: The CTS range appears independent of the anticoagulant and may be useful to create functional reference ranges to guide anticoagulation therapy. The small sample volume required allows for more frequent testing, reducing the risk of anticoagulation-related complications and iatrogenic blood loss.

PB1260 | Assessing Anticipatory Guidance Needs in Adolescents with Bleeding Disorders

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Background: In 2007, RSA 's survey of Adult Transition and Health (SATH) found that only over one half of youth ages 19-23 years reported receiving health care transition suggesting a large portion not receiving anticipatory guidance to support transition care to adult care from their pediatric health care providers. Transition, defined as the purposeful & planned process of educating patients to competently manage their own chronic conditions, is a topic of vital importance to the HTCNV's lifelong comprehensive care model. Operationally, success is determined by an adolescent's ability to independently manage their own care by the age of 19.

Fig. 2a – Overview & Description of Capture Rate

Dates 2018-08-24 – 2018-11-24	#	%
Total eligible patients	**24**	
Total patients assessed	**19**	*79.2*
Patients by age & sex		
10-14y, female	3	
10-19y, female	5	
Subtotal	**8**	*42.1*
10-14y, male	9	
10-19y, male	2	
Subtotal	**11**	*57.9*
Patients by diagnosis		
FVIII def., mild	4	*21.0*
FVIII def., moderate	2	*10.5*
FVIII def., severe	1	*5.3*
FX def., mild	1	*5.3*
FX def., severe	1	*5.3*
vWD, type 1	7	*36.8*
vWD, type 2B	1	*5.3*
vWD, type 2M	1	*5.3*
Platelet disorder	1	*5.3*

FIGURE 1 2A

Aims: to efficiently implement a standardized multidisciplinary anticipatory guidance tool & process without the need of additional staff/resources

Providing focused anticipatory guidance towards medical independence implies better medical outcomes in adulthood

Methods: patients aged 10-19 years who have been diagnosed with a bleeding disorder were evaluated using the Transition Assessment to determine ease of implementation during clinic operations (Capture Rate) and transition assessment and understanding

Results: From the time period 2018-08-24 to 2018-11-24, team members achieved a 79.2% capture rate which exceed target 75%. 19 of 24 patients were assessed. During this timeline 74 pediatric patients were seen in clinic, only 24 of those visits were eligible

Conclusions: Overall, HTCNV able to capture 79.2% of eligible patients, far exceeds the deficits identified by the SATH survey. An analysis of assessment scores found that age and severity of diagnosis may be predictors of successful transition. A severe diagnosis may imply a greater likelihood of clinical interaction. We did not put into consideration of patients with possible learning disorders or limitations. It is possible that certain topics covered in the tool may be more relevant to a young adult (i.e. 18-25 yo) than adolescent but unknown with further data on nature and quality of the educational intervention themselves.

PB1261 | Impact of Intravenous Iron Therapy in Post-menarchal Adolescents Hospitalized with Acute Heavy Menstrual Bleeding

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Background: Acute heavy menstrual bleeding (HMB) in post-menarchal adolescents often leads to iron deficiency (ID) and anemia. Literature on the impact of intravenous (IV) iron therapy in such patients is lacking.

Aims: To evaluate the impact of IV iron therapy in post-menarchal adolescents hospitalized for acute HMB.

Methods: This was a retrospective cohort study of post-menarchal adolescents admitted to a tertiary care children's hospital for acute HMB, approved by the institutional review board. Clinical/laboratory data from initial admission/follow-up were collected. The Wilcoxon rank-sum test, Fisher's exact test or Chi-square test were used to test associations between variables. Logistic regression was used to test associations and estimate odds ratios with 95% confidence intervals.

Results: Eighty-six patients were admitted for acute HMB. Table 1 and 2 summarizes the patient demographic and characteristics. When compared to patients who did not receive, patients who received IV iron had significantly shorter median time to hemoglobin normalization (77 vs. 29 days, p = 0.007) and ferritin normalization (74 vs 27 days, p = 0.006). Hemodynamic instability (heart rate > 100/minute, systolic blood pressure (BP) < 90 mmHg or diastolic BP < 60 mmHg) noted in 66/85 (78%), was not associated with red blood cell (RBC) transfusion (p = 0.122), with 16/19 (84%)

TABLE 1 Patient Demographic

	N Total	N (%)
Hispanic	86	49 (57.0)
Non-Hispanic: Caucasian	86	8 (9.3)
Non-Hispanic: Black	86	24 (27.9)
Non-Hispanic: Other	86	5 (5.8)

TABLE 2 Patient Characteristics

	N Total	Mean (SD)
Age at Menarche	85	11.5 (1.5)
Years from Menarche to HMB diagnosis	85	2.6 (1.8)
	N Total	N (%)
Bleeding disorder present	86	9 (10.5)
Anemia (Hb < 12 g/dl)	86	85 (98.8)
Iron deficiency (Ferritin < 20 ng/ml)	61	56 (91.8)
RBC Transfusion	86	80 (93.0)
IV Iron therapy	84	18 (21.4)

hemodynamically stable patients receiving RBC. Hemoglobin < 7 g/dl (p = 0.503), ferritin < 20 ng/ml (p = 0.311) or hemodynamically instability (p = 0.481) were not associated with receiving IV iron.

Conclusions: Our study shows that IV iron therapy is effective for anemia/ID management in post-menarchal adolescents hospitalized for acute HMB, with significantly faster normalization of hemoglobin/ferritin. RBC transfusion and IV iron use were independent of hemodynamic stability. Majority of hemodynamically stable patients received RBC; according to current recommendations, these patients could have been treated with IV iron alone, thereby avoiding RBC exposure. Future efforts to utilize IV iron therapy for hemodynamically stable patients will help to minimize transfusion requirement/exposure.

PB1262 | Use of 'Virtual Clinics' Telehealth Model in Pediatric Patients with Hemophilia and Inherited Bleeding Disorders: Experience from a Tertiary Care Center

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Background: Virtual Clinics (VCs) enterprise is being utilized widely by health systems, especially applicable for patients with chronic diseases such as Hemophilia and other inherited bleeding disorders requiring frequent interaction with care providers. Pediatric Non-Malignant Blood Disorders (NMBDs) section at King Faisal Specialist Hospital and Research Centre, Riyadh is the main tertiary referral center in the Kingdom of Saudi Arabia for patients with Inherited bleeding disorders.

Aims: To optimize access to specialized care to patients in the peripheries of the Kingdom by increasing VCs utilization facilitating comprehensive care delivery.

Methods: Children (≤14 years) with NMBDs are scheduled twice weekly (8 slots) in VCs through Health Outreach department in coordination with Hematologist and Clinical Nurse Coordinator responsible for patient selection. Dashboard created utilizing web-based system (REDCap) for tracking purposes.

Results: Ninety virtual clinics (VCs) were conducted in 2019 with 244 children seen: hemoglobinopathies, 95 (40%); bleeding disorders, 62 (25%); bone marrow failures (BMFs), 39 (16%) and working diagnosis 48 (20%). Total of 387 patient visits registered - an average of 1.5 visit / patient; majority of seen from the Southern 42% (103) and Northern 21% (52) regions of the country. Amongst bleeding disorders majority of the patients seen were Hemophilia, 27%; Rare Bleeding Disorders and Glanzman Thrombasthenia, 26% enabling timely laboratory test result reviews, factor replacement refills, patient education, vaccinations and dental referrals. In terms of hospital-metrics a total of 387 follow-up slots were made available for 'in-house" physician clinics improving outpatient efficiency by 10% and associated revenue, while logistic costs were completely eliminated.

Conclusions: In our experience patients and families were content with the VCs as personal logistic costs (transportation, accommodation and vocational) were lower while maintaining the quality of communication and consultation time, improving access to caregivers. Preliminary financial assessments underlines cost-effectiveness of adapting VCs model however a longer follow-up is warranted.

PB1263 | Prevalence and Management Approach to the Newborn with Classical and Late Vitamin K Deficiency Bleeding (VKDB) in LMIC

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Background: Vitamin K deficiency bleeding (VKDB) remains a serious but preventable problem worldwide with a high risk of mortality or permanent disability especially among exclusively breastfeeding infants.

Aims: This was a cross sectional hospital-based study to determine the prevalence and management approach to the newborns with classical and late VKDB that occur after second day of life (DOL).

Methods: All the newborns delivered from January-June 2018 in NICU of Aga khan university hospital; Karachi Pakistan and presented with active bleed after second DOL, were enrolled in study. Prothrombin time (PT) and international normalized ratio (INR) were monitored after 24 hours of birth, at active bleed and after VK therapy. VK 1 mg prophylactic dose given within 1-6 hours of delivery irrespective of birth weight (BW). Intravenous VK 3 mg and 5 mg was given once daily for 3 days to neonates with BW up to 2000 g and > 2000 g respectively.

Results: All neonates received prophylactic VK. Total of 105(19%) neonates of 552 had active bleed after second day of life. 98(93%) neonates presented with prolonged PT and INR at active bleed. 38 (36%) of up to 2000 g BW received 3 mg and 67(63%) with > 2000 g received 5 mg therapeutic doses. 38(36.2%) received fresh frozen plasma too. PT & INR normalized in 90 neonates with 3 days VK therapy. 7 were given second cycle of VK therapy with in 14 DOL. Only total of 12 neonates with active bleed were diagnosed with other than VKDB. Statistically the number of neonates with > 2000 g was significantly high with VKDB. No correlation was found between the GA and VKDB.

Conclusions: VK deficiency is highly prevalent among newborn babies in the population studied. These findings further highlight the importance of regular screening of neonates for VK deficiency and may be higher prophylactic doses required specially in neonates with > 2000 g BW.

PB1264 | Pediatric Heart Transplantation and Mechanical Circulatory Assistance: A Challenge for Hemostasis

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Background: Patients undergoing cardiac surgery requiring cardiopulmonary bypass and mechanical circulatory assistance in the immediate postoperative period may experience a series of changes in hemostasis.

Aims: To report a clinical case of postoperative heart transplantation with extracorporeal membrane oxygenation to treat post-transplant primary cardiac graft dysfunction, presenting changes in hemostasis and fibrinolysis.

Methods: The information contained in this work was obtained by reviewing medical records, interviewing with the patient, photographic record of the methods diagnoses to which the patient was submitted and literature revision.

Results: Patient, female, 4 years old, with congenital heart disease, who underwent a heart transplant requiring extracorporeal membrane oxygenation to treat post-transplant primary cardiac graft dysfunction. Presenting massive pulmonary hemorrhage on the 12th PO, with an urgent surgical approach to control hemostasis. During a surgical approach, she presented an incoagulable coagulogram, with an RNI 7.3, fibrinogen 131 mg/dl and a significant drop in hemoglobin from 11 to 5 g/dl. Evolving with hemodynamic instability and severe general condition. Red blood cell and platelet concentrate, cryoprecipitate, tranexamic acid, prothrombin complex concentrate and protamine were necessary to control hemostasis. Thromboelastogram was performed showing an increase in CFT (clot formation time),195 seconds, suggesting platelet deficiency or fibrinogen. After hemodynamic stability, Prescribed tranexamic acid by continuous infusion for five days until pulmonary surgical approach, with no complications, with adequate control of hemostasis. Thromboelastogram was performed again, showing CT (clotting time), 65 seconds, and CFT (clot formation time), 135 seconds, within normal values.

Conclusions: Risk factors such as longer mechanical circulatory support time and reduced platelet count increase the incidence of excessive bleeding in this age group. Changes in the control of hemostasis in the intraoperative and early postoperative periods occur as a consequence of the activation of the coagulation cascade and fibrinolysis, being still a challenge in clinical practice in view of the need for emergency measures.

PB1265 | Use of Prolong Parenteral Lipid Emulsions in NICU and Hematologic Disorders

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Background: Concerns have been raised regarding long-term administration of parenteral nutrition(PN) with intravenous lipid emulsion (IVLE)-induced hyperactivation of the monocyte-macrophage system with hematologic abnormalities, including recurrent thrombocytopenia with reduced platelet lifespan and hemophagocytosis in bone marrow.

Aims: To determine the occurrences of thrombocytopenia in very low birth weight(VLBW) neonates with prolong IVLE in PN.

Methods: All neonates with birth weight < 1500 g and platelets count (PC) > 150 × 103 at 24 hours were included in study. Neonates who received 1-3 g/kg/day IVLE in PN for at least 14 days finally formed study group of 56 during January-June 2019 in NICU of Aga khan university hospital, Karachi Pakistan. Recorded PC variation with 14 days of IVLE administration.

Results: During the first 7 days 41/56 had PC = 120 × 103-130 × 103. Total 17/41 had severe sepsis. In 8-14 days 20/56 had PC = 80 × 103-110 × 103 and 15/20 had severe sepsis. In 8-14 days 5/56 had PC < 50 × 103 and required Platelet transfusion, these all 5 neonates had severe sepsis and 2 were treated for fungal infection. All neonates with severe sepsis received meropenem plus vancomycin/meropenem plus colistin. Statistical analysis showed insignificant correlation between the thrombocytopenia and IVLE administration. IVLE was not stopped due to thrombocytopenia and it resolved in all patients with clinical improvement.

Conclusions: These findings suggest that 2 weeks IVLE in 1-3 g/kg/day doses has no measurable impact on neonatal platelet count and support its use in neonates even in presence of infection or thrombocytopenia, to provide the adequate energy and essential fatty acids supply in very crucial days of neonatal life.

THROMBOSIS IN NEONATES AND CHILDREN

PB1266 | Asymptomatic Catheter Related Thrombosis in Neonates: The Role of Thrombophilia Testing

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Background: The role of inherited thrombophilia testing in predicting a recurrence of deep vein thrombosis (DVT) after an incident of the first catheter related thrombosis in neonates remains unclear

Aims: To investigate the association between inherited thrombophilia and DVT recurrence.

Methods: We conducted a retrospective regional study of all consecutive ICD10 codes of venous thromboembolism (VTE) in neonate < 28 days (January 1, 2000, to December 31, 2012) in a regional catchment area of southern Sweden using an electronic diagnosis registry. Eligible subjects were children aged 0- 28 days presenting with asymptomatic catheter related thrombosis diagnosed with imaging, and having underwent complete inherited thrombophilia workup after the first DVT event, excluding central nervous thrombosis. Of the 25 patients diagnosed with DVTs, 17 were eligible for inclusion.

Results: A total of 17 patients had inherited thrombophilia workup after the incident of the first asymptomatic catheter related thrombosis. Inherited thrombophilia was found in 47% of patients, the prevalence of minor thrombophilia among these 17 patients was 41% (n = 7), whereas major thrombophilia accounted for 12% (n = 2) of abnormal results. Anticoagulants as treatment were administered in 60% of the first DVT. The majority of the cohort treated with anticoagulation therapy for 42-90 days. Both patients with major inherited thrombophilia were treated with anticoagulation therapy for 180 days. There was no report for pulmonary embolism, propagation or thrombosis recurrence after their incident of the first DVT in 3 years minimum follow up time in both treated with anticoagulation therapy and observed groups.

Conclusions: Inherited thrombophilia was not predictive of recurrent DVT among our cohort patients. Our findings suggest that Inherited thrombophilia testing may not predict risk for the recurrence neonatal catheter related thrombosis. Anticoagulation therapy should be evaluated on a case-by-case basis after careful assessment of the risk factors for thrombosis and bleeding.

PB1267 | Pediatric Hospital-Acquired Venous Thromboembolism: Mayo Eugenio Litta Children's Hospital Experience

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Background: Pediatric hospital-acquired venous thromboembolism (HA-VTE) events have increased over the last two decades. There is

TABLE 1 Patient Demographics

Variable	N=68 (%)
Sex	
Female	32 (47)
Male	36 (53)
Age (years)	
Median	2
0 to 5	41 (60)
6 to 10	6 (9)
11 to 15	13 (19)
>15	8 (12)
Disease Categories	
Cardiac	15 (22)
Infectious	15 (22)
Respiratory	11 (16)
Hematologic/Oncology	8 (12)
Other	19 (28)
Primary Service	
Pediatric ICU	46 (68)
Cardiovascular ICU	12 (18)
General Pediatrics	3 (4)
Hematologic/Oncology	3 (4)
Surgery	4 (6)
LOS (days)	
Range	0.66 to 240
Median	15.5
Number of Risk Factors*	
0-2	5 (7)
>3	63 (93)
Type of Line#	
PICC	22 (32)
Central	45 (66)
Location of Line$	
Upper Extremity	38 (56)
Lower Extremity	29 (43)
Presence of Additional Central Lines	37 (54)
Presence of >2 lumens	60 (95)

*Risk factors defined by ISTH recommendations

#One event occurring without presence of CVL

$One event occurring not in extremity

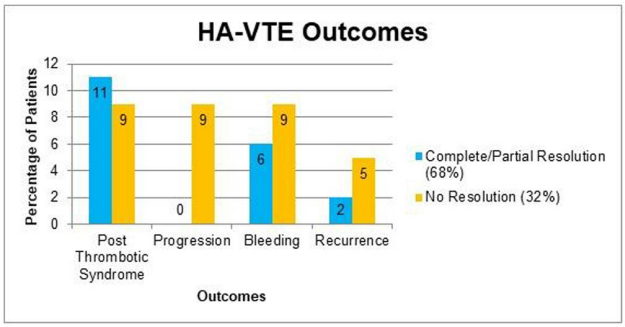

FIGURE 1 Pediatric Hospital-Acquired Venous Thromboembolism Outcomes

a need to better risk-stratify pediatric patients for HA-VTE in order to prevent acute and long-term complications.

Aims: We aim to better characterize pediatric HA-VTE events and outcomes at our institution using the International Society of Thrombosis and Hemostasis defined thrombotic risk factors, bleeding complications, and thrombosis outcomes in order to explore potential prophylactic VTE interventions.

Methods: This was a retrospective observational cohort study. All HA-VTE events occurring in children, 0 - 21 years old, admitted to our children's hospital between 2011-2019 were analyzed. Patient demographics, central venous line (CVL) characteristics, VTE characteristics, and clinical outcomes were collected using the electronic medical record. Statistical analysis was descriptive with the use of logistic regression when comparing risk factors and thrombosis outcomes.

Results: In an eight year period, 68 HA-VTE unique patient events occurred, 98% associated with CVLs. Patient demographic and clinical data are outlined in Table 1. Site of VTE was distributed evenly between upper and lower extremity with 75% of VTE events located at the CVL site. Patients < 5 years old contributed 60% of the VTE events. Sixty-eight percent of patients achieved complete or partial resolution (CR/PR), while 32% did not. Only 82% of patients were anticoagulated. Thrombosis outcomes and complications are summarized in Figure 1. For patients achieving CR/PR, 19% developed complications versus 32% in those who did not achieve resolution (p = 0.12).

Conclusions: In our study, the majority of HA-VTE events occurred in younger, critically ill patients and related to a CVL. While the incidence of pediatric HA-VTE is rare, many patients experienced acute and long-term complications related to the thrombotic event or anticoagulation used. Improved prophylactic measures for those at greater risk for VTE is needed to prevent VTE events and their associated complications.

PB1268 | Self-Management of Oral Anticoagulation with Vitamin K Antagonist Programs Progressively Improves Time in Therapeutic Range in Pediatric Population

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Background: Anticoagulant treatment with vitamin K antagonist is increasing in children diagnosed with congenital heart diseases. As international normalized ratio fluctuations are frequent in pediatric population, a suboptimal time in therapeutic range (TTR) has been usually described. Self-management programs have demonstrated to be useful increasing TTR in this field.

Aims: The aim of this study is to evaluate the sustainability and the improvement of TTR over time by using self-management of oral anticoagulation with vitamin K antagonist in a pediatric series.

TABLE 1 TTR ranges for each period in all patients and in those cases initiating the self-management program. Patients whose TTR < 60% was explained b

	Period 1 Jul-Dec 16	Period 2 Jan-Jun 17	Period 3 Jul-Dec 17	Period 4 Jan-Jun 18	Period 5 Jul-Dec 18	Period 6 Jan-Jun 19
Patients TTR 80-100% (n,%)	10 (47.7)	20 (43.5)	27 (52.9)	30 (53.6)	25 (42.4)	37 (61.7)
Patients TTR 60-80% (n,%)	7 (33.3)	18 (39.1)	16 (31.4)	17 (30.3)	27 (45.7)	20 (33.3)
Patients TTR 40-60% (n,%)	2 (9.5)	7 (15.2)	8 (15.7)	7 (12.5)	6 (10.2)	3 (5)
Patients < 40% (n,%)	2 (9.5)	1 (2.2)	0	2 (3.6)	1 (1.7)	0
TRR < 60% explained by medical conditions (n)	1	3	6	4	2	3
Educational program reinforcement when TTR < 60% (n)	3	5	2	4	4	2
Patients who improve (TTR > 60%) following 2nd educational program (n)	3	4	2	2	2	-
Number and TTR of patients initiating self-management program	n = 21 75.04 + / - 20.1	n = 25 72.24 + / - 19.7	n = 5 65.56 + / - 17.9	n = 5 63.74 + / - 21.7	n = 3 79.68 + / - 7.0	n = 1 -

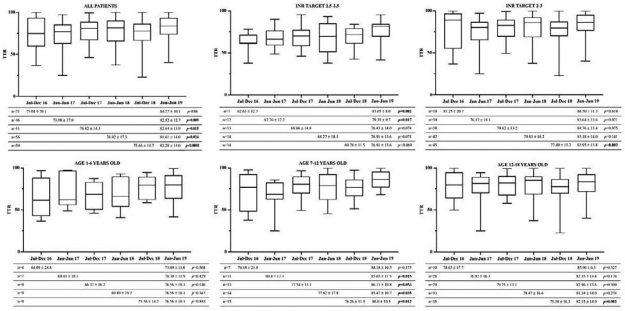

FIGURE 1 TTR for all patients and in the different INT target and age groups of patients

Methods: A prospective cohort study was conducted in a series of pediatric patients during three years in order to evaluate the evolution of TTR over time. Filiation data, anticoagulant treatment indication and data from self-management program were collected. Physicians and nurses evaluated TTR, which was calculated in periods of six months. When bad compliance was suspected, an educational reinforcement appointment was performed.

Results: A total of 60 patients were evaluated. Total follow up was of 148.28 patient-years and mean (SD) follow-up was 2.36 ± 0.65 years (range 0.42 - 3.00 years). The overall incidence of serious complications was 1.34% patient-year (CI 1.1 - 1.94). Global TTR evaluation showed a statically relevant improvement > 80% ($p < 0.05$), but this result was not achieved in small children (1-6 years old) and in patients whose INR target was 2.5 to 3.5, final TTR in those groups was > 73% (figure 1). Table 1 shows the number of patients with TTR below 60% due to medical conditions. When bad adherence to treatment or to self-management was suspected, a reinforcement of the educational program was scheduled. TTR in the following period was specifically evaluated in those cases.

Conclusions: Self-management is an adequate tool to control vitamin K antagonist, but periodically assessment of self-management program helped patients to improve the efficacy of anticoagulation.

PB1271 | Pharmacokinetic Modelling and Simulation Support the Age and Body-Weight-Adjusted Dosing of Dabigatran Etexilate in Paediatric Patients with Venous Thromboembolism

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Background: Dabigatran etexilate (DE), a direct oral thrombin inhibitor effective for the treatment of venous thromboembolism (VTE) in adults, is currently being evaluated for the treatment of VTE in children using several formulations across the age spectrum from birth to < 18 years.

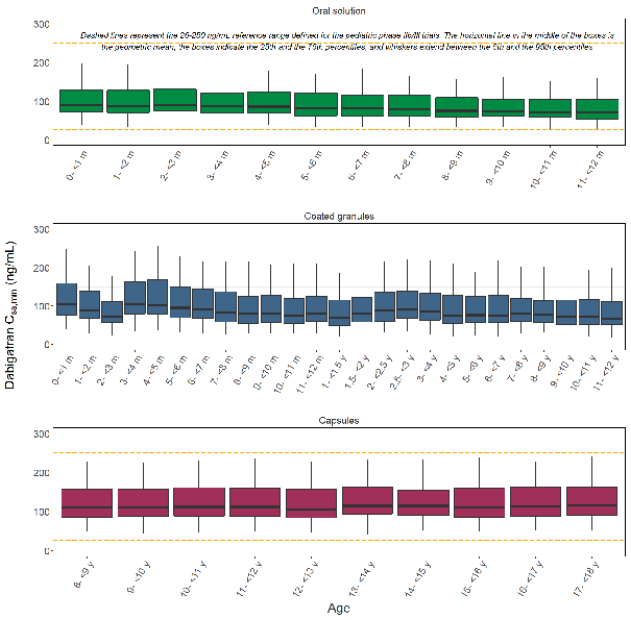

FIGURE 1 Plasma levels of dabigatran following administration to paediatric patients as oral solution, coated granules, or capsules

Aims: This study evaluated the pharmacokinetics (PK) of dabigatran in paediatric patients with VTE following administration of DE as oral solution, coated granules, or capsules. The appropriateness of a dabigatran paediatric dosing algorithm was assessed using simulations based on a population PK model.

Methods: Paediatric data from three phase IIa and two phase IIb/III paediatric studies (N = 392) as well as data from 32 healthy males (< 40 years old), were used for the analysis (consent obtained, ethics committee approved). A population PK model was fitted to the data, using non-linear mixed-effects modelling. The potential influence of co-variates, such as age, body weight and renal function, was evaluated using a stepwise co-variate model-building procedure. The final population PK model was used for evaluating the performance of the dabigatran paediatric dosing algorithm by comparing the simulated paediatric trough exposure with adult reference exposure.

Results: The population PK of dabigatran was adequately described by a two-compartment model with first-order elimination and first-order absorption. All the disposition parameters increased with increasing body weight in accordance with allometric scaling. The apparent clearance increased with increasing age and decreased with decreasing renal function. The original paediatric dosing algorithms for DE, based on body weight and age, were confirmed by simulations. The impact of dose titration was minimal.

Conclusions: A population PK model of dabigatran for paediatric patients with VTE was developed. Simulation demonstrated that the final dosing algorithms for DE were appropriate for all formulations. No dose titration is needed when DE is administered to paediatric patients as oral solution, coated granules, or capsules (Figure).

PB1272 | Pharmacokinetic-Pharmacodynamic Relationship between Dabigatran Plasma Concentrations and Coagulation Laboratory Parameters in Paediatric Patients with Venous Thromboembolism

L. Mitchell[1]; D. Roeshammar[2]; F. Huang[3]; M. Albisetti[4]; L. Brandão[5]; L. Bomgaars[6]; E. Chalmers[7]; J. Halton[8]; M. Luciani[9]; D. Joseph[3]; I. Tartakovsky[10]; S. Gropper[11]; P. Reilly[12]; M. Brueckmann[10]; J. Stangier[13]; on Behalf of the Study Investigators

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Background: Dabigatran etexilate, a direct oral thrombin inhibitor effective for treating venous thromboembolism (VTE) in adults, is being evaluated for the treatment of VTE in paediatric patients.

Aims: This study characterized the population pharmacokinetic/pharmacodynamic (PK/PD) relationships between dabigatran total plasma concentrations and coagulation laboratory parameters (measured by central lab: activated partial thromboplastin time [aPTT], diluted thrombin time [dTT] and ecarin clotting time [ECT]) in the paediatric VTE patient population, including a comparison with adults.

Methods: Paediatric PK/PD data from three phase IIa and two phase IIb/III paediatric studies (N = 382 for aPTT, N = 343 for dTT, N = 381 for ECT) were compared to data from adult patients with VTE (N = 1881 for aPTT, N = 702 for dTT, N = 1179 for ECT), and healthy adult subjects (N = 97); consent obtained, ethics committee approved. Separate PK/PD models were developed for each of the coagulation laboratory parameters, using non-linear mixed-effects modelling. Co-variates, such as sex and age, were assessed on baseline and drug-effect parameters, using a stepwise co-variate model-building procedure.

Results: Graphical explorations of observed data showed similar PK/PD relationships in children and adults for all three laboratory coagulation parameters. aPTT increased non-linearly with increasing plasma total dabigatran concentration (E_{max} [maximum effect] relationship). dTT and ECT increased linearly with increasing plasma total dabigatran concentration (Figure). On average, baseline ECT and aPTT was estimated slightly higher for children below

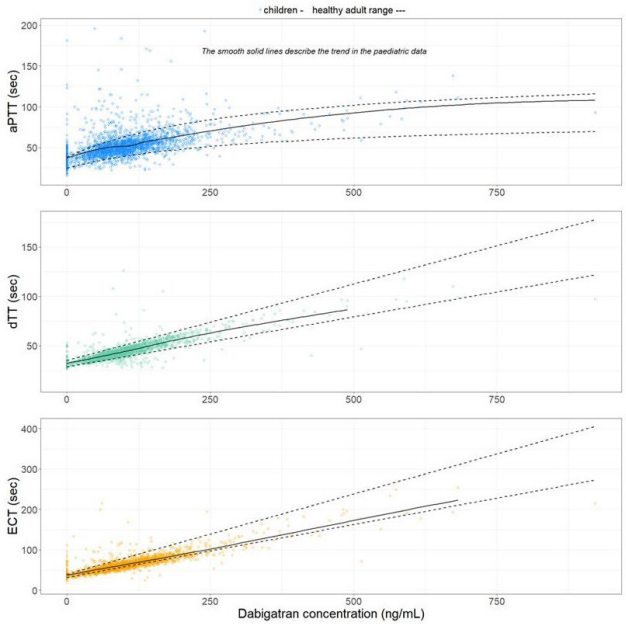

FIGURE 1 PK/PD relationships between dabigatran total plasma concentrations and aPTT, dTT and ECT

approximately 5 months than for other children. Overall, model predictions showed similar PK/PD relationships in children and healthy adults for all three laboratory coagulation parameters across all paediatric age groups.

Conclusions: Population PK/PD models have been developed for aPTT, dTT and ECT in paediatric patients with VTE. PK/PD response of dTT and ECT was linear with less variability than aPTT. PK/PD relationships were similar in paediatric and adult patients with VTE, as well as in healthy adults.

PB1274 | Improve the Quality of Life in Children with Thrombosis and Need for Oral Anticoagulation: The "Coagulin Project"

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Background: Anticoagulated children require successive clinical and analytical monitoring, with multiple hospital visits, which causes a deteriory in their quality of life and their families.

"Coagulín project"combines an innovative process of treatment,maintaining a more humane relationship and giving voice to patients, evaluating the reported health and experience results based on *PROMS"Measures of results reported by the patient" and PREMS"Measures of experience reported by the patient"*.

Aims: Evaluate safety and efficacy of treatment with antivitamin-K drugs in children with thrombotic pathology through computer applications.

Evaluate the degree of satisfaction reported health and experience results based on PROMS/PREMs.

Methods: We include children under16 who require oral anticoagulation.After an initial evaluation,parents are instructed in the management of the program and in the control of coagulation at home with portable coagulometers.The results obtained are sent via web through a personal link of the patient with the hospital.The hematologist receives and validates the result and sends the new treatment schedule and the date of the next control.

A direct mail is provided for technical complications.We evaluate quality of care results (efficacy/clinical safety) and humanization (degree of family satisfaction).

Results: n = 17.Age:1-14 years.All with thrombotic events.

Clinical/safety results: 93%controls in range.No emergency visits. No problems related to the reliability of controls, devices or software.Quality of life results:100% report improvement of their children/families with 98%satisfaction with the program, highlighting the time gained by their children for other activities,without loss of school days or parent work.The perceived improvement in family conciliation,personalized follow-ups and the children most involved in their illness were the best qualified elements.

Conclusions: "Coagulín Project"is an innovative program that prioritizes the quality of life, humanizes analytical control and favors efficient and sustainable management of resources. The results based on the patients experience(PREMS/PROMS)demonstrate a high degree of satisfaction with the program,obtaining adequate and safe controls,without relevant complications,and with an evident improvement in the life.

PB1275 | Heparin Induced Thrombocytopenia in a 9 Years Old Child: A Case Report

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Background: Heparin-induced thrombocytopenia (HIT) is a rare, life-threatening complication of heparin exposure caused by antibodies against the heparin-PF4 complex. The condition is associated with a high risk of thrombosis and is extremely rare in children.

Aims: We present a 9-years old boy with HIT.

Methods: The patient had unrecognized protein S deficiency and presented with extensive proximal deep vein thrombosis of the lower extremity and pulmonary embolism, probably associated with previous varicella infection. He started LMWH therapy immediately. Due to the extent of the thrombosis and its remarkable clinical manifestation and with the accessibility of experienced radiologists, the 3rd day from the presentation, local thrombolysis with subsequent mechanical thrombectomy was performed with a very good effect. During the procedure, the patient was on unfractionated heparin, afterwards treated with LMWH with adequate antiXa levels. Ten days after the thrombectomy, the local finding on the limb worsened, and Doppler ultrasound revealed re-thrombosis. At that time the first decrease of platelets was noticed, although platelets were still remaining within the normal range. The second attempt of local thrombolysis and thrombectomy was performed the same day with satisfactory effect. Less than 24 hours after the end of thrombolysis, a second re-thrombosis was diagnosed, at that time with further platelet decrease. HIT was suspected, heparin was stopped, and therapy with fondaparinux was initiated. HIT was later confirmed with HIT antigen test and flow cytometry. Despite the fondaparinux level was within a therapeutical range, 13 days later DUS showed progression of the thrombosis to the contra-lateral lower extremity. The therapy was switched to rivaroxaban without any further worsening of the thrombosis extent.

Results: One year after the event, the patient has partial recanalization of the involved venous system.

Conclusions: We present a rare case of a pediatric patient with HIT and difficulties with adequate therapy in children.

PB1276 | The Role of Hereditary Elevated Lipoprotein (a) as a Thrombophilic Risk Factor in Two Siblings with Cerebral Vein Thrombosis

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Background: Lipoprotein a (Lp(a)) > 30 mg/dl increases the risk of venous thromboembolic events in children by factor 7.2. It has structural homologies to plasminogen which explains its antifibrinolytic and prothrombotic characteristics. Lp(a) levels show an interindividual variability and are genetically determined.
Aims: We highlight the importance of inherited elevated Lp(a) as a thrombophilic risk factor in children.
Methods: A Caucasian family with five children is described. Two of them suffered from cerebral venous thrombosis. At the age of 14 days twin 1 developed a large thrombosis of the sinus sagittalis superior. Consequently he suffers from spastic cerebral palsy and developmental disability to this day. Years later the oldest daughter was diagnosed with a long-distance cerebral vein thrombosis at the age of 16 years. The thrombosis could be resolved via systemic anticoagulation without any remaining defects.
Results: A thrombophilia screening revealed considerably elevated levels of Lp(a) in several family members (figure). The highest values were detected in the two children suffering from cerebral venous thrombosis (girl 16y, twin 1).
In all siblings but one sister as well as in the mother heterozygous mutation of the prothrombin gen (G20210A) was detected.
Looking at the medical history of the non-consanguineous parents, one finds that the father was earlier diagnosed with four deep vein thrombosis. Furthermore there is a history of thromboembolisms in the paternal family. In contrast no thrombotic event has been observed in the mother.
Conclusions: Increased Lp(a) concentration is an important, independent risk factor for venous thromboembolisms, especially in family-related clustering events characterized by additional underlying thrombophilic disorders. Therefore, it should be included in thrombophilia screening in children.

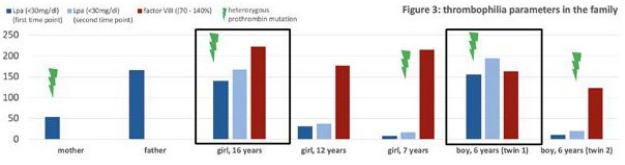

FIGURE 1 Thrombophilia parameters in the family

Generally the management of these patients can be challenging since no specific Lp(a)-lowering therapy is available to this day. Therefore, other risk factors such as LDL cholesterol levels (target level: < 100 mg/dl) need to be addressed.

PB1277 | Right Atrial Thrombosis in Children: Patient Characteristics, Outcomes, and Interrater Reliability of Echocardiographic Characterization

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Background: Pediatric right atrial thrombosis (RAT) carries potential for morbidity and mortality. However, the benefit of anticoagulation is unknown. Risk-stratification utilizing RAT echocardiographic characteristics has been suggested. RAT clinical outcomes with anticoagulation treatment based on published risk factors have not been reported. Furthermore, interrater reliability (IRR) of RAT characterization by echocardiography has not been studied.
Aims:
1) Describe RAT outcomes managed with and without anticoagulation.
2) Measure IRR of echocardiographic RAT characterization.

TABLE 1 Study patient demographics and outcomes. Multiple adverse outcomes were seen in several patients

		Total (n = 131)
Demographics	Median (observed range) in age	25d (2d-20y)
	Male (%)	67 (51)
	Weight (Kg)	3.1 (0.6-109.6)
Outcomes	No Adverse Outcome (%)	99 (76)
	Thrombus Progression	7
	Death due to Thromboembolism or Bleeding	0
	Pulmonary Embolism	2
	Clinically Relevant Bleeding	9
	Other	11

TABLE 2 Interrater reliability assessment of RAT characterization by echocardiography. Pulmonary valve dimension is a control measurement

Categorical Measure	n Patients	*Fleiss Kappa*	95% CI	Agreement
Acoustic Reflection	42	0.62	(0.47, 0.78)	Moderate
Mass Location	42	0.51	(0.41, 0.61)	Weak
Mobility	42	0.2	(0.07, 0.32)	None
Continuous Measure	**n Patients**	***Carrasco et al's CCC***	**95% CI**	**Agreement**
Longest Dimension	44	0.76	(0.64, 0.85)	Poor
Orthogonal Dimension	44	0.78	(0.67, 0.86)	Poor
Longest Adherence Dimension	43	0.6	(0.43, 0.73)	Poor
Pulmonary Valve Dimension	39	0.94	(0.9, 0.96)	Moderate

Methods: Retrospective single-center cohort study at The Johns Hopkins Hospital. Patients 0 to 21 years with RAT from 2013 to 2018 were included. Anticoagulation was initiated based on discretion of treating clinicians, but informed by presence/absence of echocardiographic RAT characteristics. The medical record was reviewed for demographics, treatments and outcomes. Adverse thromboembolic (TE) outcomes included RAT progression, pulmonary embolism (PE), clinically-relevant bleeding (ISTH-defined), and death due to TE or bleeding. For IRR of RAT characteristics, echocardiograms from 50 randomly selected subjects were reviewed by three mutually-blinded pediatric cardiologists.

Results: We identified 131 patients with RAT (Table 1). Median age was 25 days. A provoking clinical factor was present in 125 (95%). Anticoagulation was given in 67 (51%) patients. Enoxaparin was the most commonly used anticoagulant (46) followed by unfractionated heparin (21). Adverse outcomes included RAT progression in 7, PE in 2, and clinically relevant bleeding in 9. There were no deaths related to TE or bleeding.

IRR assessment demonstrated that all RAT characteristics (Table 2) had poor reliability except acoustic reflection (moderate agreement; K = 0.62 [95%CI, 0.47-0.78]).

Conclusions: We report large single-center outcomes of pediatric RAT. While echocardiographic assessment was used in decision-making regarding anticoagulation therapy, our study showed poor reliability for RAT characteristics other than acoustic reflect. Future research must focus on improving risk stratification in pediatric RAT, in order to inform interventional studies of treatment paradigms, including use and duration of anticoagulation.

PB1278 | Management and Outcomes of Newborns at Risk for Inherited Antithrombin Deficiency at the Karolinska University Hospital, Stockholm, Sweden

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Background: Since levels of antithrombin (AT) are low at birth, diagnosing inherited AT deficiency in newborns is challenging. In Stockholm, Sweden, pregnant women with known AT deficiency are referred to the Karolinska University Hospital, where local guidelines for management of newborns at risk for inherited AT-deficiency have been established. Data on pregnancies, obstetric and neonatal outcomes are included in a registry.

Aims: We aimed to evaluate the current practice at the Karolinska University Hospital on managing deliveries of these newborns (Table 1), the predictive value of AT levels at birth and the neonatal outcomes of newborns with AT deficiency.

Methods: All children born to mothers with AT deficiency at the Karolinska University Hospital 2003-2018 were identified from the registry and included in the study. Data was collected from the medical records and the registry. AT activity had been measured postnatally and after 6 months of life.

Results: The total study cohort comprised of 41 newborns. There was a significant association between low AT values postnatally and after 6 months of life (p = 0.001; Figure 1). Half (21/41) of the children were diagnosed with AT deficiency; two suffered from sinus thrombosis which presented at 10 days of age. Both children with sinus thrombosis were delivered using vacuum extraction.

Conclusions: The current guidelines are successful in identifying the children with AT deficiency and diagnosing thrombosis. AT levels at birth are predictive for inherited AT deficiency. The risk for

TABLE 1 Local guidelines for management of newborns born to mothers with known antithrombin deficiency

Heredity for antithrombin deficiency during the perinatal period. If the newborn has antithrombin deficiency there is an increased risk of thrombosis including stroke. The following guidelines for the delivery and management of the child are recommended
1. If possible, avoid instrumental delivery such as forceps or vacuum extraction to avoid bleeding that could trigger the coagulation system and lead to secondary thrombosis
2. With instrumental delivery or distressed child with impaired APGAR and / or need for intravenous access early measurement of antithrombin level is recommended
3. With unaffected child and normal delivery, the antithrombin level of the child is tested in connection with the PKU test. Early departure is discouraged
4. Brain ultrasound is recommended before discharge, with distressed child as soon as possible. If the antithrombin level of the child is above 0.5 kIE/L, ultrasound can be omitted
5. Breastfeeding should be successful before discharge to avoid dehydration, which could lead to increased thrombosis risk.
6. Parents are instructed to contact the hospital with difficulties in breastfeeding, vomiting, lethargy or decreased general condition which could indicate stroke or thrombosis
7. Inform the pediatric coagulation center of the antithrombin level daytime if the child is well; with distressed child contact the coagulation consult immediately to discuss further investigation and treatment including antithrombin substitution

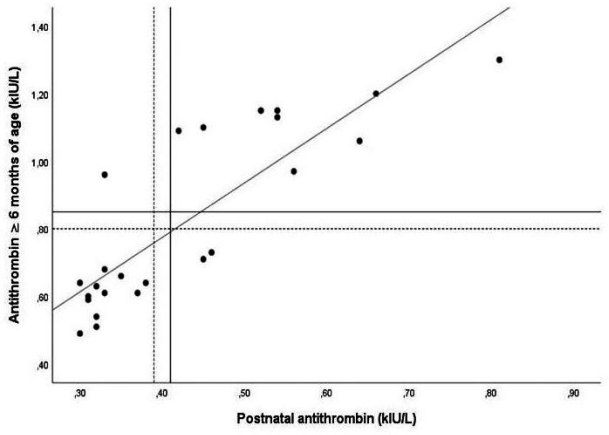

FIGURE 1 Antithrombin levels during first 10 days after birth and after 6 months of life

thrombosis during the neonatal period is accentuated by the use of instrumental delivery.

PB1279 | Polygenic Risk Score-Analysis of Thromboembolism in Patients with Acute Lymphoblastic Leukemia

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Background: Thromboembolism (TE) is a severe toxicity of acute lymphoblastic leukemia (ALL) treatment; but genetic studies on TE in patients with ALL are often underpowered. Attempts to extrapolate from the genetics of TE in adults have been many and the results conflicting.

Aims: We investigated whether there is common genetic etiology between TE in the general adult population and TE in patients with ALL, using polygenic risk scores (PRS).

Methods: We prospectively registered TE events and collected germline DNA in patients 1.0-45.9 years with ALL treated according to the NOPHO ALL2008 study (2008-2016). The study was approved by national ethical review boards and required signed informed consents. Based on summary statistics from two large genome-wide association studies from the INVENT consortium and the UK Biobank on TE in adults, we performed PRS analysis on individual level data in NOPHO. We gradually expanded the PRS by increasing the p-value threshold to capture polygenicity, with power > 80% for PRS including up to 40,000 SNPs.

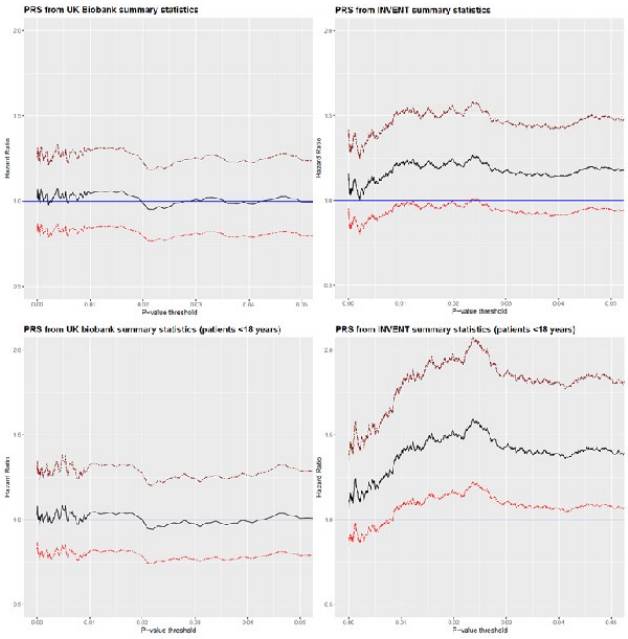

FIGURE 1 HRs of PRS from UK Biobank and INVENT summary statistics on TE in the NOPHO cohort (top), with subgroup analysis of children 1.0-17.9 years (bottom)

Results: Eighty-nine of 1252 patients with ALL developed TE, cumulative incidence 7.2%, at a median 12.7 weeks from diagnosis. PRS of genome-wide significant SNPs from INVENT and the UK Biobank were not significantly associated with TE in ALL, HR 1.16 (p 0.14) and 1.02 (p 0.86) respectively. Expanding the PRS to include more SNPs did not reveal polygenic overlap. In subgroup analysis of children < 18 years (n = 1125), there was evidence of significant polygenic overlap with the INVENT data, though effect sizes were small, HR 1.60 (p < 0.001) for the best fit PRS (18,964 SNPs, p-value threshold 0.02).

Conclusions: Thus, genetic factors known to affect risk of TE in the general adult population may influence risk of TE in children with ALL, but we consider this effect to be small in the context of cancer, chemotherapy and a coagulation system under development.

PB1280 | Ischemic Strokes in Children

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Background: Ischemic strokes in children are based on many reasons such as genetic mutations, chronic diseases, environmental factors, lifestyle.

Aims: To demonstrate the multifactorial etiology of ischemic strokes in children.

Methods: There were examined 3 boys with ischemic strokes.

Patient A, 1,5 years old, perinatal ischemic stroke. A child from the first pregnancy proceeding against placental insufficiency. Childbirth occurred at 32 weeks of gestation with muscle hypotension, hyporeflexia, anisocoria.

Patient B, ischemic stroke at 11 months. A child from the first pregnancy. Childbirth occurred at 38 weeks without any complications. At 11 months, amid complete health, lethargy, face asymmetry, tilt of the neck to the right, paresis of the right upper and lower extremities were found.

Patient C, Ischemic stroke at 7 years old. It was preceded by measles/mumps/rubella revaccination, acute gastroenteritis with degree II excision, toxicosis and a long period of immobilization.

All patients underwent MRI, examination for DNA genes of thrombophilia, coagulological research, determination of homocysteine level.

Results: The diagnosis of ischemic stroke in all three patients was confirmed by MRI. The coagulological research data did not go beyond the reference values, the level of natural anticoagulants was within the normal range, AFA were not detected. The homocysteine level in patient A was 5.5 μmol /L, patient B - 11.2, patient C - 10(0.0-5.0).

Genetic analysis of patient A - heterozygous polymorphisms: F2, F7, F13, MTRR. In the homozygous state: ITGA2, PAI-1. Mother is the carrier of the ITGA2 mutation.

Genetic analysis of patient B - homozygous polymorphisms: ITGB3, MTRHFR.

Genetic analysis of patient C - heterozygous polymorphisms: MTHFR, MTRR, PAI-1, FGB, ITGA2-α2.

Conclusions: In the pathogenesis of ischemic stroke in children, the role of several factors is discussed, including the combination of thrombophilia genes, an increase in homocysteine levels, and the impact of trigger factors.

PB1281 | Impact of a Perioperative Venous Thromboembolism Prophylaxis Screening Tool for Pediatric Orthopedic Surgical Patients

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Background: Venous thromboembolic events (VTE) pose a significant challenge in pediatric orthopedic surgical patients (POSP). Since universal thromboprophylaxis is not recommended, there is a need for a validated screening tool to identify high-risk patients and optimize thromboprophylaxis. A VTE thromboprophylaxis screening tool for POSP was developed and implemented at the IWK Health Centre in October 2016 (Figure 1).

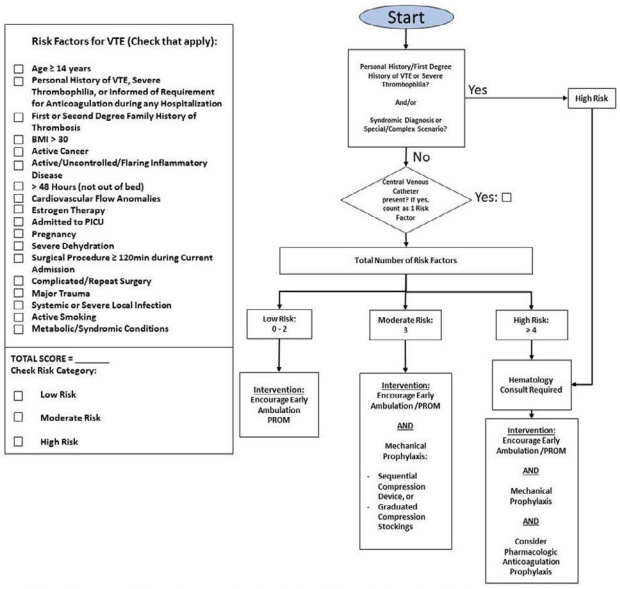
Aims: To evaluate the impact of the VTE thromboprophylaxis screening tool on use of thromboprophylaxis and VTE in POSP.

Methods: This study (duration: December 1, 2014 to June 1, 2019) was approved research ethics board. Using the tool, all POSPs were screened, scored, and categorized into low, moderate, and high risk

TABLE 1 Risk Factor and Thromboprophylaxis Correlation Analysis

VTE Algorithm Factors	Correlation to Anticoagulation (Thromboprophylaxis) Correlation Coefficient	P-Value
Risk Score	0.336	<0.00
Risk Level	0.732	<0.00
Age ≥ 14	0.258	<0.00
BMI > 30	0.288	<0.00
Limited/Altered Mobility > 48 Hours	0.484	<0.00
Surgical Procedure > 120 Minutes during Current Admission	0.281	<0.00
Complicated/Repeat Surgery	0.369	<0.00

*Correlation is Significant at the 0.01 Level (2-Tailed) using Spearman correlation



K. Padhye, R. El-Hawary, V. Price, S. Stevens, B. Branchford and K. Kulkarni, "Development of a Perioperative Venous Thromboembolism Prophylaxis Algorithm for Pediatric Orthopedic Surgical Patients," *Pediatric Hematology and Oncology*, 2019. DOI: 10.1080/08880018.2019.1695030

FIGURE 1 Perioperative Venous Thromboembolism Event Thromboprophylaxis Screening Tool [1]

groups. Pharmacological thromboprophylaxis was recommended in high risk patients (risk score > 4) in consultation with pediatric hematology. Thromboprophylaxis use and VTE were the primary study outcomes. Cases (all patients post implementation of screening tool) and controls (all patients pre implementation of screening tool) were compared.

Results: The VTE thromboprophylaxis screening tool was used in 62.6% (n = 411) of the 657 POSPs since October 2016. Of the 684 control patients, 28 (4.1%) received thromboprophylaxis. The cases and controls were comparable in age, gender and diagnoses. Compared to the controls, there was a significant (p = 0.046, 47.9%) reduction in use of thromboprophylaxis. No patients had VTE before or after implementation of the algorithm. Compliance with the screening tool was excellent with 100% of the patients in the high-risk category receiving thromboprophylaxis. High-risk patients were more likely to have a BMI > 30 (35.7%), limited/altered mobility (57.1%), and to undergo complicated/repeat Surgery (64.3%) (Table 1).

Conclusions: This study demonstrates successful implementation of a VTE thromboprophylaxis screening tool that resulted in a significant reduction in thromboprophylaxis use in POSPs with no increased VTE. Further studies are needed to assess the impact of this tool on incidence of VTE and ensure universal implementation.

PB1282 | Prevalence, Characteristics and Risk Factors of Peripherally Inserted Central Catheter-Related Venous Thromboembolism in a Pediatric Intensive Care Unit: A Cross-Sectional Retrospective Study

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Background: Central venous catheterization represents an important risk factor for venous thromboembolism (VTE) in critically-ill children. Peripherally inserted central catheters (PICCs) are used with increasing frequency in this population due to their ease of insertion and removal with little or no sedation. Recently, it has been demonstrated that PICC placement in children carries a significant risk of VTE. There is limited data on characteristics and predictive risk factors of PICC-related VTE in critically-ill children.

Aims: Report prevalence, characteristics and risk factors of PICC-related VTE in critically-ill children who were admitted to the pediatric intensive care unit (PICU) at our center.

Methods: We identified all patients ≤ 18 years of age with no prior VTEs who were admitted to PICU (2013-2018) and required PICC placement by searching radiology reports using MONTAGE

TABLE 1 Significant clinical characteristics

Characteristic	No PICC-VTE (N = 323)	PICC-VTE (N = 27)	P-value
PICU stay > 30 Days	47/323 (15%)	11/27 (41%)	0.0016
Sepsis	81/323 (23%)	13/25 (52%)	0.0082
Viral infections	91/323 (28%)	13/27 (48%)	0.0460
Non-infectious medical risk factor	20/323 (6%)	9/27 (33%)	<0.0001
No. of additional risk factors	1 (1-2)	2 (1-3)	0.0147
PICC days	14 (8-25)	20 (8-55)	0.0423
PICC dysfunction	12/323 (4%)	5/27 (19%)	0.0061
Blood product transfusions	145/323 (45%)	20/27 (74%)	0.0044

TABLE 2 Multivariable logistic regression analysis for PICC-related VTE

Putative risk factor	Odds ratio (95% confidence interval)	P-value
Non-infectious medical risk factor	6.03 (2.19 to 16.38)	0.0004
PICC dysfunction	4.36 (1.18 to 14.46)	0.0192
Blood product transfusions	3.34 (1.34 to 9.26)	0.0134
Prolonged PICU LOS (>30 days)	3.25 (1.28 to 8.15)	0.0119
Viral infections	2.67 (1.06 to 6.84)	0.0370

AUC = 0.82 (P < 0.0001); Hosmer-Lemeshow statistic 4.431 (P = 0.8163)

Search and Analytics software followed by chart reviews to confirm cases for inclusion in the study. Clinically-symptomatic and radiologically-confirmed PICC-related VTEs were identified from a prospectively-maintained institutional thrombosis database. To determine independent risk factors, univariate and multivariable logistic regression analyses were performed comparing patients with PICC-related VTE and patients experiencing no PICC-related VTEs. The results of multivariable modeling are presented as odds ratios, 95% confidence intervals and P-values.

Results: During the study period, 350 patients were admitted to the PICU and required a PICC. Twenty-seven patients developed PICC-related VTEs at median of 5 days (range 1-93 days) yielding a prevalence of 7.7% and 27.9 VTEs per 1000-PICC-days. Significant characteristics are summarized in Table-1. Non-infectious medical risk factor, PICC dysfunction, blood product transfusions, prolonged PICU stay and viral infections were identified as independent, statistically-significant risk factors for PICC-related VTEs by multivariable modeling (Table-2).

Conclusions: PICC placement was associated with increased risk of PICC-related VTE in our study population. We identified several significant factors for assessing the risk of PICC-related VTEs in critically-ill children which warrant prospective validation.

PB1284 | Homocystinuria in the Etiology of Sinüs Vein Thrombosis Presented with Focal Seizure: A Case Report

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Background: Homocystinuria in the etiology of sinüs vein thrombosis presented with focal seizure.

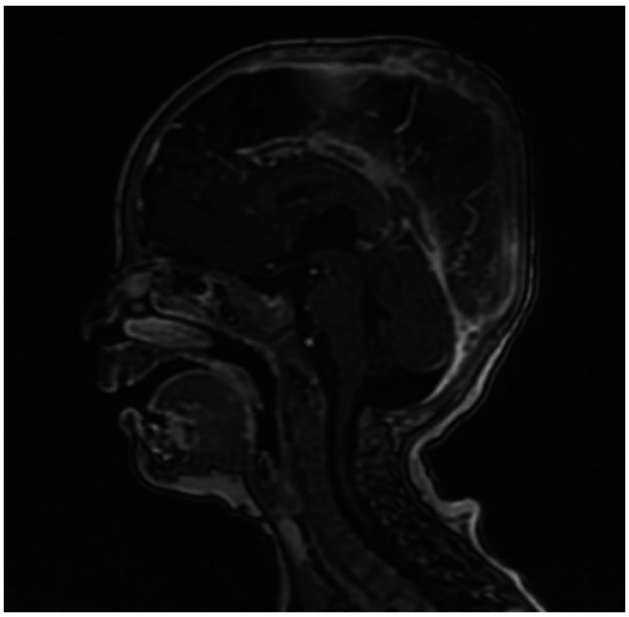

FIGURE 1 Cranial Magnetic Resonance Image 1

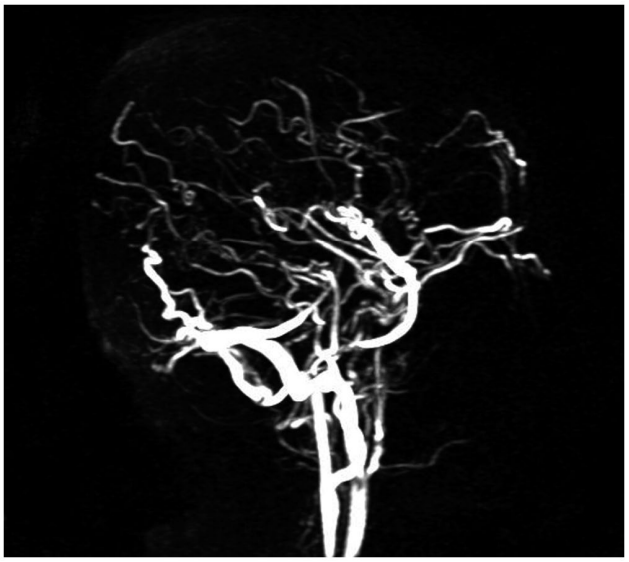

FIGURE 2 Cranial Magnetic Resonance Image 2

Aims: Homocystinuria is a multisystemic metabolic disorder with an autosomal recessive transition with a frequency of 1/100.000-200.000. Main findings are arterial and venous thrombosis, myopia and lens dislocation in many organs. Thrombotic complications may lead to different neurological findings in homocystinuria. Especially in sinüs thrombosis, partial or generalized seizures may ocur which are treatment-resistant.

Methods: 11 months old male child admitted to the emergency department with focal seizure and revealed thrombosis in the superior sagittal sinüs by cranial imaging. Serum homocysteine level was found high in the examinations. In genetic analysis, the diagnosis of homocystinuria was confirmed upon the detection of homogenous p.Arg379Gln (c.1136G>A) variant in the methylenetetrahydrofolate reductase heterozygous mutation and cistationin beta synthase gene (NM_000071).

Results: We wanted to share the importance of considering neurometabolic diseases in terms of early diagnosis and treatment in the differential diagnosis of cases with cranial thrombosis presenting with seizures.

Conclusions:

PB1285 | Pediatric Thrombosis: A Retrospective Analysis of the Incidence, Characteristics and Risk Factors in a Spanish Center

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Background: Pediatric thrombosis is an increasingly common problem. We hypothesized that it occurs most commonly in the hospital environment with associated illnesses.

Aims: To study the incidence, characteristics and risk factos of pediatric thrombosis, both venous and arterial.

Methods: A retrospective analysis of children with both venous and arterial thrombosis in a two year period was performed.

Clinical characteristics of this patients under 14 years were examined. All of them were attended in our center, the Pediatric and Maternal hospital of Málaga (Spain).

Results: Thirty two children were diagnosed in our center, in a two year period since 1-8-2017 until 1-8-2019. Almost half of the patients revised were older tan two years of age (46,8%), 31,2% were infants and 15,6% newborns.

The vast majority of events where venous, with only five arterial cases. Two children had simultaneous venous and arterial thrombosis.

Twelve children had catheter related thrombosis, six had cerebral venous sinus thrombosis. We found only one abdominal event, and four present an ischemic stroke. The rest had thrombosis of the upper o lower limbs.

In all cases, the event occurs during the admission period. Most of the children had underlaying diseases, and almost a half had simultaneous infectious diseases. Most of them were infected with gram positive microorganisms, three of them present gram negative infections and four don't have any microbiological.

We don't do thrombophilia studies systematically, but we knew that one child was positive for the prothrombin mutation in heterozygosis.

Conclusions: Thrombosis is mostly diagnosed in hospitalized children, especially in those who had chronic or acute underlying diseases. In general the presence of an gram positive infectious disease, a central venous catheter of both, are the most important risk factors.

In conclusion, pediatric thrombosis, as hospitalized children pathology, need to be studied to fin primary prevention strategies and better management.

PB1286 | Coagulation Activation Via Extrinsic Pathway Is the Major Cause of Hypercoagulation in Newborns during First Days of Life

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Background: Newborns during first days after birth have decreased concentration of plasma proteins; yet, their hemostasis is shifted to hypercoagulation detected by global hemostasis assay thrombodinamics, and is represented by increased clot growth rate and spontaneous clotting.

Aims: We aim to identify the causes of the observed neonatal hypercoagulation.

Methods: 22 newborns were enrolled into the study. All parents gave their written informed consent. Cord and peripheral blood was drawn into 106 mM sodium citrate tubes with or without 50 μg/ml corn-trypsin inhibitor (CTI) immediately after birth. Clot growth rate (V) and spontaneous clotting (T_{sp}) were assessed using thrombodynamics. To find out the origins of hypercoagulation we added either 100 nM of factor VIIa with inhibited active site (FVIIai) to block extrinsic tenase or 100 nM of activated factor IXa inhibitor nitrophorin 2 (NP2) to block intrinsic pathway.

Results: We revealed hypercoagulation both in cord and peripheral blood samples with no significant difference between sample types. In 95% of cord samples spontaneous clotting was observed. CTI had no significant effect on hypercoagulation (Median (25-75%) μm/min: V=49 (40-60) μm/min; V (CTI)=41 (39-50) μm/min; the reference range was 20-29 μm/min) or spontaneous clotting (T_{sp}=26 (19-33) min; T_{sp}(CTI)=24 (15-37) min; no spontaneous clotting was observed in adult healthy donors). Same tendencies were observed in peripheral blood.

Addition of NP2 slightly shifted hemostasis towards normal coagulation in cord blood (T_{sp} was prolonged from 24 (16-37) min up to 38 (20-54) min; P=0.004) and had no impact in peripheral blood. Addition of FVIIai dramatically prolonged T_{sp} both in cord blood (T_{sp}=24 (16-37) min; T_{sp}(FVIIai)=60 (43-60) min; P=0.001) and in peripheral blood (T_{sp}=18 (14-57) min; T_{sp}(FVIIai)=51 (23-60) min; P=0.001).

Conclusions: Hypercoagulation in newborns is caused by activation of coagulation predominantly via extrinsic pathway.

PB1287 | Impact of Inherited Thrombophilia on Risk of a First Perinatal Arterial Stroke and Futher Course or Recurrence Rates: A Systematic Review and Meta-Analysis of Observational Studies

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Background: A clear understanding of the association of inherited thrombophilias (IT) with perinatal arterial stroke (PAS) and its course has yet to be established. Recent case-control studies only reported small numbers of patients and grouped together different stroke entities.

Aims: Estimation of the impact of IT: on a first PAS and: on the further course using a combined end point describing neurological outcome and thromboembolic recurrence.

Methods: Systematic search in electronic databases (PubMed, EMBASE, OVID, Web of Science, Cochrane Library) for studies published from 1986 to August 2019. Abstraction of data on year of publication, study

design, country of origin, number of patients/ control subjects, ethnicity, stroke type. Calculation of summary odds ratio (OR) and 95% confidence interval (CI) with the fixed-effects- or random-effects-model.

Results: Inclusion of 23 of 113 references. Analysis of 1027 patients and 2594 control subjects. We found no significant heterogeneity or publication bias. OR/ CI (fixed-effects-model): Factor V G1691A mutation 2.72/ 2.0 – 3.7; factor II G20210A variant 1.4/ 0.90 – 2.2; MTHFR T677T genotype 1.6/ 1.26 – 2.04.

Evaluation of 4 of 23 studies on the combined study endpoint poor neurological outcome and recurrence rates with IT present: OR/ CI (relevant publication bias, random-effects-model): 5.8/ 1.35 – 24.81.

Conclusions:

i) Factor V G1691A mutation and MTHFR T677T genotype represent risk factors for PAS. In our analysis factor II G20210A variant does not yield a higher risk for a first PAS.

ii) Using the combined end point approach we found a statistically significant association of poor neurological outcome and recurrence rates with the presence of at least one inherited thrombophilia.

PB1288 | The Frequencies of Thrombophilic DNA Polymorphisms in Children with Hemolytic Uremic Syndrome from the North-Western Region of Russia

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Background: Hemolytic uremic syndrome (HUS) affects approximately 2-3 of 10 000 children annually. This is a life-threatening condition with high probability of poor clinical outcome in late period. Kidney insufficiency in HUS patients is accompanied by disturbances of hemostatic system which reflect the presence of thrombotic microangiopathy. The role of thrombophilic DNA polymorphisms in pathogenesis of HUS is not clear.

Aims: To determine genotype frequencies for several prothrombotic DNA polymorphisms in children with HUS originated from the North-Western region of Russia (NWR).

Methods: We studied 15 children with HUS from 1.0 to 4.8 years old (mean age of the group - 2.8±1.2 years). "Typical HUS" was diagnosed in 14 (93.3%) children, whereas 1 (6.7%) patient had atypical duration of disease ("atypical HUS"). Genotyping for the factor V (FV G1691A), FII G20210A and PAI-1 -675 4G/5G variations was performed by PCR-RFLP. Genotype frequencies were detected by direct counting.

Results: FV Leiden mutation was identified in 2 (13.3%) children. The 4 years old girl with severe acyclic duration of "atypical HUS" and high anti-protein H IgG levels had heterozygous FV G1691A mutation, whereas 4.8 years old boy with post-vaccination HUS, characterized by both nephritic syndrome and hematuria, was homozygous for the FV Leiden. Prothrombin G20210A gene mutation was not found in the studied group. PAI-1 4G/4G genotype was detected in 7 (46.7%) patients.

Conclusions: FV Leiden mutation and PAI-1 4G/4G genotype were more frequently found in children with HUS than in the healthy population of NWR. DNA polymorphisms associated with prothrombotic state could be involved in development of HUS in children.

PB1289 | The Throm-PED Registry: The Cornerstone of the International Pediatric Thrombosis Network

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[1]*Erasmus MC University Medical Center Rotterdam MC Sophia Children's Hospital, Rotterdam, the Netherlands*; [2]*University Children's Hospital, zurich, Switzerland*; [3]*McMaster Children's Hospital, Hamilton, Canada*; [4]*Hospital de Pediatría Prof. Dr. Juan P. Garrahan, Buenos Aires, Argentina*; [5]*Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of*; [6]*Children's Hospital Colorado, Aurora, United States*; [7]*Royal Hospital for Children, Glasgow, United Kingdom*; [8]*Charité University Medicine, Berlin, Germany*; [9]*Children's Hospital, Los Angeles, United States*; [10]*Medical University of Vienna, Vienna, Austria*; [11]*Royal Children's Hospital, Melbourne, Australia*; [12]*University Hospital Schleswig-Holstein, Kiel, Germany*; [13]*Children's Hospital of Philadelphia, Philadelphia, United States*; [14]*Shaare Zedek Medical Center, Jerusalem, Israel*; [15]*Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*

Background: Since the first prospective national registries of pediatric thromboembolic events (TEs) in the nineties, little progress has been made in advancing the knowledge about the management of this potentially life-threatening complication. Due to the relative rarity and heterogeneity of the disease, most management guidelines in children are still based on low evidence studies and mostly extrapolated from adult trials. International research collaboration is needed, which is provided by the International Pediatric Thrombosis Network (IPTN) consisting of pediatric thrombosis centers across the world. The cornerstone of the IPTN is the Throm-PED Registry,

TABLE 1 Treatment of 165 patients with venous VTE

Treatment	n (%)
Low-molecular-weight heparin	111 (66.1)
Unfractionated heparin	32 (19)
Vitamin K antagonists	34 (20.2)
Direct oral anticoagulants	13 (7.7)
Fondaparinux	11 (6.5)
Bilivarudin	3 (1.8)
Thrombolysis	8 (4.8)
No treatment	15 (8.9)

a prospective disease-based registry, established to increase knowledge about pediatric TEs in general. This basic registry can flexibly be supplemented with datasheets for additional sub-studies in the future.

Aims: To evaluate the epidemiology, risk factors, and management of arterial and venous pediatric TEs.

Methods: Members of the IPTN register consecutive pediatric patients with venous and arterial TEs in the Throm-PED registry, which is facilitated by the International Society on Thrombosis and Haemostasis (ISTH) installation of REDCap. Following data are prospectively collected in the basic registry: age at diagnosis of TE, gender, type, location, risk factors, and treatment.

Results: Since 2017, 70 centers in 27 countries became IPTN members. Eight centers have obtained Research Ethics Committee approval so far. Hitherto, the Throm-PED registry includes 165 patients with venous TE (male 45%, median age 5.3 years). 31.5% of patients are less than 1 year. Most frequent locations include upper (35.1%) or lower (23.8%) extremity. Most frequent risk factors include central venous catheter (44%), infection (24.4%), and surgery (18.5%).

Conclusions: In a short period, the Throm-PED registry has collected prospective epidemiological data of consecutive pediatric TE patients worldwide. This registry will serve as basis for conducting both epidemiological and cross-sectional, parallel cohort and intervention studies in children with TEs to advance the field of pediatric thrombosis.

PB1290 | Thrombosis and Anticoagulant Therapy among Pediatric Patients with Active Cancer: Real Life Data

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Background: Venous thromboembolism in children is rare, but on the rise. The rates of thrombosis in childhood cancers are much lower than in adults. Therapy for childhood cancer is often intense and associated with significant thrombocytopenia. Data on thrombosis and anticoagulation in children with cancer is limited.

Aims: In this study, we aim to report our experience with anticoagulant therapy in children with cancer.

Methods: Databases were reviewed for children who received any form of anticoagulation. Only patients with a diagnosis of cancer and radiologically-confirmed diagnosis of thrombosis were included. Clinical characteristics, cancer specific treatment and type and duration of anticoagulants used were reviewed.

Results: Between January 2011 and December 2018, a total of 45 patients (49% males) fulfilled the inclusion criteria. Median age at diagnosis was 10.7 years (range, 0.8-17.9 years). Leukemia was the most common underlying diagnosis (n=12, 26.7%) followed by lymphoma and bone tumors (n=6 each, 13.3%). Seven (15.6%) patients had surgery, 25 (55.6%) were on chemotherapy within 4 weeks of thrombosis and 8 (17.8%) had central line at time of thrombosis. Majority (n=37, 82.2%) developed the clot within 30 days of hospitalization and only 8 (17.8%) had it while ambulatory. Thrombosis involved the extremities in 23 (51.1%) patients (including 2 with upper extremity DVT), sagittal vein in 12 (26.7%) and pulmonary embolism in 4 (8.8%). Remarkably, 9 patients (75.0%) with sagittal sinus thrombosis were on steroids at the time of thrombosis; 5 with leukemia had just received intrathecal triple therapy and asparaginase, as well. All patients were treated with low-molecular weight heparin (LMWH). Anticoagulation was complicated by bleeding in 3 (6.7%) patients; one minor and 2 majors.

Conclusions: Unlike adults, thrombosis in children with cancer relates more to recent hospitalization and mostly related to chemotherapy. LMWH is safe and effective in the management of cancer-related thrombosis in pediatric patients.

PB1291 | Thrombosis and Laboratory Monitoring in Children with Acute Lymphoblastic Leukemia

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Background: The children with Acute Lymphoblastic Leukemia (ALL) have the high risk of thrombosis (around 36%) while asymptomatic thrombosis presents in nearly 70% of ALL patients. The use of CVCs and chemotherapy can affect the development of thrombosis. The assessment of the hemostasis in children with ALL is still important and actual problem.

Aims: To evaluate the state of hemostasis system in children with ALL to identify the high-risk group.

Methods: Seventy three patients (45 boys, 28 girls, 1-17yr, median 5yr) with ALL were enrolled in this study. The patients were treated using ALL-MB-2015 protocol. APTT, TT, PR, fibrinogen, ATIII, D-dimer levels, Thromboelastography (TEG), Thrombodynamics (TD), thrombomodulin (TM) and endothelin-1 (ET-1) levels were used to assess coagulation state in patients before and during the treatment.

Results: TEG parameters and clotting tests were normal (nearly 66%) or in hypocoagulation (nearly 34%) area during the treatment. Fibrinogen and ATIII lowered during the treatment in 72% and 41% of patients respectively. Thrombosis occurred in 40 patients (55%). TD revealed hypercoagulation in 80%. We've divided patients in two groups: 1st with low ATIII level and 2nd with normal ATIII level. There was 53% of thrombosis in 1st group if patients had hypercoagulation by TD and normal D-dimer levels, but only 16% if they had normal TD or high D-dimer levels. Even there was 45% of thrombosis in 2nd group with normal ATIII also if patients had hypercoagulation by TD and low D-dimer levels. There was no thrombosis in both groups with TD in normal area. Interesingly, we see threefold increase of

TM and ET-1 levels in patients with thrombosis (p< 0.001) compared to patients without thrombosis.

Conclusions: The endothelium dysfunction confirmed by high TM and ET-1 levels and the reduced lysis potential confirmed by low ATIII, normal D-dimer levels and hypercoagulation by TD is the hypothetic group of parameters to predict thrombotic complication in children with ALL.

PB1292 | Bivalirudin as the Primary Anticoagulant for Pediatric Patients with a Berlin EXCOR Ventricular Assist Device (VAD)

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Background: The Berlin EXCOR ventricular assist device (VAD) is used primarily in the infant/toddler population for support in heart failure. Systemic anticoagulation is used to minimize hematologic complications with VAD use. There is little published literature regarding bivalirudin use in this patient population.

Aims: To describe patient outcomes with bivalirudin as the primary anticoagulant in the setting of Berlin EXCOR VAD use.

Methods: Retrospective case series of pediatric patients with VAD and bivalirudin as their primary anticoagulant. Data from initial insertion of the VAD and initiation of bivalirudin until VAD removal and bivalirudin discontinuation was collected. Patient demographics, dose, monitoring, and adverse reactions to bivalirudin were recorded.

Results: Eight patients were identified having bivalirudin as the primary anticoagulant during VAD use. Ages ranged from 2.5wks to 8 years. Four patients had complex congenital heart disease; four had cardiomyopathy/cardiac failure. Median days on VAD was 129 (range 24-351). Six patients had bivalirudin started within 2 days of VAD placement with two patients starting bivalirudin on day 7 after initially starting on heparin. All patients reached therapeutic range after 5 dose adjustments with 5 patients needing none. Median number of dose adjustments to stay within therapeutic range was 14.5 (range 9-84). Median percentage of days within therapeutic range was 98.9%. Two patients required device change due to thrombus. Six patients had no bleeding events, one patient had 3 bleeding events (GI bleed x2 and mediastinal bleed), and one had one bleeding event. Five patients received heart transplants, two patients had VAD removed due to needing ECMO support, and one patient died with VAD in place. Cause of death was hemorrhage, though not attributed to anticoagulation.

Conclusions: Bivalirudin was effectively used in patients with VAD. Dose adjustments were infrequently needed, and most patients did not have any significant device thrombus or bleeding events.

PB1293 | Expanding the Spectrum of Heterozygous Antithrombin Deficiency Type II HBS? Unusual Presentation of Thromboembolic Events in Two Children

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Background: Recent geno-phenotype analyses show a low risk for thromboembolic events (TEE) in patients with heterozygous AT deficiency type II, especially in carriers of variants affecting the Heparin binding site (HBS). Unusual presentations like abdominal thrombosis or arterial thromboembolic events in young children are rare.

Aims: To compare two new cases with unusual presentation with literature data.

Methods: Case report and literature review.

Results: The first patient is a 12 year old boy from Bosnia who presented with intestinal bleeding and a painful right leg. He had a history of short bowel syndrome due to several resections of the intestine performed of unclear reason in the neonatal period. Ultrasound revealed deep vein thrombosis (DVT) of both iliacal and the right femoral vein. An MRI angiography showed agenesis of the infrarenal cava vein with pelvic and lumbar collaterals. Because of

TABLE 1 Patient Demographics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Age at VAD Placement	11mo	13mo	3mo	5wks	8yo	2.5wks	15mo	12mo
Sex	Male	Female	Male	Female	Female	Male	Male	Female
Cardiac Diagnosis	Complex Congenital Heart Disease	Dilated Cardiomyopathy	Heart Failure Secondary to Aortic Stenosis	Dilated Cardiomyopathy	Complex Congenital Heart Disease	Enterovirus Myocarditis	Complex Congenital Heart Disease	Complex Congenital Heart Disease
VAD Removal Reason	Heart Transplant	Heart Transplant	Heart Transplant	Death due to infected VAD cannula site hemorrhage	Transitioned to ECMO	Heart Transplant	Heart Transplant	Transitioned to ECMO

persistent AT deficiency of 60% genetic testing was initiated and a heterozygous missense mutation in the SERPINC1 gene was found, which is causing AT type II HBS, known as AT-Rouen I.

The second child was admitted at the age of 4 years due to acute hemiparesis during an episode of acute respiratory infection. MRI and the clinical course of the disease were consistent with hemorrhagic encephalitis. Persistent decreased AT activity of 68% was observed. Genetic testing showed a heterozygous mutation in the SERPINC1 gene, known as AT type II HBS Basel.

Conclusions: We cannot prove that there is a causal relationship of AT deficiency and the unusual and early clinical presentation in our patients. We speculate that, in the first case, vena cava thrombosis in the perinatal period caused perfusion problems of the gut. Although the second case did not reveal typical radiological signs of arterial thromboembolic stroke we assume that AT deficiency together with hyperinflammation triggered thrombosis in small cerebral vessels.

PB1294 | Venous Thromboembolism (VTE) in Children with Community-acquired Staphylococcus Aureus Bacteremia (CA-SAB) in Children: Risk Factors and Prevalence Study from a Single Pediatric Tertiary Care Center in Argentina

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Background: VTE associated with CA-SAB in previously healthy children is increasingly recognized. Inherited and acquired prothrombotic risk factors in this setting have been scarcely investigated.

Aims: To identify risk factors associated with VTE in a cohort of children with CA-SAB at a single pediatric tertiary care center and to assess its prevalence. To describe clinical characteristics, laboratory findings and outcomes of the cohort with VTE.

Methods: From January-2013 to December-2019 consecutive previously healthy children< 18 years admitted with CA-SAB were registered by epidemiological surveillance study in our hospital and patients with confirmed VTE were prospectively recruited. From January-2018 to December-2019, patients with CA-SAB without VTE were also included for laboratory testing (LT). Clinical and laboratory risk factors from both cohorts were statistically analyzed. (STATA 12.0). Level of significance: 0.05.

Results: During a seven-year period, 334 children with CA-SAB were admitted, VTE was confirmed in 39 of them. VTE prevalence was 11.5% (CI 95% 8.2.-15.3). Figure1 describes clinical characteristics and outcomes of VTE patients.

LT was consented in 28/89 recruited patients of the cohort with CA-SAB without VTE. No statistical differences in clinical variables were observed between the 28patients available for LT and the remaining 61patients. Figure2 summarizes univariate analysis of risk factors in patients with and without VTE.

VTE location	Catheter related, n (%)	5 (13)
	VTE adjacent to infectious foci, n (%)	33 (85)
	Upper venous system, n (%)	5 (13)
	Inferior venous system, n (%)	32 (82)
	Pulmonary embolism, n (%)	0
	Other locations, n (%)	3 (8)
Antithrombotic therapy	Unfractionated heparin, n (%)	6 (18)
	Enoxaparin, n (%)	39 (100)
	Vitamin K antagonist, n (%)	32 (82)
	Duration (month), median (range)	4.6 (0.9 - 13.6)
Outcomes	Progression, n (%)	3 (8)
	Recurrence, n (%)	0
	Major bleeding, n (%)	1 (2.5)
	All-cause mortality, n (%)	0
Recanalization	Complete, n (%)	9 (23)
	Partial, n (%)	16 (59)
	None, n (%)	14 (36)
Post-thrombotic syndrome	Present, n (%)	9 (23)
	Absent, n (%)	16 (59)
	Not evaluated, n (%)	14 (36)
Follow-up	Time (years), median (range)	1.2 (0.3 - 6.0)

FIGURE 1 Patients with VTE: Clinical characteristics and outcomes

Conclusions: This is the largest prospective pediatric cohort assessing VTE in the setting of CA-SAB. VTE prevalence was similar to published data.

In this study, older children, severe infection, pulmonary infection and a longer hospital stay were associated with significant higher risk of developing VTE. No association with either acquired or hereditary thrombophilia was found.

A very low frequency of catheter-related thrombosis was observed and there was neither thrombosis recurrence nor VTE-related death.

	Patients with VTE n=39	Patients without VTE n=28	*p-value*	OR IC95%
Age, years (median, range)	**8,6 (0.1-14.9)**	**5,3 (0.5 - 14,6)**	**0,0306**	**----**
Male, n (%)	28 (72)	19 (68)	0,7237	1,2 (0,4-3,5)
***S. aureus:* Methicillin-resistant, n (%) / Methicillin-sensitive, n (%)**	35 (90) / 4 (10)	20 (71) / 8 (29)	----	----
Time from symptoms onset to infection diagnosis, days (median, range)	3 (0-11)	2.5 (0-11)	0,154	----
Severe infection*, n (%)	**33(85)**	**15 (54)**	**0.0053**	**4,8 (1,5-14,9)**
Persistent bacteremia (≥5 days), n (%)	17 (44)	7 (25)	0.1102	2,3 (0,8-6,7)
Pulmonary infection, n (%)	**31 (80)**	**6 (21)**	**<0,001**	**14,2 (4,3-46,8)**
Osteomyelitis, n (%)	24 (62)	17 (57)	0.6804	1,04 (0,4-2,8)
Length of hospital stay, days (median, range)	**43 (14-106)**	**18 (7-76)**	**0.0004**	**----**
Acquired thrombophilia: transient positive aPL, n (%)**	24 (61)	18 (64)	0.8027	0,9 (0,3-2,4)
Lupus Anticogulant, n (%)	20 (51)	15 (54)	0,8084	0,9 (0,3-2,4)
aCL IgG positive, n (%)	3 (9)	4 (15)	0,4478	1,5 (0,3-8,7)
aCL IGM positive, n (%)	4 (10)	2 (7)	0,6681	1,1 (0,2-6,9)
anti-β2GPI IGG positive, n (%)	3 (10)	2 (7)	0,6681	0,7 (0,04-11,9)
anti-β2GPI IGM positive, n (%)	1 (3)	1 (4)	0,8242	0,95 (0,4-2,4)
Simple positivity, n (%)	19 (49)	14 (50)	0,9356	0,9 (0,4-2,5)
Doble positivity, n (%)	2 (5)	4 (14)	0,1987	0,9 (0,06-1,9)
Triple positivity, n (%)	2 (5)	0 (0)	0,2298	----
Inherited thrombophilia*, n**	1 (heterocygous FVL)	0	----	----

* According to CDC guidelines
**aPL (antifosfholipid antibodies): lupus anticoagulant and/or anticardiolipin antibodies (IgG/IgM) and/or anti-β2GPI antibodies (IgG/IgM)
***Inherited thrombophilia included: Antithrombin, Protein C, free and total Protein S, activated PC resistance, factor V Leiden (FVL), Prothrombin G20210A

FIGURE 2 Clinical and laboratory risk factors

Página 1

In this setting, most of the VTE were adjacent to infected foci; these findings suggest physiopathological mechanisms of thrombosis which are yet to be elucidated.

PB1295 | Pulmonary Embolism in Pediatric Age: Retrospective Analysis of 10 Year Period in a Tertiary Hospital

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Background: Pulmonary embolism (PE) in the pediatric population is relatively rare when compared to adults. However, the associated potential morbidity and mortality is substantial and the incidence is increasing. An accurate and timely diagnosis is critical. Identifiable risk factors are usually present but vary across the pediatric age range.
Aims: To evaluate incidence, demographic characteristics, associated risk factors, treatment and outcome of PE in children, diagnosed in our hospital from 2008 to 2018.

TABLE 1 Risk factors identified in pediatric patients with PE - most patients had more than one risk factor

Risk Factors in PE	n
Inherited thrombophilias	5* (n= 1 combined thrombophily: protein S deficiency and factor V Leiden mutation (heterozigothy); n= 2 protein C deficiencies; n= 2 prothrombin gene mutations (heterozigothy).
Antiphospholipid syndrome	1
Central venous catheter	4
Vascular malformation	2
Oral contraception	9
Immobilization	8
Inflammatory/infectious disease	9
Other underlying disorder (cianotic cardiopathy, neoplasic)	2
Obesity	3

Methods: A retrospective analysis was performed of all cases of pediatric PE diagnosed in our hospital, to evaluate incidence, demographic characteristics, associated risk factors, treatment and outcome of PE in children younger than age 18 years, from 2008 to 2018. We excluded short admissions (< 24hours) transferred to other hospitals.
Results: Twenty-two cases of PE were identified in 20 patients (11 female), median age 15,5 years-old, with 17 adolescents patients. Eight patients with classic symptoms had a recent admission in emergency departments with discharge without definitive diagnose. Risk factors were identified in all patients (table 1), 9 of the females were taking oral contraceptive pills, 6 patients presented thrombophilias, 2 patients had May-Thurner Syndrome and 6 patients were in-patients with severe co-morbidities and reduced mobilization.
Definitive diagnosis was performed by CT pulmonary angiography or intravenous contrast CT. D-dimers were negative in 2 patients with chronic PE. Four patients received fibrinolytics due to right ventricular dysfunction, one patient was in venoarterial ECMO. All patients received anticoagulation, for treatment and secondary prevention. Two patients died during the hospitalization episode for PE.
Conclusions: Pediatric PE is a rare but potentially fatal condition, that often is undiagnosed due to devaluation of symptoms or its attribution to underlying conditions. A high clinical suspicion index and the establishment of specific pediatric guidelines are important to develop, in order to improve diagnosis and management of pediatric PE.

PB1296 | Arterial Ischemic Stroke in Greek Infants, Children and Adolescents: a Single-Centre Experience

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Background: Childhood arterial ischemic stroke (AIS) is a rare but serious event.
Aims: To investigate the clinical background of the childhood AIS in a reference Thrombosis Centre in Greece.
Methods: Data were retrospectively collected for children aged 28 days-18 years old with AIS referred between 2009 and 2019. Patients with cancer or CVL related AIS were excluded from the study.
Results: Overall, 48 children were reported [mean age: 7.5±4.4 years, 54.5% males]. Most children (85%) presented with focal symptoms, including hemiparesis (67%), speech disturbances (29%) and facial weakness (23%), but also with headache (31%), convulsions (31%) and loss of consciousness (29%). Diagnosis was based on MRI/MRA (53%), followed by CT. Affected vessels were middle cerebral artery (46%), carotid (14.6%), basilar (8.3%) and anterior cerebral artery

(6.2%). Dissection was confirmed in 10.4%. Lateralization was right in most cases, followed by left side in 40% and both sides in 8.3%. Transient ischaemic attack was assumed in 10.4%.

Trauma (27%), infection (18.8%), cardiac disorders (18.8%), arteriopathies (16.6%) and prothrombotic states (12.5%) were the most frequently identified risk factors, while a quarter had no apparent underlying cause. Heterozygosity for FVLeiden and FII20210A mutations and homozygosity for MTHFR-C776T were found in 4.5%, 5% and 10.9% of patients respectively, without statistical significance.

The majority of children (98%) were discharged home (rehabilitation clinic: 2%). At time of discharge most common neurological symptoms were hemiparesis (35%) and speech disturbances (16.6%), while 31% of children had no neurological sequelae. Almost three quarters of patients received anticoagulation (42% aspirin, 15% warfarin, 15% warfarin and then aspirin), while 56% received anticoagulation for >2 years. After long-term follow up, 46% had no neurological residual, 8% had attention deficits and 8% cognitive decline. No child died.

Conclusions: This study provides population-based data for childhood AIS, which may be useful for further research.

PB1298 | Pulmonary Embolism in Pediatric Patients: A Ten-year Experience from a Tertiary Center in Brazil

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Background: Although rare, Pulmonary Embolism (PE) is a potentially life-threatening condition with significant morbidity and mortality and likely underdiagnosed due to non-specific symptoms and a low index of suspicion.

Aims: To evaluate clinical characteristics, diagnosis and treatment of PE in pediatric patients followed at our center in Brazil.

Methods: Retrospective data analysis from 2009 to 2019 of a tertiary center to identify patients < 18 years with PE, objectively diagnosed radiologically. Patient demographics and clinical course were recorded.

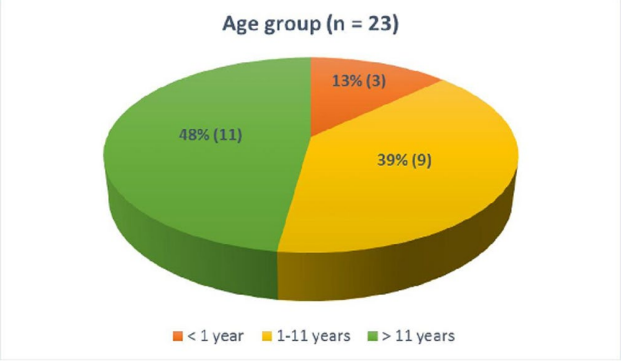

FIGURE 1 Age of patients at PE diagnosis

Results: The incidence of PE was 3.2 per 10,000 hospitalized patients. Of the 23 cases included, 69.6% occurred during the hospitalization and 52.2% were males. The median age was 11 years (2 days - 17 years) (Figure 1). Sixteen patients (69.5%) presented symptoms. The most common was dyspnea (56.2%), chest pain (50.0%) and hypoxemia (43.7%). The median time from symptoms to diagnosis of PE was one day (0-11 days). Risk factors were central venous catheter (39.1%), malignancy (34.8%) and recent surgery (34.8%). Two or more risk factors were present in sixteen (69.5%) patients. PE was diagnosed by computed tomography pulmonary angiogram in twenty-one patients (91.3%). One patient was ineligible for anticoagulation therapy. Twenty-two patients (95.6%) received unfractioned heparin or low molecular weight heparin. Thrombolysis was not performed. Five patients (22.7%) lost to follow-up and twelve of the remaining seventeen (70.6%) received anticoagulation therapy during 3 to 6 months. Two patients presented bleeding complications: one pulmonary hemorrhage (day 9 of treatment) and one fatal intracranial hemorrhage (day 30 of treatment).

Conclusions: At least one risk factor was identified in all patients diagnosed with PE, which emphasizes the importance of increasing awareness of high-risk pediatric patients. The presence of comorbidities such as malignancy and recent surgery associated with central venous catheter should raise the clinical suspicion for PE even without the classic symptomatology.

PB1299 | Hospital Acquired Thrombosis in Pediatric Population

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Background: Venous thrombo -embolism (VTE) is no longer considered a disease of adults as its frequency in pediatric population is increasing. An alarming increasing incidence of 70% was noted for pediatric VTE in hospitalized children in United States during 2001-2007.

We hereby studied VTE in pediatric patients in an academic tertiary care center in Pakistan.

Aims: This study aimed at determining the risk factors for hospital acquired venous thrombo -embolism (H-VTE) in pediatric patients.

Methods: This was a retrospective study conducted at the Aga Khan University, Pakistan during January 2011 to June 2019. We used 2015 ICD-9-CM Diagnosis Code 557.0 to identify admitted patients with deep venous thrombosis (DVT) and pulmonary embolism (PE). Medical charts and computerized data were reviewed to collect demographics, risk factors, clinical features, laboratory/radiological investigations, medical/surgical interventions and mortality.

Results: There were 134617 pediatric patients who were admitted during the study period. VTE rate was high at 15.9/10,000 hospital admissions for children. A total of 215 pediatric patients were identified through ICD coding for various types of thrombotic disorders. Of these, 109 patients had either DVT (n=76) or PE (n=31) or both DVT and PE (n=2). There were 66 males and 43 females with

a median (IQR3-1) age of 13 years (16.5-5). H-VTE was observed in 41 patients (37.6%) with 61% males and 54% adolescents with a rate of 3/10,000. Predominant risk factors were catheter placement (51.2%), various infections (41.5%) and prolonged hospital stay with a mean (±SD) of 17.6 (±17.2) days. Anticoagulation was given to 25 patients (61%). Mortality of H-VTE was 19.5%.

Conclusions: A significant high rate of H-VTE was observed in pediatric age group. Similar to adults, children should also be assessed for thrombosis at the time of hospital admission and appropriate thromboprophylactic measures should be taken.

PB1300 | Heparin Resistence in Pediatric Patient with Homozygous Antithrombin Deficiency, Treated with Dabigatran: A Case Report

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Background: Antithrombin (AT) deficiency is the most severe thrombophilia and is caused by various mutations on the SERPINC1 gene. The functional defect affecting the heparin binding site (HBS) of AT in homozygous individuals confers high risk of venous thrombembolism (VTE).

Aims: To evaluate the response to anticoagulant therapy.

Methods: We present a case of a 15-year-old boy with iliofemoral phlebothrombosis with extension to the vena cava inferior. He was diagnosed in early childhood with epilepsy syndrome and cerebral palsy and is on multiple antiepileptic drugs (AEDs). The thrombophilia screening showed only a slight decrease in AT activity. After administration of weight-based therapeutic dose low molecular weight heparin (LMWH), the measured peak anti-Xa level was very low. AT deficiency was suspected and genetic analysis was performed. Because of potential drug interactions of acenocoumarol with AEDs and difficulties in regular laboratory monitoring in this patient, it was decided to start off-label use of DOACs (Apixaban 2 x 2.5 mg or Dabigatran 110 mg once daily).

Results: AT activity was 74% (Berichrom® AT III, range 79.4% - 112%). Anti-Xa level measured 4 hours after administration of Clexane 30 mg twice daily was < 0.1 U/mL. DNA analysis showed homozygous mutation - p.Leu131Phe at the HBS of AT (Illumina MiSeq platform). We found trough plasma level of Apixaban 16 ng/mL (DiXal®, range 42 - 140 ng/mL). The plasma level of Dabigatran measured 12 hours after administration was 86 ng/mL (Hemoclot®, trough level 45 - 220 ng/mL). There was neither VTE recurrence nor bleeding events during one year of follow-up.

Conclusions: As previously described, molecular testing identifies mutations that can be missed by traditional activity assays. Therapeutic LMWH doses can't attain sufficient anticoagulation in patients with homozygous HBS AT deficiency. Dabigatran seems to be an appropriate alternative to conventional anticoagulants in these patients.

PB1301 | Qualitative and Quantitative Characterization of Plasma Exosomes from Neonates and Adults

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Background: Neonatal platelets, compared to those of adults, are hyporeactive in response to most agonists and show impaired granule release, which have been associated, among others, with defects in SNARE machinery proteins. These proteins are involved in the biogenesis of exosomes: extracellular vesicles (30-100 nm) that, coming mainly from platelets, participate in numerous pathophysiological processes, including immunity and inflammation.

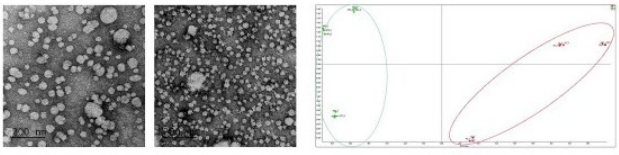

Figure 1A. Representative images of plasmatic adult (left) and neonatal (right) exosomes by transmission electron microscopy (N=3/group; scale bars= 200 nm). The images were processed with MIP4 software.

Figure 1B. PCA analysis of the DE proteins detected by SWATH MS in adults (red) and neonatal (green) exosomes.

Pathway	Proteins
Hemostasis	RAP1A; YWHAZ; KNG1; FLNA; GNAI2; ITGA2B; VWF...
Formation of Fibrin Clot (Clotting Cascade)	KNG1; A2M; F5; PF4; FGA; FGB; ...
Integrin cell surface interactions	RAP1A; ITGA2B; ITGB3; THBS1; VTN; TLN1; ...
Complement cascade	MASP1; C3; C1R; C7; C6; C4B;
β3 integrin cell surface interactions	ITGA2B; ITGB3; THBS1; VTN; FGA; FGB; ...
Common Pathway	F5; PF4; FGA; FGB; FGG;
Platelet activation, signaling and aggregation	RAP1A; YWHAZ; FLNA; GNAI2; ITGA2B; ITGB3; VWF...
...	...

Figure 1C. Representative table of comparative study of the gene enrichment analysis within each age group performed with IPA software.

FIGURE 1 (1A-1C)

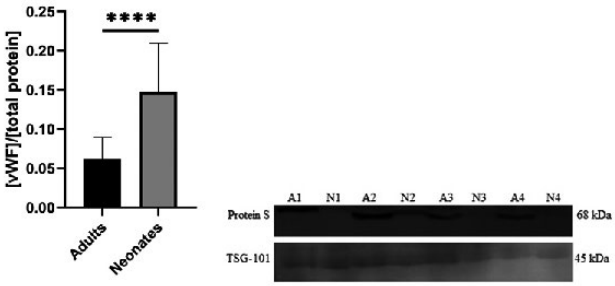

Figure 2A. vWF levels in plasma exosomes from neonates and adults detected by ELISA (N=10/group; p<0.0001).

Figure 2B. Western blot of Protein S in plasmatic exosomes from adults (A) and neonates (N). TSG-101 (Tumor susceptibility gene 101 protein) was evaluated as exosome specific marker.

FIGURE 2 (2A-2B)

Aims: To investigate the potential differences between newborn and adult plasma exosomes.

Methods: Exosomes were isolated from platelet-free plasma (PRP) by ultracentrifugation. PRP was extracted from umbilical cords blood of full-term neonates (N=14) or from peripheral blood of healthy adults (N=12). Exosomes characterization included: microRNA expression by analysis of placental- (miR-141, miR-299-5p) and platelet (miR-199, miR-146a)-specifics microRNA; size determination by transmission electron microscopy; amount of total protein by BCA; quantitative proteomic analysis by SWATH-MS in 1 pool of 6 samples/group (10 technical replicates/group); PCA and gene enrichment analysis by Ingenuity Pathways. Validation assays of two of the differently expressed (DE) proteins (FCh>2) were done by ELISA or immunoblotting.

Results: microRNA expression studies in neonatal exosomes showed similar platelets-specifics microRNA expression levels similar to adults exosomes, but no expression of placental- specifics microRNA. Plasma exosomes from neonates are smaller (Fig 1A) and contain 44% less protein than those of adults ($p< 0.05$). SWATH-MS analysis showed 113 DE proteins: 81 overexpressed and 52 underexpressed in exosomes from neonates compared to adults. PCA analysis showed two populations according to age group (Fig 1B). The greatest differences were found in proteins related to complement activity, immunoglobulins and hemostasis (Fig 1C).

Assays validation confirmed the overexpression of vWF ($p\leq0.001$, Fig 2A) and underexpression of protein S (Fig 2B) in neonatal (*vs* adult) exosomes.

Conclusions: Plasma exosomes from neonates are qualitatively and quantitatively different from those of adults. Main differences are found in proteins related with platelet function and immunity.

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PB1302 | A Rare Case of Phlephlebitis; An Unusual Underlying Reason for Portal Venous Thrombosis

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Background: Pylephlebitis is a septic thrombophlebitis of the porto-mesenteric veins, an extremely rare complication of intraabdominal infections especially in the region drained by the portal systems or in the structure contiguous to the portal vein, It is associated with a high mortality even early dignosis and appropriate treatment.

Aims: Here, we report a case of septic thrombophlebitis of the portal and superior mesenteric veins with multiple liver abscesses caused by ruptured appendicitis.

Methods: A case is presented.

Results: A 17 years old girl was referred to our hospital with an initial diagnosis of acute hepatitis because of prolonged fever, abdominal pain, hyperbiluribinemia and elevated liver enzymes. However virali nfections which can cause hepatitis was found to be negative. Complete blood count showed leukocytosis (WBC:18900/mm3)with increased number of immature leukocytes. Abdominal CT showed portal and superior mezentheric vein thrombosis with multiple liver abscess. Moreover, there was an irregularity of the cecum wall and appendix which was compatible with a ruptured appendicitis. She was treated with long-term antibiotics and anticoagulation.

Conclusions: This rare complication is reported to be associated with up to a 50% mortality rate.Early diagnosis with abdominal CT is the key point of the treatment. Moreover, this complicatıon may also result in a portal hypertension and chronic liver disease even treated with an appropriate approach.

PB1303 | The Prevalence of Lupus Anticoagulant in Children and Adolescences Referred to the Reference Coagulation Laboratory of Iranian Blood Transfusion Organization

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Background: Lupus anticoagulant (LA) is an acquired IgG or IgM autoimmune antibody against platelet membrane phospholipids. LA is an important cause of aPTT prolongation in children.

Aims: Determination of LA prevalence in children and adolescences that were referred to the coagulation laboratory of IBTO.

TABLE 1 Demographic data and chief complaint of 51 patients with positive LA

Age Median (range)	Sex F/M	Chief Complaint: Incidental finding before surgery or during hospitalization	Mucocutaneous bleeding	History of Thromboembolic events	No clinical data
3y (4 months-18y)	21/30	26	19	2	4

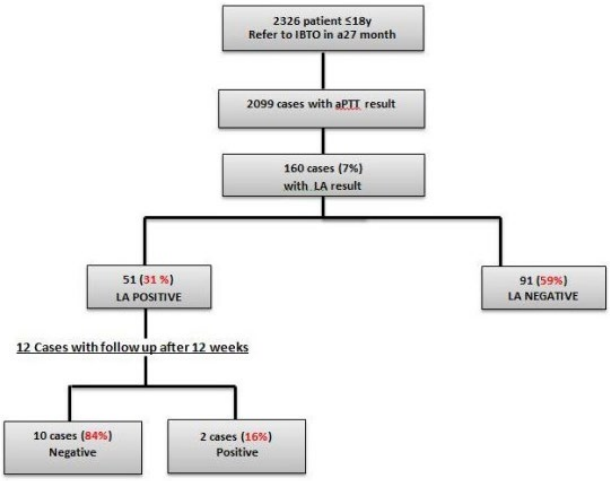

FIGURE 1 Flow-chart 1

Methods: During a period of 27 months (March 2014 to July 2016), all patients ≤ 18 years old who referred to our lab and had results of aPTT and lupus anticoagulant (LA) were evaluated. LA test panel was including screening tests (aPTT-LA and DRVVT screen) and as needed, the mixing study and confirmatory tests (Hexagonal assay and/or DRVVT confirm) all performed by STAGO reagents and instruments. In cases with positive LA, their follow-up refer to our lab were evaluated after 12 weeks.

Results: Our data revealed a LA prevalence of 2.5% in all referred patients ≤ 18 y with aPTT result and 31% in those who LA test was performed for them (flow-chart 1). In more than half of the LA positive patients, the main reason for referring them to the lab was incidental finding of aPTT prolongation noticed before surgery or during hospitalization (table 1). Interestingly, 90% of patients with positive LA, referred to the lab without any LA request by physician.

Conclusions: LA is not infrequent in children and adolescence however in most cases it is a transient problem without any significant clinical findings. About 90% of our cases were not suspected for LA by their physicians so it may be frequently missed in children clinically.

PB1304 | Clinical and Radiological Outcome in a Single Center Cohort of Pediatric Patients with Focal Cerebral Arteriopathy (FCA). Safety of Antithrombotic Prophylaxis and Therapy

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Background: FCA predominates among vascular causes of pediatric ischemic arterial stroke (AIS). Acute anticoagulation treatment and early initiation of low dose aspirin appear to reduce progression of the vaso-occlusive process and recurrence of ischemic episodes; the duration of antiplatelet secondary prevention is suggested for at least 2 years. To date there are no clinical studies on safety of antiplatelet therapy in children.

Aims: Analyze the etiology, choice and timing of diagnostic investigations, treatments, complications and outcome of patients with FCA observed at Gaslini Institute.

Methods: A retrospective observational study of pediatric patients with AIS secondary to FCA/isolated FCA selected according to CASCADE criteria from January 2005 to December 2019. We analyzed the index episode, neuroradiological evolution, clinical outcome (Pediatric Stroke Outcome Measure score, cognitive level,

TABLE 1

Antithrombotic therapy	Number/Total (%)
Acute therapy:	28/33 (84.8%)
Heparin followed by aspirin/other antiplatelet drug	14/28 (50%)
Directly Aspirin	10/28 (35.7%)
Other additional therapy (thrombectomy)	3/28 (10.7%)
Antiplatelet prophylaxis:	33/33 (100%)
Single antiplatelet (only Aspirin)	29/33 (87.9%)
Double antiplatelet (Aspirin + Clopidogrel)	1/33 (3%)
Switch from Aspirin to Clopidogrel	3/33 (9.1%)
Duration antiplatelet prophylaxis (months) [N=33]	24 (0.5 – 175.2) Median (min - max)

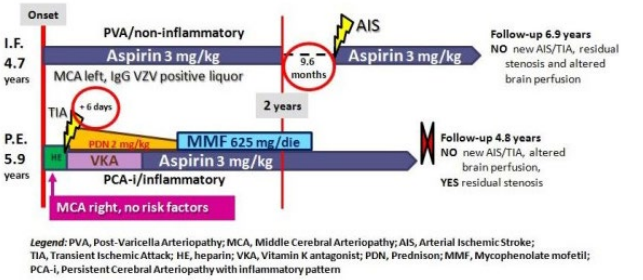

FIGURE 1

executive functions) and therapy used (acute phase, prophylaxis, etiological).

Results: 154 children with AIS and vasculopathy were enrolled of whom 33 were classified as AIS post-FCA/isolated FCA. Median follow-up was 2.3 years. In 17/33 cases an infectious etiology was recognized, particularly Varicella. All patients underwent Magnetic Resonance Angiography, 7 patients required diagnostic angiography. Vascular stenosis persisted after 6 months from onset in 71% of 31 cases with adequate follow-up. Four cases of arteriopathy relapse were observed in patients with previously unrecognized PVA. The outcome shows moderate impact of neurological deficit, headache, impaired cognitive and executive functions. A wide variety in approach to antithrombotic therapy was seen (Table 1). Compliance was excellent with antiplatelet therapy which was safe without major adverse events. Recurrence of clinical ischemic event occurred in 2 patients (Figure 1).

Conclusions: Improving neuroimaging by applying a score able to monitor arterial disease over time could be useful. It may be appropriate to extend antiplatelet prophylaxis beyond 2 years in PVA. Despite a small sample size, our study provides important insights for better management of FCA in pediatrics.

PB1305 | A Child with Ischemia of the Glans Penis after Circumcision

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Background: Circumcision is the most frequent surgical procedure performed worldwide. Although considered relatively safe, circumcision is associated with several complications. Ischaemia of the glans penis is a rare complication of circumcision, which has been reported to be associated with dorsal nerve block with local anesthetics, cauterisation, tight suture line/dressing, inadequate surgical procedure, or other underlying diseases.

Aims: We describe a ten-year-old obese boy developing severe ischaemia of the glans penis 20 h after circumcision.

Methods: We treated with 10 mg/kg oral pentoxifylline (PTX) for eight days, topical and intravenous antibiotic, and dressing.

Results: Clinical improvement was seen as the colour of the penile glans gradually returned to normal. The patient was discharged at the end of the tenth day.

Conclusions: Glans ischemia should be treated urgently to prevent and avoid permanent damage. Conservative treatment should always be considered as an option. Our single case experience showed that oral pentoxifylline may be recommended as a treatment modality in ischemia of the glans penis with a satisfying result.

PB1306 | Global Hemostasis, Procoagulation and Anticoagulation Proteins Derangements in Children and Adolescents with Hemoglobin H and Beta-Thalassemia/ Hemoglobin E Diseases

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Background: Thalassemia disease is associated with thrombosis due to hypercoagulable state caused by several hemostatic derangements. Rotatory thromboelastometry (ROTEM®), a global hemostasis test, has been demonstrated hypercoagulable state in β-thalassemia/hemoglobin E disease (β/E). However, there are a few evidences in hemoglobin H disease (Hb H).

Aims: To compare the changes in ROTEM®, procoagulants and anticoagulants among children and adolescents with Hb H, β/E and controls.

Methods: We enrolled 38 subjects with Hb H, 22 subjects β/E with 12 age-matched controls. ROTEM®, D-dimer, procoagulants [factor VIII clotting activity (FVIII:C) and von Willebrand factor antigen (vWF:Ag)] and anticoagulants [antithrombin (AT), protein C (PC) and free protein S (FPS) activities] were performed in all subjects.

Results: The median age of all subjects was 13 years (min-max 5-21) and 54% were male. PC and FPS levels were significantly lower in Hb H compared with β/E and controls (83.5 VS 70.2 VS 112, $P< 0.01$; 57.6 VS 45.2 VS 86, $P< 0.01$, respectively). ROTEM® parameters were not statistically different among the all groups but maximum clot firmness (MCF) in EXTEM and INTEM was still significantly higher in splenectomized subjects than non-splenectomized subjects and controls ($P< 0.01$). PC and FPS levels in splenectomized patients were significantly higher than non-splenectomized subjects ($P< 0.01$) and were correlated with total and direct bilirubin and serum ferritin (r=-0.44, -0.35 and -0.41 for PC and r=-0.59, -0.38 and -0.54 for FPS, respectively; $P< 0.01$).

Conclusions: ROTEM® parameters were not different among Hb H, β/E and control groups in this study. Hypercoagulable state was more commonly found splenectomized subjects. Low PC and FPS likely caused by liver injury was frequently found in β/E than Hb H

and control groups but were not associated with hypercoagulable state.

PB1307 | Risk Factors for the Development of Hospital-acquired Pediatric Thromboembolism - Dealing with Potentially Causal and Confounding Risk Factors Using Direct Acyclic Graph (DAG) Analysis

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Background: Hospital-acquired venous thromboembolism (HA-VTE) in children comprises multiple risk factors that should not be evaluated separately due to collinearity and multiple cause/effect relationships. This limitation may be overcome by the use of Directed Acyclic Graph (DAG) analysis.

Aims: To evaluate thrombotic risk factors in a pediatric hospital setting using an alternative statistical methodology to differentiate exposures and confounders.

Methods: Retrospective, case-control study with 24 cases of objectively confirmed HA-VTE and 76 controls matched by age, sex, unit of admission, and period of hospitalization. Descriptive statistics were used to define distributions of continuous variables, frequencies, and proportions of categorical variables, with a comparison between cases and controls. Due to a large number of potential risk factors of HA-VTE, a DAG model was created to identify confounding, reduce bias, and increase precision on the analysis. The final model consisted of a DAG-based logistic regression. The study was approved by the institutional review board and consent was waived.

Results: In the univariable model, the following variables were selected as potential risk factors for HA-VTE: length of stay (LOS, days), immobility, ICU admission in the last 30 days, LOS in ICU, infection, central venous catheter (CVC), number of CVCs placed, L-asparaginase, heart failure, liver failure and nephrotic syndrome (NS). Using the DAG methodology, confounders and exposures were differentiated (Fig. 1).The final model

Figure 1. Directed acyclic graph (DAG) model of hospital-acquired venous thromboembolism (HA-VTE) in children.

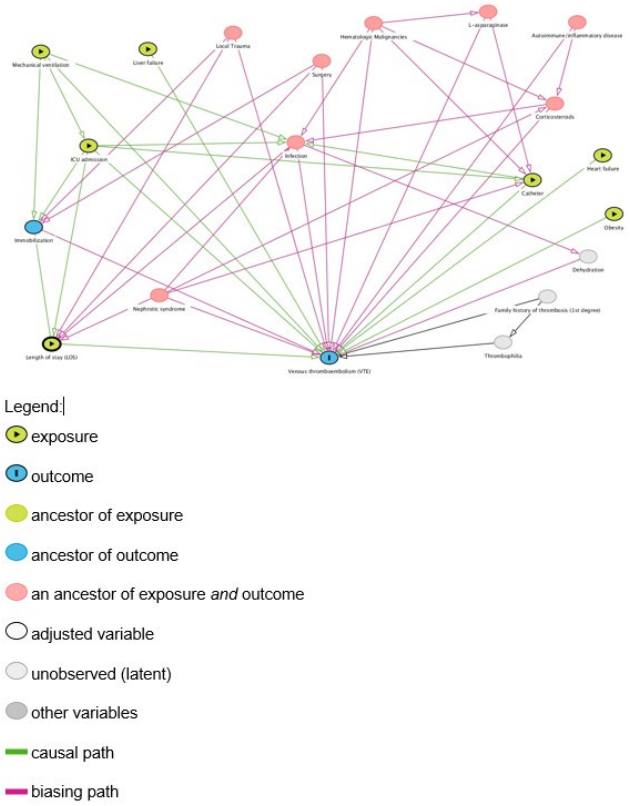

FIGURE 1 Directed acyclic graph (DAG) model of hospital-acquired venous thromboembolism (HA-VTE) in children

TABLE 1 Multivariable regression models

Variable	Full Model OR (95%CI)	Full Model p-value	Final Model OR (95%CI)	Final Model p-value
Length of stay	1.066 (1.016-1.119)	0.010	1.054 (1.024-1.084)	<0.001
Liver failure	15.736 (0.773-320.276)	0.073	8.891 (1.017-77.725)	0.048
L-asparaginase	621.588 (4.352-88,770.419)	0.011	23.493 (3.353-164.588)	0.001
Nephrotic syndrome	227.560 (4.416-11,726.072)	0.007	36.096 (3.218-404.846)	0.004
ICU admission	14.290 (0.705-289.568)	0.083		
Catheter	16.177 (0.849-308.342)	0.064		
Obesity	5.450 (0.388-76.452)	0.208		
Hematologic malignancies	0.022 (0.000-1.135)	0.058		
Immobilization	0.672 (0.044-10.272)	0.775		

(Table 1) revealed LOS (OR=1.05, 95%CI=1.02-1.08, p< 0.001), liver failure (OR=8.90, 95%CI=1.02-77.73, p=0.05), L-asparaginase (OR=23.49, 95%CI=3.35-164.59, p=0.001), and NS (OR=36.10, 95%CI=3.22-404.85, p=0.004) as independent risk factors.

Conclusions: The DAG-based approach was useful to clarify the influence of confounders and multiple causalities of HA-VTE. Interestingly, CVC placement, a known thrombotic risk factor, was considered a confounder, while liver failure, LOS, L-asparaginase use and nephrotic syndrome were confirmed as risk factors to HA-VTE. Large confidence intervals are related to the sample size, however the results were significant.

PB1308 | Risk Factors for Symptomatic Venous Thromboembolism in Children and Adolescents with Lymphomas

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Background: Symptomatic venous thromboembolism (SVTE) often complicates cancer and its treatment. SVTE is well studied in children with acute lymphoblastic leukemia, but there is little data about this complication in other malignancies.

Aims: To evaluate risk factors of SVTE in children and adolescents with lymphomas.

Methods: We analyzed the impact of age and volume of mediastinal mass (VMM) in 272 children and adolescents (0-18years) with primary lymphomas who were treated in our center. 83 of them had Hodgkin's lymphoma, 189 - with NHL. ROC analysis identified optimal cutoff. Competing risk-regression was based on Gray's test. P value < 0.05 considered significant.

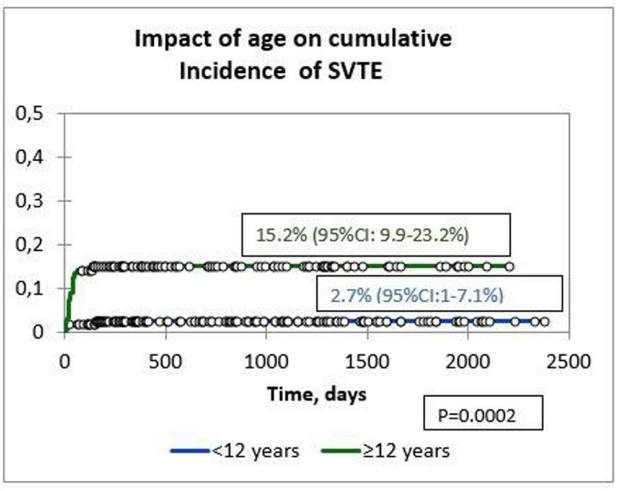

FIGURE 1 Impact of age on cumulative Incidence of symptomatic venous thromboembolism

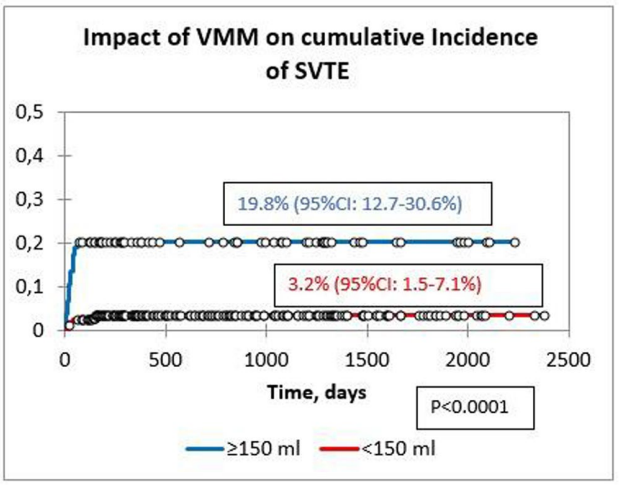

FIGURE 2 Impact of the volume of mediastinal mass (VMM) on cumulative Incidence of symptomatic venous thromboembolism

Results: 24 episodes of SVTE were diagnosed in 22 patients (8.1% 95%CI:4.8-11.3%). Most of SVTE occurred in the basin of vena cava superior (VCS, n=13) and vena cava inferior (VCI, n=5). Pulmonary embolism was found in 3 patients, cerebral venous sinus thrombosis - in 2 patients. 1 patient had combined SVTE in VCS and VCI. The median time from diagnosis of lymphoma to VTE was 22 days (IQR 14-41 days).

The median age of patients with SVTE was 11 years (IQR 6.5-14.9 years). Optimal cutoff point for age was 12 years. Cumulative incidence of SVTE in patients < 12 years and ≥12 years was 2.7% (95%CI:1-7.1%) and 15.2% (95%CI: 9.9-23.2%) respectively, p=0.0002. (figure1)

Mediastinal lymphadenopathy was found in 102 of 272 patients. The median VMM was 332 ml (IQR179-646 ml). Optimal cutoff point for VMM was 150 ml. Cumulative incidence of VTE in patients with VMM < 150 ml and ≥150 ml was 3.2% (95%CI: 1.5-7.1%) and 19.8% (95%CI: 12.7-30.6%) respectively, p< 0.0001. (figure2)

Conclusions: Age ≥12 years and VMM ≥150 ml are risk factors for SVTE in patients with lymphomas. These risk factors could be potentially used in a primary antithrombotic prophylaxis.

PB1309 | Moyamoya Disease - A European Clinical Case Report

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Background: Moyamoya disease is a chronic progressive cerebrovascular condition characterized by bilateral stenosis/occlusion of the arteries around the circle of Willis, with prominent arterial collateral circulation. Etiology is unknown, but high incidence among

Asian population and familial clustering in 10-15% suggest genetic predisposition.

Aims: Description of an occidental familial Moyamoya disease.

Methods: Collection of clinical data in SClínico® application.

Results: Male patient, 11 years-old, without Asian ancestry, with migraine antecedents, progressively presented, over the previous 24 hours, hypoesthesia and paresis of right upper limb, dysarthria, dysphagia to liquids and labial commissure deviation to the left side. Computed tomography and magnetic resonance imaging revealed recent ischemic injury in the left middle cerebral artery territory and alterations suggestive of Moyamoya disease. Therapeutic dose of enoxaparin was prescribed, with improvement of neurological deficits. Since discharge, patient has been accompanied by Neuropediatrics, Immunohemotherapy and Cardiology. Cerebral angiography confirmed diagnosis. Excepting patent *foramen ovale* (PFO) and interatrial communication (IAC), no other thrombotic risk factors were identified. Anticoagulation was maintained until percutaneous closure of PFO, posteriorly replaced by antiaggregation. Revascularization surgery was also performed.

The only sibling, male, 5 years-old, also with migraine history and no reported ischemic events, was evaluated. Likewise his brother, PFO, IAC and radiological alterations suggestive of Moyamoya disease were detected without other thrombotic risk factors. Antiaggregation was initiated.

Conclusions: Long-term antiaggregation is recommended for both asymptomatic and ischemic presentations. Anticoagulation as secondary prophylaxis remains controversial, due to increased bleeding risk, both in children (greater risk of falling) and adults (hemorrhage as predominant manifestation). This case emphasizes the importance of holistic management of thrombosis in Moyamoya disease (due to identification of PFO, anticoagulation was extended).

There are few studies that include non-Asian patients. Therefore, regional variants with prognostic/therapeutic implications cannot be excluded, highlighting the importance of reporting these cases and suggesting that multicenter trials may be needed.

PB1310 | Effectiveness and Safety of Primary Thromboprophylaxis in Pediatric Renal Transplant: a Systematic Review of the Literature and Meta-Analysis

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Background: Graft thrombosis is one of the most frequent and potentially preventable causes of early allograft loss following pediatric renal transplant. There is conflicting evidence regarding the role of primary thromboprophylaxis.

Aims: To determine the effectiveness and safety of thromboprophylaxis in prevention of renal graft thrombosis among children (0-21 years) undergoing renal transplant.

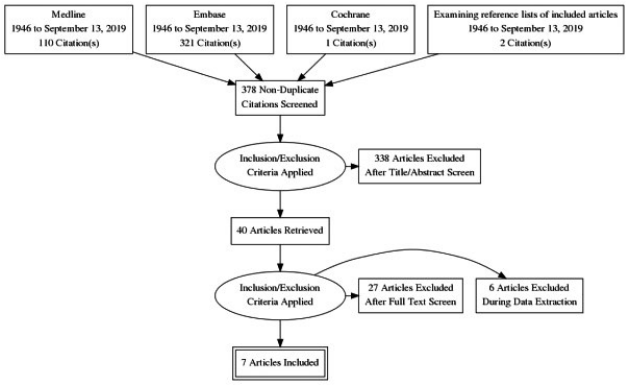

FIGURE 1 Search results for thromboprophylaxis in pediatric renal transplants

Methods: A systematic literature review was conducted searching MEDLINE®, EMBASE and Cochrane Libraries from inception until September 2019. Primary outcomes were objectively proven graft thrombosis and major bleeding, defined as need for surgical exploration or PRBC transfusion. In the absence of randomized studies, non-experimental studies comparing patients with or without thromboprophylaxis were included in the analysis.

Results: Seven studies (5 retrospective, 2 prospective; Figure 1) describing 1012 patients (592 cases, 420 control) were included (Figure 2). Meta-analysis demonstrated thromboprophylaxis

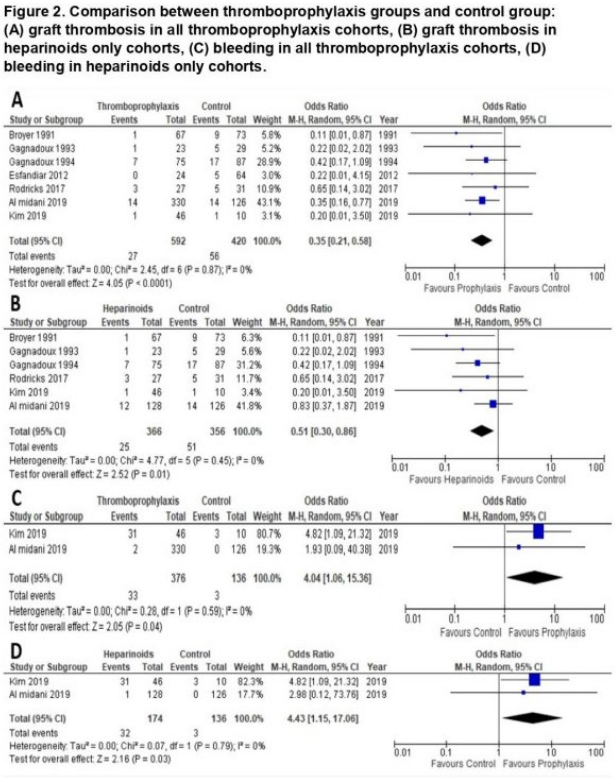

FIGURE 2 Comparison between thromboprophylaxis and control group

efficacy in prevention of graft thrombosis (odds ratio (OR) 0.35; 95% confidence interval (CI) 0.21 - 0.58). Subgroup analysis of patients treated only with heparinoids confirmed the protective effect of thromboprophylaxis (OR 0.51; 95% CI 0.30 - 0.58). There was a low degree of heterogeneity among the studies (I^2=0). Major bleeding in relation to thromboprophylaxis was extracted from two studies and demonstrated an increased risk of bleeding (OR: 4.04; 95% CI 1.06 - 15.36). Subgroup analysis of major bleeding in heparinoid only cohorts showed similarly increased risk (OR 4.43; 95% CI 1.15 - 17.06).
Conclusions: Current evidence suggests that primary thromboprophylaxis is effective in preventing renal graft loss in pediatric recipients. This benefit is offset however by increased risk of bleeding (no fatal cases). Further research is required to determine optimal treatment regimen and duration in pediatric patients with diverse allograft needs.

PB1311 | A Rise in Plasma Coagulability During the First 3 Months Following Provoked Venous Thromboembolism is Associated with the Development of Post-Thrombotic Syndrome in Children and Young Adults: Analysis of the Kids-DOTT Multicenter Trial-Derived Biobank

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Background: Elevated plasma levels of markers of coagulation and inflammation, including D-dimer, factor (F)VIII, interleukin (IL)-6) have been identified as prognostic factors for post-thrombotic syndrome (PTS) in adults.
Aims: 1) To determine whether elevated plasma cytokine levels and a rise in plasma coagulability over the first 3 months post-diagnosis of deep venous thrombosis (DVT) in children are prognostic of PTS; 2) To investigate the relationship between elevated cytokine levels and increased plasma coagulability in pediatric patients with DVT.
Methods: We analyzed banked plasma biospecimens from NHLBI-sponsored multinational trial of anticoagulant treatment for provoked VTE in patients < 21 years old. All patients with an extremity DVT and PTS assessment at 1 year were included. Plasma coagulability was measured using the Clot Formation and Lysis (CloFAL) assay and cytokines levels were measured by multiplex immunoassay on plasma samples obtained at 6 weeks and 3 months post-DVT. Univariable and multivariable logistic regression were performed to test prognostic associations with PTS. Pearson correlation was used to test the association between cytokine levels and CloFAL assay measures.
Results: The study population consisted of 80 patients, median age 12.7 years. PTS developed in 34% (Table 1). After adjustment in a multivariable model, increase in area under the curve in the CloFAL assay (AUC60) between the 6 week and 3 month time points post-DVT remained a candidate (*P*=0.06) independent prognostic factor for PTS (OR=1.436, 95%CI=0.99-2.084). Levels of C-reactive protein, IL-6 and interferon gamma at 3 months post-VTE were significantly associated with increase in plasma coagulability as measured by CloFAL AUC60.
Conclusions: We have identified a rise in plasma coagulability during the first 3 months post-DVT diagnosis, as a candidate prognostic factor for PTS which may be driven by the time course of inflammatory response, as evidenced by elevated plasma levels in several cytokines.

PB1312 | Antithrombin Activity Level below 60% in Children Has Implications for Heparin (LMWH) Dosing

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Background: Antithrombin (AT) is a natural anticoagulant inhibiting thrombin and factor Xa and thus accelerating the neutralisation of coagulation proteins. LMWH is responsible for accelerating AT interaction with thrombin and Xa. AT deficiency (acquired or congenital) reduces the ability to achieve the desired anticoagulant effect using LMWH. The anti-Xa test measures the activity of heparin/LMWH against the activity of activated coagulation factor X.
Aims: Determination of the Antithrombin activity levels having an effect on LMWH dosing in a paediatric cohort.
Methods: AT activity levels were prospectively measured (2y period) in patients < 1 y of age pre-operatively scheduled for LMWH post operatively (n= 335).
The cut-off AT activity level affecting Anti-Xa levels was determined by performing *in vitro* heparin recovery.
Anti-Xa assay type was evaluated; AT supplementation (AT+) and without (AT-) on heparin recovery (n=54).
Results: Patients (n=54) receiving LMWH therapy showed that the LMWH assay (AT+) had greater heparin recovery compared with the LMWH (AT-) assay. In vitro analysis showed that the heparin recovery was dependent on AT levels for both types of anti Xa assays. There was a clinically significant marked reduction in the amount of heparin recovered when AT activity levels fell below 60%. *Heparin levels remained within the sub therapeutic range (0.25-0.5 IU/ml).*
210/335 patients were found to have AT activity less than 0.60 IU/ml (mean 0.40 IU/ml) with the majority of patients (n= 209) < 1 month old. Mean AT activity levels of >0.60 IU/ml were found in patients > 1 month of age.
Conclusions: LMWH requires the presence of AT which may be limiting in determining anti-Xa levels in AT deficient patients. The cut-off concentration at which AT had an effect on Anti-Xa levels was 60%, in this study 210 patients had AT activity < 0.60 IU/ml. This finding has implications for LMWH dosing in neonates.

PB1313 | Impact of Blood Group on the Incidence of Thrombosis in Neonates

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Background: The incidence of thrombosis peaks in the neonatal period because of the developing coagulation system, comorbidities and use of venous and arterial catheters. Additional risk factors studied to date include sepsis, surgery, elevated hematocrit and inherited thrombophilia. Non-O blood group is associated with increased risk of thrombosis in adults and in pediatric leukemia patients. To date, the association between blood group and thrombosis has not been explored in the neonatal population.

Aims: To evaluate the association of blood group with incidence of thrombosis in neonates.

Methods: This is a single-center retrospective study using the Canadian Neonatal Network database. Patients diagnosed with thrombosis between January 1, 2014 and September 30, 2018 were included in the study. Health records were reviewed to extract demographic variables, blood group, thrombosis details and risk factors.

Results: There were 4860 neonatal intensive care admissions during the study period. Of these, 190 developed thrombosis (arterial 23, venous 155, mixed 11, unspecified 1) giving an incidence of 39 per 1000 admissions. Among 190 admissions with thrombosis, 88 (46%) had non-O blood group (A - 63, B - 19, AB - 6), 98 (52%) had O blood group and 4 (2%) didn't have blood group data available (Table 1). Surprisingly, non-O blood group patients had a lower risk of developing thrombosis than patients with O blood group (odds ratio 0.7; confidence interval 0.52-0.93; $p < 0.001$).

Conclusions: The incidence of thrombosis was much higher in our study than previously reported. However, previous epidemiologic studies are approximately 20 years old and neonatal care has changed significantly with more survivors who have a higher incidence of co-morbidities. Second, non-O blood group was associated with a lower risk of developing thrombosis in our study population. Further studies are required to replicate these findings.

TABLE 1 Distribution of blood group in admissions with and without thrombosis

Blood Group	Admissions with thrombosis, n (%)	Admissions without thrombosis, n (%)	All admissions, n (%)
A	63 (33)	1522 (33)	1585 (33)
B	19 (10)	576 (12)	595 (12)
AB	6 (3)	184 (4)	190 (4)
Total non-O Blood Group	98 (52)	2282 (49)	2370 (49)
O Blood Group	88 (46)	1769 (38)	1867 (38)
Blood group unavailable	4 (2)	619 (13)	623 (13)
Total admissions	190 (100)	4670 (100)	4860 (100)

PB1314 | Bivalirudin Administration in Pediatric Ventricular Assist Devices and in Pediatric ECMO

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Background: Children supported with mechanical support devices (MCS) experience bleeding and thrombotic complications that may depend upon anticoagulation strategies.

Aims: Our study aimed to evaluate the effects of bivalirudin administration on activated prothrombin time (Ptts), Lactate dehydrogenase (LDH) and free Hemoglobin (fHb) levels.

Methods: Fourteen (14) children were analyzed: 4 Berlin Heart (BH), 3 Infant Jarvik 2015 (IJ) and 7 extracorporeal membrane oxygenation (ECMO). Bivalirudin was prescribed to a Ptts target between 80 and 100 seconds.

Mean patients' age and weight was 49.8 months (60.9) and 13.8 Kg (10.8), respectively.

Results: 1746 bivalirudin doses were retrospectively recorded. Mean (SD) dose of bivalirudin was 0.8 (0.4) mg/kg/h and mean (SD) Ptts was 81 (20) seconds. Requirement of bivalirudin dose varied between ECMO, BH and IJ (BH: 1 (0.41), IJ: 0.82 (0.48), ECMO 0.69 (0.45) mg/kg/h; p=0.0001). A significant correlation between Ptts values and bivalirudin doses was found in all patients, with the highest values in ECMO patients (r:0.43, p=0.0001). Over time, patients showed some variations on Ptts values that appeared rather stable around the target values with occasional oscillations in bivalirudin doses. Quartiles of Ptts targets (60-70, 70-80, 80-90, >90) were associated with a significant progressive anticoagulant increase (p=0.0001). LDH and fHb correlation was different between ECMO (r: 0.27, p=0.0001), IJ (r: 0.33, p= 0.0001) and BH (r: -0.24, p=0.003). The lowest levels of both markers were found in BH patients (LDH 642.8 (244.3) mMol/L; fHb 58.05 (41.11) g/L) vs ECMO (LDH 779.5 (712.4) mMol/L, fHb 77.6 (53.1) mg/L) vs IJ (LDH 1517 (915.2) mMol/L, fHb 103.3 (121.2) g/L) (p=0.0001).

Conclusions: Bivalirudin warrants stable Ptts levels with slightly different behavior among different MCS. It prevented severe thrombotic and hemorrhagic complications.

PLATELET DISORDERS AND VON WILLEBRAND DISEASE

ACQUIRED THROMBOCYTOPENIAS

PB1315 | Can we Predict the Clinical Course of Children with ITP by MPV? A Preliminary Study

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Background: Mean platelet volume (MPV) is considered as a marker of platelet function, and is known to be increased in immune thrombocytopenia (ITP). However, the clinical implication of children with ITP has not been understood.
Aims: We analyzed MPV difference and MPV change between healthy controls and children with ITP.
Methods: We reviewed medical records of children aged under 18years with ITP (n=36) and healthy controls (n=36) from June 2010 to November 2018 at Kyung Hee University hospital. These subjects were stratified into the followings:
(i) Healthy controls [group I, n=36];:
(ii) Newly diagnosed ITP(nITP) and Persistent ITP(pITP) [group II, n=24]; and
(iii) Chronic ITP(cITP) [group III, n=12].
Several hematological indices were measured using Advia 2120 instrument (Siemens Healthcare Diagnostics Inc., Tarrytown, NY).
Results: The median MPV at diagnosis among groups I, II, and III was 7.20, 8.15, and 8.65fL, respectively (P< 0.05). Initial MPV cut off value between group I and group II+III was 7.6fL, between group II and group III was 9.0fL. We also analyzed changes of MPV over time in treated patients (n=28); nITP and pITP (n=22); and cITP (n=6). We compared MPV values:
(i) at diagnosis,
(ii) after treatment,:
(iii) after 1 month, and
(iv) after 3 months.
The results showed that MPV difference ($\Delta MPV_{\text{at diagnosis - after 3mo}}$) was 3.00fL in nITP and pITP, compared with 1.64fL in cITP (P< 0.05). In contrast to nITP and pITP, the MPV was decreased only to a small extent and even increased again after 3 months in cITP.
Conclusions: This study showed that MPV at diagnosis is statistically higher in children with ITP than healthy controls. MPV changes, as well as initial MPV value, can be regarded as a prognostic marker to predict disease course of ITP. This retrospective study is the first to investigate MPV values and its changes in ITP children and future large prospective studies are required to confirm our findings.

PB1316 | Megakaryocytic Hyperplasia in Bone Marrow Biopsy as a Novel Predictor of Response in Patients with Immune Thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is an acquired autoimmune disorder characterized by increased platelet destruction. The recent introductions of Thrombopoietin Receptor Agonists (TPO-RAs) had increased response rate of persistent e chronic disease and moved interest in response predictor. Previous studies showed a correlation between thrombopoietin level and response probability to TPO-RAs. Bone marrow biopsy is not required for ITP diagnosis, but it is suggested in relapsed/refractory disease, before the start of a second line therapy.
Aims: This work aim is to evaluate if megakaryocytic hyperplasia, typical characteristic of ITP, could influence response rate to TPO-RA in a cohort of primary immune thrombocytopenic patients.
Methods: From a total of 191 patients contained in our Center database, 44 persistent and chronic ITP were retrospectively enrolled; all patients performed bone marrow biopsy before starting a TPO-RAs as second or greater than second line therapy; each histological section was revised by a single pathologist to assess the grade of megakaryocytic hyperplasia. The cohort was analyzed by two subgroup, based on median number of MGK/high power magnification (6 Megakaryocytes/40x): 21 out of 44 patients (48%) showed low-grade megakaryocytic hyperplasia and 23 out of 44 (52%) moderate/severe-grade megakaryocytes hyperplasia.

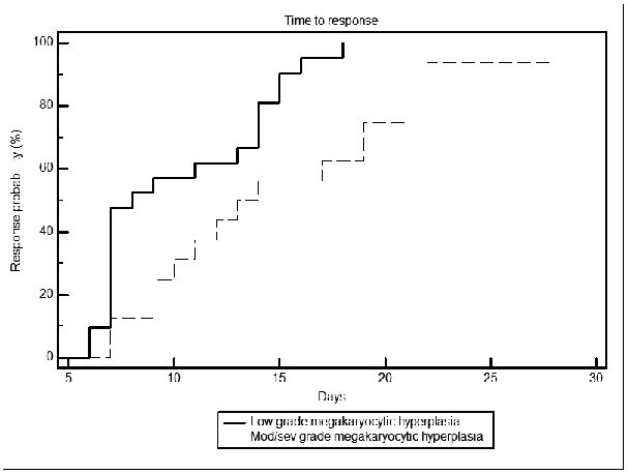

FIGURE 1 Difference in time to R to TPO-RAs between low (8 days, black) and mod/sev grade (16 days, gray) megakaryocytic hyperplasia (p 0.0003)

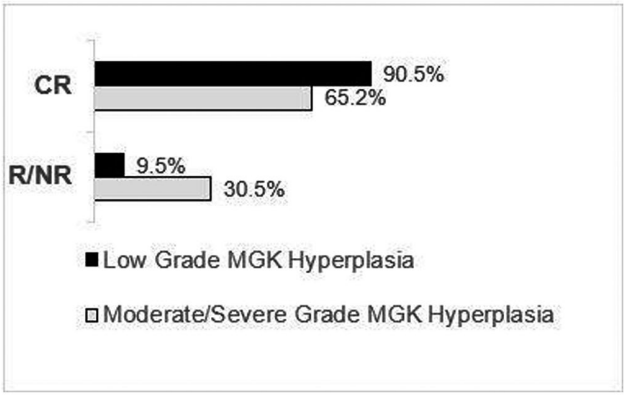

FIGURE 2 Difference in complete response rate between low (black) and mod/sev grade (gray) megakaryocytic hyperplasia. (p 0.04)

Results: Complete Response rates were significantly higher in patients with low grade megakaryocytic hyperplasia (90.5% vs 65.2%, chi-square, p 0.04), with similar rates of Loss of response (47.6% and 34.8%, p ns); this group of patients also showed shorter median time to response (8 days vs 16 days, p 0.0003, HR 0.4 CI 95% 0.2-0.77). Time to Loss of Response was 9 and 2 months respectively (p. ns).
Conclusions: ITP patients with lower grade of megakaryocytic hyperplasia showed higher CR rate and shorter time to response to TPO-RAs. Low grade megakaryocytic hyperplasia probably reflects lower thrombopoietin level and could be a useful tool to predict response to TPO-RAs.

PB1318 | Phase I/II, Open-Label, Ongoing Study of PRN1008 (Rilzabrutinib), an Oral Bruton Tyrosine Kinase Inhibitor, in Patients with Heavily Pretreated Immune Thrombocytopenia (ITP)

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Background: Despite significant therapeutic advances in ITP over the past 15 years, a subset of patients is refractory to existing options and durable remission remains elusive. PRN1008 (rilzabrutinib) is an oral, reversible, covalent inhibitor of Bruton tyrosine kinase targeting underlying disease mechanisms of platelet destruction. Initial phase I/II dose-escalation results support the 400 mg BID dose as optimal for clinical activity and safety (Kuter. ASH 2019).
Aims: Subset efficacy/safety analysis of rilzabrutinib at optimal 400 mg BID dose.
Methods: This ongoing, open-label study examines rilzabrutinib in heavily-pretreated ITP adults with baseline platelet counts < 30,000/μL, including patients on stable doses of concomitant corticosteroids/thrombopoietin-receptor agonists who had inadequate response. Primary endpoint: ≥2 consecutive platelet counts ≥50,000/μL and increase of ≥20,000/μL from baseline without requiring rescue medication.
Results: As of 13Nov2019, 16 patients initiated rilzabrutinib 400 mg BID (14/16 ongoing). Median baseline age: 44.5 years (range, 21-74); all primary ITP for a median of 7.2 years (range, 0.8-40.3) and median of 6 prior therapies (range, 1-41; 25% prior splenectomy). Median baseline platelet count was 12,050/μL (range, 3500-27,670/μL). Related treatment-emergent AEs (all grade 1/2) were similar to the overall study population; most frequent were nausea, diarrhea, and

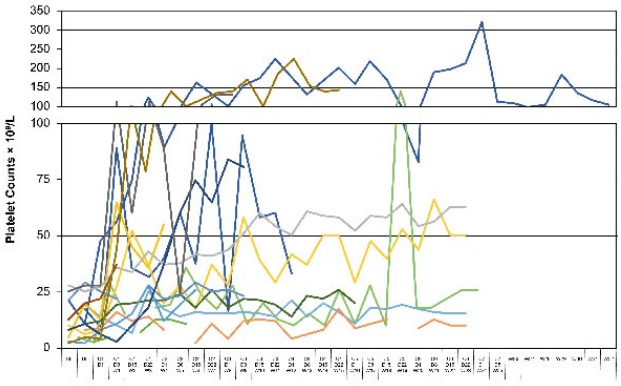

FIGURE 1 Platelet Counts Over Time With Rilzabrutinib 400 mg BID in Immune Thrombocytopenia (N=16)

TABLE 1 Platelet Counts With Rilzabrutinib 400 mg BID in ITP

	2 Consecutive ≥50K (primary)	Any 2 ≥50K	50% of Counts ≥50K	4 of Final 8 ≥50K	4 of Final 6 ≥50K
Started on 400 mg BID (N=16)	6 (38)	6 (38)	5 (31)	6 (38)	6 (38)
Completed ≥12 weeks of 400 mg BID (n=10)	5 (50)	6 (60)	4 (40)	5 (50)	5 (50)

Data cut-off: 13Nov2019. All data are presented as n (%).

abdominal distension. With a median of 12.1 weeks (range, 1.3-28.9) of rilzabrutinib, 6/16 patients (38%) achieved the primary endpoint (Table 1); none required rescue medication. In 10 patients treated ≥12 weeks, 5 (50%) achieved the primary endpoint. Platelet counts increased to ≥30,000/μL by day 8 for 100% of responders (n=6/16; Figure 1) and this increase is predictive of achieving the primary endpoint and durable response.

Conclusions: Rilzabrutinib 400 mg BID showed rapid, durable clinical activity, and a positive day 8 predictive value, alongside a well-tolerated safety profile. Enrollment has expanded at the optimal 400 mg BID starting dose to further demonstrate magnitude and durability of clinical benefit.

PB1319 | Characteristics of Patients with Cyclic Thrombocytopenia: An Observational Cohort Study

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Background: Cyclic thrombocytopenia (CTP) is characterized by periodical fluctuations of the platelet counts. Most cases are misdiagnosed as immune thrombocytopenia (ITP). The pathogenesis is incompletely understood and response to specific ITP treatments is poor.

Aims: To study the pathogenesis, risk factors, course of disease and response to treatment.

Methods: Between 1991 and 2018 all records of patients treated for isolated thrombocytopenia were screened. Blood counts, clinical chemistry, bone marrow aspiration, comorbidities, co-medications, complications and specific therapies were collected for patients who were not responding to ITP treatment and had the typical platelet count fluctuations. Data are presented as a descriptive analysis.

Results: We identified 10 patients of whom 8 were female (Figure 1). All patients were initially diagnosed as ITP. At presentation, 4 patients had mild mucocutaneous bleeding. The time interval until CTP diagnosis ranged from 2 months to 13 years. Platelet nadirs ranged from 4 to 46 G/l and cycle duration from 14 to 35 days. 9 patients had a disease of the thyroid gland. T-cell receptor beta and/or gamma rearrangement was found in 7 patients. Until CTP diagnosis patients were unsuccessfully treated with corticosteroids (all patients), intravenous immunoglobulins (6), TPO-receptor agonists (4), rituximab (5) and splenectomy (6). Upon CTP diagnosis, 7 patients received cyclosporine. One patient each developed T-cell large granular lymphocytic leukemia and acute myeloid leukemia. One patient had pulmonary embolism during TPO agonist therapy. One patient had intracranial bleeding at a platelet count of 1 G/l. A remission occurred in 6 patients.

Conclusions: CTP has to be considered in patients with isolated thrombocytopenia who do not respond to ITP treatment. CTP predominantly occurs in women and is often associated with a disease of the thyroid gland. Transformation into malignant hematological disorders can occur. Remission rates are high.

Clinical and laboratory characteristics of 10 patients with cyclic thrombocytopenia

Pt #	Sex	Age at ID (yrs)	Time until ID (yrs)	Mean cycle duration (days)	Bleeding during follow-up	Comorbidities	Complications	TCRR
1	F	43	1.5	23	Intracranial Mucocutaneous Gynecological	Hypothyroidism	ICB	clonal
2	F	55	2.5	14	Mucocutaneous	Hypothyroidism		clonal
3	F	65	10	28	Mucocutaneous	Hypothyroidism	PE	clonal
4	F	47	2.5	28	Mucocutaneous	Euthyroid struma nodosa		N/A
5	F	36	4	26	Mucocutaneous	Hypothyroidism		none
6	F	57	1.5	28	Mucocutaneous	Hypothyroidism, polymyalgia rheumatica	AML	clonal
7	M	41	3	24	Hematuria Mucocutaneous			clonal
8	F	53	0.3	35	Mucocutaneous	Hypothyroidism, DM I	T-LGLL	clonal
9	F	60	4	34	Mucocutaneous	Euthyroid struma nodosa		none
10	M	48	13	28	Mucocutaneous	Hypothyroidism		clonal

Pt #...patient number; F... female; M... male; ID... initial diagnosis of CTP; DM I... diabetes mellitus type I; ICB...intracranial bleeding; PE... pulmonary embolism; AML... acute myeloid leukemia; T-LGLL... T-cell large granular lymphocytic leukemia; TCRR... T-cell receptor rearrangement; N/A...not assessed;

FIGURE 1 Clinical and laboratory characteristics of 10 patients with cyclic thrombocytopenia

PB1321 | Pathogenic Mechanisms Contributing to Thrombocytopenia in Patients with Systemic Lupus Erythematosus

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Background: Systemic lupus erythematosus (SLE) is an autoimmune condition developing thrombocytopenia in about 10-15% of cases.

Aims: To investigate possible causes of low platelet count, including platelet apoptosis and desialylation as well as inhibition of platelet production.

Methods: Twenty-five SLE patients with and without thrombocytopenia were included. Platelet apoptosis was studied by phosphatidylserine exposure, loss of mitochondrial membrane potential ($\Delta\Psi m$), and active caspase 3 measurement; platelet activation by PAC-1 binding and P-selectin externalization;. plasma induction of normal platelet desialylation by *Ricinus communis* agglutinin I and peanut

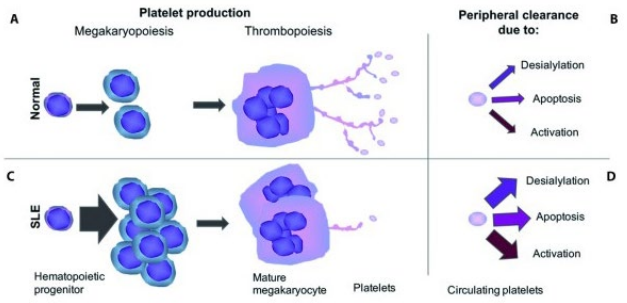

FIGURE 1 Proposed new mechanisms of thrombocytopenia in SLE

agglutinin binding; all by flow cytometry. Effect of SLE plasma on megakaryopoiesis and thrombopoiesis were evaluated using CD34+ hematopoietic progenitors isolated from normal blood cord.

Results: Platelet apoptosis was found in platelets from 4 thrombocytopenic patients. Six patients had *ex vivo* platelet activation, three of them also displaying platelet apoptosis. Besides, plasma from SLE patients induced desialylation of normal platelets. While megakaryopoiesis was increased, thrombopoiesis (proplatelet count) was decreased in the presence of SLE plasma. Depletion of anti-platelet autoantibodies from SLE plasma reverted the inhibitory effect on thrombopoiesis. Overall, these abnormalities were more frequently observed in patients with active disease (SLEDAI>4) than in those patients with inactive disease (SLEDAI < or = 4).

Conclusions: Platelet clearance due to apoptosis and desialylation, as well as inhibition of platelet production due to impaired thrombopoiesis, could be relevant mechanisms leading to thrombocytopenia in SLE. On the contrary, increased megakaryopoiesis, could help maintain normal platelet count in spite of the deteriorated thrombopoiesis in some SLE patients.

PB1322 | Elevated BAFF Promote B Cell Survival and Autoantibody Production through Upregulating Let-7b in Immune thrombocytopenia

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Background: The pathogenesis of immune thrombocytopenia (ITP) has not yet been completely elucidated. It has been shown that B cell activating factor BAFF is overexpressed ITP patients and dysregulated miRNAs contribute to ITP pathogenesis. However, it is unclear that whether BAFF can affect B cell function through regulating miRNA levels in ITP.

Aims: We aimed to investigate whether and how BAFF affect B cell homeostasis through regulating miRNA levels in ITP.

Methods: The $CD19^+$ B cells were isolated from peripheral mononuclear cells of ITP patients and healthy controls using immunomagnetic microbeads. The miRNA levels were detected by real-time quantitative PCR. The function of miRNAs in B cell were verified in primary human B cells and in a splenocytes transferring model by transfecting miRNA mimics or inhibitors. B cell survival and antibody production were evaluated by flow cytometry and enzyme-linked immunosorbent assay, respectively.

Results: Our results showed that plasma BAFF was significantly elevated in ITP patients with positive platelet autoantibodies and the expression of let-7b was significantly increased in B cells from ITP patients. We found that plasma BAFF was positively correlated with the let-7b level in B cells in ITP patients. Transfection of let-7b mimics into healthy primary B cells enhanced BAFF-induced B cell survival and antibody production *in vitro*, while transfection of let-7b inhibitors showed opposite effects. We also found that transfection of let-7b inhibitors into immunized mouse CD61 knockout splenocytes which were then transferred to SCID mice, can abolish platelet antibody production *in vivo*.

Conclusions: Elevated plasma BAFF can promote B cell survival and autoantibody production through upregulating let-7b levels in ITP.

PB1323 | Role of Bone Marrow-Derived Mesenchymal Stem Cell Defects in CD8+ CD28- Suppressor T-Lymphocyte Induction in Patients with Immune Thrombocytopenia and Associated Mechanisms

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Background: Numeric and functional defects of Ts cells and immune regulation abnormalities of mesenchymal stem cells (MSCs) are involved in immune thrombocytopenia (ITP) pathogenesis. Recent studies showed that MSCs can promote Ts cell differentiation.

Aims: Comparing the Ts cell induction abilities of bone marrow-derived MSCs (BM-MSCs) of patients with ITP and normal controls (NCs) to examine the causes of Ts cell defects, MSCs immune regulation abnormalities and the reason behind this difference.

Methods: BM-MSCs were isolated from 16 ITP patients and 10 normal controls, after co-cultured of CD8+ T cells with BM-MSCs for 3 days, CD8+CD28- percentages, FasL, IL-10, CD25, CD69 expression on Ts or effector T cells, along with cell apoptosis and proliferation were analyzed by FACS; and inhibition function of Ts cell was detected by multi-lymphocyte reactions. Moreover, UC-MSCs were co-cultured with Ts cells (1:20) from ITP patients to determine whether they can repair Ts defects in ITP patients.

Results: BM-MSCs elevated Ts cell percentage and function, but the efficiency was lower in patients with ITP than in NCs. Neutralization or blockade experiments showed that blockade of interleukin (IL)-6 partially reversed Ts cell induction by BM-MSCs. Addition of exogenous IL-6 down-regulated Ts cell apoptosis. Moreover, BM-MSCs enhanced IL-10 secretion from Ts cells and increased Ts cell ability to inhibit effector T cell activation. IL-6 secretion, regulatory abilities of IL-10 expression in Ts cells, and the enhanced efficiency of Ts cells to inhibit effector T cell activation by BM-MSCs were all decreased

in patients with ITP. And umbilical cord-derived MSCs (UC-MSCs) efficiently improved ITP Ts cell numbers and dysfunction.

Conclusions: Defects in BM-MSCs involved in Ts cell induction play a role in ITP pathogenesis, and exogenous UC-MSCs may be useful for ITP therapy.

PB1325 | Platelet Surface GPIbα, Platelet Surface Activated GPIIb-IIIa and Platelet Surface P-Selectin in Veno-Arterial Extracorporeal Membrane Oxygenation Patients

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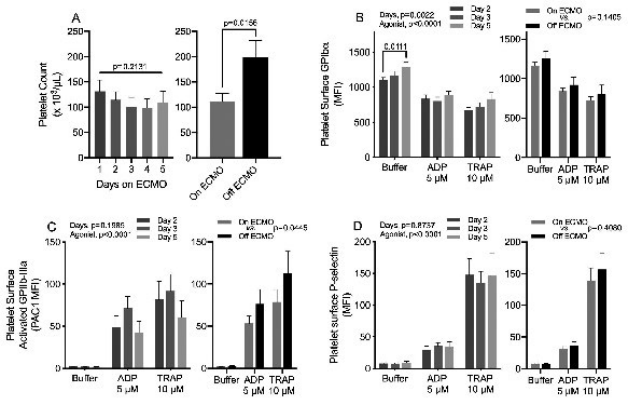

FIGURE 1 Platelet counts, GPIbα, activated GPIIb-IIIa, and P-selectin on ECMO and after discontinuation

Background: Patients on veno-arterial extracorporeal membrane oxygenation (ECMO) are at high risk for bleeding. This is related to systemic anticoagulation, loss of large Von Willebrand Factor multimers, reduced alpha and dense granule secretion, and platelet receptor GPIbα and GPVI shedding. Little information is available regarding changes in platelet function that may affect bleeding risk, in part because many platelet tests are invalid for thrombocytopenia.

Aims: To evaluate changes in platelet surface GPIbα and markers of platelet activation (platelet surface activated GPIIb-IIIa and P-selectin) in patients before *vs.* after discontinuation of ECMO.

Methods: Following IRB-approved informed consent, blood was collected from 10 patients (Table) on Day1,2,3 and 5 after initiation and 48-72 hours after stopping ECMO treatment. Whole blood flow cytometric analysis of platelet surface GPIbα, activated GPIIb-IIIa, and P-selectin was performed.

Results: Platelet counts were low in patients on ECMO and increased after discontinuation (Fig.A). GPIbα by mean fluorescence intensity (MFI) increased with days on ECMO (p=0.0022 mixed model, p=0.0111, Day2 vs. Day5 Dunnett's multiple comparison, Fig.B). After discontinuation of ECMO, platelet surface GPIbα did not increase significantly. In contrast, agonist (ADP or thrombin receptor activating peptide [TRAP])-stimulated platelet surface activated GPIIb-IIIa did not change with time on ECMO but did significantly increase after stopping ECMO (Fig.C). Agonist-stimulated platelet surface activated GPIIb-IIIa was more variable across subjects (CV 74%) than platelet surface GPIbα (CV 25%), with some subject's on-ECMO TRAP-stimulated PAC1 MFI below the 95th %percentile of off-ECMO values. Unstimulated and agonist-stimulated platelet surface P-selectin was not significantly altered by duration of ECMO or discontinuation of ECMO (Fig.D).

Conclusions: Changing (increasing) levels of platelet surface GPIbα over time on ECMO highlights the need for longitudinal studies. Reduced agonist-stimulated platelet surface activated GPIIb-IIIa may, in combination with heparin and the reduction in platelet counts, contribute to ECMO-related bleeding.

TABLE 1 The patients' characteristics

Variable	mean±SD or n (%)	Variable	n (%)	Variable	mean±SD or n (%)
Age in years	46 ± 17	Anti-platelet drug before ECMO		ECMO days	27 ± 31
Sex (% male)	5 (50.0)	_ Aspirin	1 (10.0)		
Hypertension	5 (50.0)	_ Clopidogrel	1 (10.0)	Weaning strategy	
Diabetes mellitus	1 (10.0)	_ Treprostinil	1 (10.0)	_ Inotropes	6 (60.0)
Hyperlipidemia	3 (30.0)	Etiology of shock		_ Transition to LVAD	1 (10.0)
Obstructive sleep apnea	1 (10.0)	_ Post cardiotomy	3 (30.0)	_ Transplantation	1 (10.0)
Tobacco use	1 (10.0)	_ Pulmonary embolism	1 (10.0)	_ Unable to wean off (died)	2 (20.0)
		_ CHF	5 (50.0)		
		_ STEMI	1 (10.0)		

PB1326 | Predictive Factors of Chronicity in Adult Idiopathic Thrombocytopenic Purpura

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Background: The epidemiology of idiopathic thrombocytopenic purpura (ITP) is not well known. Recent retrospective studies have indicated that two-thirds of adult ITP patients are at risk of developing a chronic form of the disease.

Aims: The objectives of this study are to investigate the predictive factors of chronicity in adult patients with an ITP, to describe the clinical and biological characteristics and to specify the type of first-line treatments in this population as well as their evolution.

Methods: This is a monocentric retrospective study that included all patients diagnosed and monitored for an ITP over a period of 4 years.

Results: Our cohort consisted of 163 patients with a mean age of 41.2 with a sex ratio of F / H of 2.41. In 27% of the cases, the circumstances of discovery were fortuitous. The median bleeding score for Khellaf was 4. The mean platelet count was 25.6 G / L. A myelogram was performed in 75.5% of the cases of which 100% of the more than 60 years. A "watch and wait" strategy was decided for 16 patients. 88.9% of patients received treatment with corticosteroids with a CR and failure rate of 48.5% and 18.4% respectively. Of our patients 30.7% have progressed to a chronic ITP. The predictive factors for chronicity at 12 months were platelet count ($p = 0.001$) and severity of hemorrhagic syndrome ($p = 0.05$). Indeed, a platelet count of more than 30G / L was associated with< a high probability of passage to chronicity ($p = 0.02$).

Conclusions: The adult ITP is a disease frequently associated with bleeding where one third of the patients progress to a chronic form. High platelet counts and severity of bleeding at diagnosis are associated with a risk of progression to chronic ITP.

PB1327 | Antithyroid Treatment Improves Thrombocytopenia in a Young Patient with Graves' Disease: A Case Report

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Background: Autoimmune thyroid disorders, including Graves' disease and Hashimoto's thyroiditis, were reported in patients with primary immune thrombocytopenia (ITP).

Several etiopathogenetic mechanisms connecting thyroid diseases and thrombocytopenia have been described. Thrombocytopenia is often documented in patients with Graves' disease, due to reduced platelet life span in hyperthyroidism, immune dysregulation and genetic predisposition (HLA B8 presence). Thyroid autoantibodies have been detected in 89% of patients with ITP and autoimmune thyroid disease.

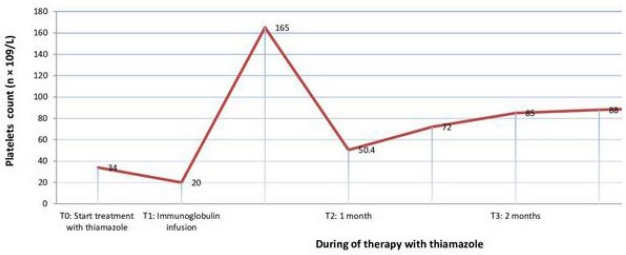

FIGURE 1 Platelet count recovery after start of antithyroid treatment

Aims: Our report broadens the knowledge about the clinical and therapeutic management of patients suffering from ITP and Graves' disease

Methods: We present the case of a 14-years old girl, who was referred to our Pediatrics Unit, because of the appearance of weight loss, profuse sweating and episodes of recurrent epistaxis. A complete health team, made up of hematologists and endocrinologists, met in consultation in order to reach a diagnosis. A suppression of serum TSH concentrations, the presence of anti-TSH receptor antibodies and at the same time an immune thrombocytopenia with positive anti-platelet antibodies, have been detected. Furthermore, a positive direct and indirect Coombs test without hemolytic anemia, ANA positivity, and a C4 consumption have been documented. The patient started treatment with thiamazole with progressive improvement of thyroid function and thrombocytopenia, requiring only an intravenous immunoglobulin infusion on one occasion. A multidisciplinary follow-up has been scheduled, in order to monitor the multi-organ immune dysregulation.

Results: This is the first report that documents a significant improvement in thrombocytopenia, following the administration of antithyroid treatment alone in a young subject suffering from Graves' disease.

Conclusions: In literature, reports of ITP responding to antithyroid treatment in patients with Graves' disease have been reported, but the peculiarity of our case is that platelet count recovery has been obtained with only antithyroid treatment, without further specific therapies for ITP, if not on one occasion the administration of intravenous immunoglobulin.

PB1328 | Apoptotic Markers May Help in Predicting the Disease Course of Pediatric Immune Thrombocytopenic Patients

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Background: Fifteen to thirty percent of pediatric immune thrombocytopenia (ITP) patients will remain chronically thrombocytopenic at one year from diagnosis. To date, none of the attempts to classify patients on diagnosis have been successful.

We hypothesized that a different pathophysiology is responsible for non-chronic versus chronic pediatric ITP. Predicting the course of ITP upon diagnosis might affect the disease course in patients with chronic disease.

Aims: In the current study, we examined whether a difference exists in the apoptotic markers' presentation at diagnosis between non-chronic pediatric patients and those with chronic disease.

Methods: Blood samples were collected from 42 patients with a clinical diagnosis of ITP whowere admitted to five pediatric departments in Israel between the years 2010-2014. All blood samples were taken prior to initiation of treatment. We incubated patients' sera with washed platelets and compared the results among three research groups: healthy controls, chronic ITP, and non-chronic ITP patients. Phosphatidylserine exposure and mitochondrial electrochemical potential were measured using flow cytometry followed by a Human Apoptosis Array (HAA).

Results: We found increased expression of five apoptotic proteins; BIM, CD40, IGFBP2, P21, and SMAC on platelets incubated with sera of non-chronic pediatric ITP patients, compared to chronic patients' sera upon diagnosis.

Conclusions: Our data suggests a different pathophysiology in non-chronic and chronic pediatric ITP patients. This indication might help to classify ITP patients into non-chronic versus chronic ones, at diagnosis. This, in turn, may enable us to tailor a more specific therapy to these seemingly different clinical entities currently being treated by identical regimens.

PB1329 | Glucocorticoid Receptor Modulates the Mitochondria Function of Myeloid-Derived Suppressor Cells in Immune Thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is an autoimmune hemorrhagic disorder in which abnormal activation of T-cells are involved. Myeloid-derived suppressor cells (MDSCs) are characterized by remarkable ability to suppress

T-cell function. Corticosteroids are involved in the first-line treatment for ITP, however, 30% of patients fail to respond or relapse. And glucocorticoid receptor (GR) can translocate into the mitochondria and regulate transcription of mitochondrial genome.

Aims: To investigate how the GR regulates the function of MDSCs in ITP.

Methods: We collected 27 ITP patients who had received high-dose dexamethasone treatment. The MDSCs were induced in vitro and CHIP-qPCR assays were performed. Seahorse XFanalyzerwas used to analyze oxygen consumption rate and parametersof mitochondrial

Figure 1

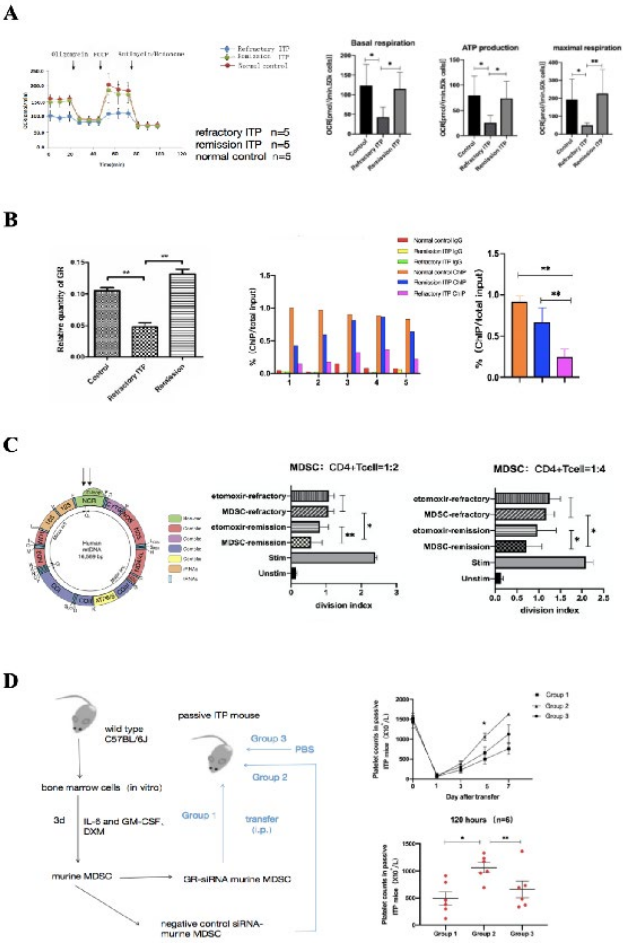

FIGURE 1 GR modulates the mitochondria function of MDSCs in ITP

function of MDSCs. MDSCs were treated with CPT-1 inhibitor, etomoxir, to explore the effect of inhibiting fatty acid oxidation. GR expression was blocked in murine MDSCs which were transplanted into ITP mice.

Results: The mRNA expression of GR and mtDNA-encoded genes (ATP8, COX1, CYTB, ND1, ND5), as well as the binding of GR to mtDNA in MDSCs were decreased in steroid-resistantITPgroupcompared to steroid-responsive ITP groupand healthy controls. MDSCs in steroid-resistant patients had decreased levels of basal respiration, maximal respiration and ATP production, suggesting that the mitochondriaof MDSCs in steroid-resistant group had lower activities of electron transport chains and oxidative phosphorylation.The function of MDSCs to inhibit T-cell proliferation was decreased in steroid-resistant group.Etomoxirreduced the ability of MDSCsto block T-cell proliferation in steroid-responsivegroup, but did not alter the suppressivefunction of MDSCs on T cells in steroid-resistant group. ITP mice transplanted with GR siRNA-treated MDSCs showed lower platelet counts than those with control siRNA-treated MDSCs, suggesting that the function of MDSCs to ameliorateITP relied on GR.

Conclusions: This study demonstrated that GR-associated MDSCs impairments are involved in the pathogenesis of ITP.It might berelated to the modulation of mitochondrial respiration and metabolism by GR.

PB1330 | Desmopressin for Procedures or Radiological Interventions (DRIVE): Participant Characteristics in a Placebo-Controlled Double-Blind, Randomised Feasibility Trial of Desmopressin in Thrombocytopenic Critically Ill Patients Prior to Procedures

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Background: Thrombocytopenia is common in critically ill patients, many of whom undergo invasive procedures putting them at risk of bleeding.

Aims: To describe the participant characteristics of thrombocytopenic patients undergoing invasive procedures recruited to the DRIVE trial (ISRCTN12845429).

Methods: Critically ill patients were eligible if they had a platelet count of less than 100 x10^9/l and were undergoing an invasive procedure, which the treating physician considered to have a bleeding

TABLE 2 Baseline clinical characteristics

	Median (interquartile range) or n (%)
Age (years)	60 (50 to 68)
MaleFemale	24 (59%)17 (41%)
Admission reason:	
Bowel obstruction	2 (5%)
Haemorrhage	2 (5%)
Sepsis	26 (63%)
Liver cirrhosis	3 (7%)
Malignancy	3 (7%)
Obstetric	1 (2%)
Trauma	2 (5%)
Other	2 (5%)
Procedure:	
Arterial line insertion	3 (7%)
Arterial line removal	5 (12%)
Central venous catheter insertion	8 (20%)
Central venous catheter removal	8 (20%)
Chest drain removal	1 (2%)
Drain insertion	2 (5%)
Lumbar puncture	1 (2%)
Vascath insertion	3 (7%)
Vascath removal	7 (17%)
Other	3 (7%)

TABLE 1 Baseline laboratory tests. *two patients randomised with platelet counts > 100 x10^9/L

	N	Median*Interquartile rangeRange*	Normal range
Platelet count*x10^9/L*	41	*5240 to 710 to 174**	150 to 400
Von Willebrand Factor antigen*IU/mL*	41	*4.84.2 to 7.41.9 to 11.3*	0.5 to 2.0
Von Willebrand Factor activity GP1bM*IU/mL*	39	*4.83.7 to 7.31.6 to 13.1*	0.5 to 2.0
Von Willebrand Factor collagen binding*IU/mL*	33	*5.93.5 to 8.21.6 to 14.1*	0.5 to 2.0
Peak thrombin generation*nM*	15	*207180 to 33142 to 337*	176 to 406
Endogenous thrombin potential*nM.min*	15	*15131247 to 1894790 to 2939*	944 to 2048
PFA200 ADP/collagen closure time*seconds*	17	*>300149 to > 30087 to > 300*	60 to 112
PFA200 P2Y closure time*seconds*	17	*248139 to > 30049 to > 300*	<106

risk. Patients with capacity were approached for informed consent. A personal or professional legal representative was approached for patients who lacked capacity (approved by Oxford C Research Ethics Committee [16/SC/0524]). Patients were randomised (1:1) to receive intravenous desmopressin 0.3 mcg/kg or matching placebo. The primary feasibility outcome was the proportion of eligible patients who were randomised and received desmopressin or placebo. Secondary outcome measures included bleeding and blood transfusion up to 24 hours, and thromboembolic events and serious adverse events up to 28 days after the procedure. Baseline blood tests (repeated 30 and 120 minutes after infusion of desmopressin or placebo) are outlined in table 1.

Results: 41 patients were recruited between 01 January 2016 and 04 July 2019 from 3 UK critical care units (Table 2). The most common cause of thrombocytopenia was sepsis 26/41 (63%). The majority of patients that critical care physicians considered for inclusion were undergoing insertion or removal of a central venous catheter, VasCath or arterial line 34/41 (83%). VWF antigen, activity and collagen binding were elevated up to 7x the upper limit of the normal range at baseline (Table 1).

Conclusions: The DRIVE trial results will be reported this year and will inform the design of clinical trials to prevent bleeding in critical care. Baseline VWF levels were significantly elevated and sepsis was the most common cause of thrombocytopenia for critically ill patients undergoing invasive procedures.

PB1331 | Atorvastatin Restores the Imbalance of CD4$^+$ T cells in Immune Thrombocytopenia via Ras/MEK/ERK and mTOR/PI3K/AKT Pathways

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Background: Immune thrombocytopenia (ITP) is an autoimmune hemorrhagic disease, in which the overactivation of

T cells is crucial in the pathogenesis. Atorvastatin (AT), a lipid-lowering medicine, has shown promising immunomodulatory properties in some autoimmune diseases, such as rheumatoid arthritis. However, the immunoregulatory role of atorvastatin in ITP remains elusive.

Aims: To investigate the effect of atorvastatin in the treatment of ITP.

Methods: CD4$^+$ T cells were isolated from ITP patients and cultured with different dosages of AT for 3-5 days, with or without (±)-Mevalonolactone (MA). Cell growth and function markers, as well as the activation of cell signal pathways were evaluated. AT (10 mg/kg/qod) was administered intragastrically to the ITP mice for 5 weeks to investigate the effect of AT in vivo.

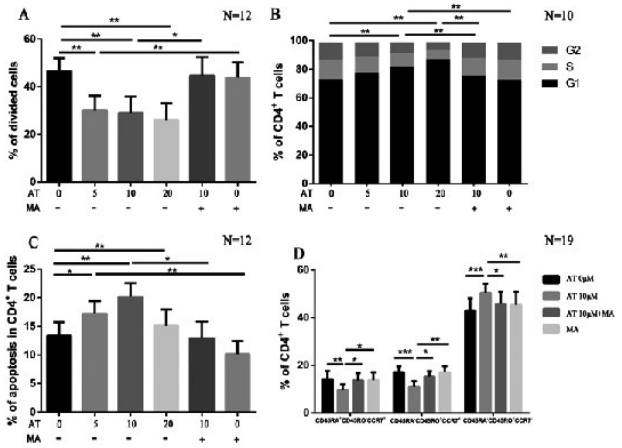

FIGURE 1 Cell proliferation (A), cell cycle (B), cell apoptosis (C) and the expression of CCR7 on T cells (D)

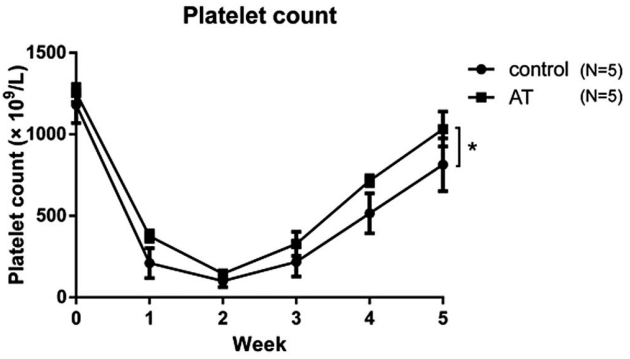

FIGURE 2 Platelet count of ITP murine models

Results: AT significantly inhibited cell proliferation, led to cell cycle arrest at the G1 phase, and induced apoptosis of CD4$^+$ T cells in vitro. Besides, the expression of CD25, CTLA-4, CD69, HLA-DR and CCR7 on CD4$^+$ T cells, CD4$^+$CD45RA$^+$CD45RO$^-$ naïve T cells and CD4$^+$CD45RA$^-$CD45RO$^+$ memory T cells or CD4$^+$CD25$^+$ effector T cells were significantly decreased after AT treatment.

In ITP models, the AT group had significant higher platelet counts at week 5 and a tendency to reduced expression of CD25 and CD69 on CD4$^+$ T cells of spleen and thymus.

Besides, the phosphorylation of mTOR, Akt and Erk, as well as activation of Ras were reduced dramatically after AT treatment in vitro. The inhibition effect of AT could be offset by adding MA in vitro.

Conclusions: Our study showed atorvastatin could reinstate the functions of CD4$^+$ T cells by inhibiting the excessive activation, proliferation and survival of CD4$^+$ T cells in ITP via Ras/MEK/ERK and mTOR/PI3K/AKT pathways. In summary, atorvastatin could be used as a potential therapy in ITP via restoring the over-activated cellular immune.

PB1332 | Use of Eltrombopag in Children with Chronic Immune Thrombocytopenia (ITP): A Real Life Retrospective Multicenter Experience of the Italian Association of Pediatric Hematology and Oncology (AIEOP)

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Background: The thrombopoietin receptor agonist eltrombopag has been shown to be safe and effective for children with chronic immune thrombocytopenia (ITP).

Aims: The aim of the present study was to characterize eltrombopag use in current clinical practice.

Methods: This is a retrospective multicentre study conducted in 17 centres affiliated to the Italian Association of Pediatric Haematology and Oncology (AIEOP). The primary objective of the study was to determine the prevalence of eltrombopag use in Italian children affected by chronic ITP, after European Medicines Agency authorization for pediatric age. The secondary objective was to assess efficacy in the first 6 months and safety during the whole period of eltrombopag treatment in current clinical practice. Signed informed consent has been acquired from patient's parents. A total of 386 children with chronic ITP were retrospectively enrolled and eligible for analysis. Among these patients, 71 received eltrombopag.

Results: The prevalence of eltrombopag use was 19% (95% CI 0.15 - 0.23). Thirty-one patients (44%) were male and forty patients (56%) were female. The median age at the first dose of eltrombopag was 12 years (3-17 years). The median duration of eltrombopag treatment was 11 months (1-32 months) and the median starting dose was 50 mg/day (12.5-75 mg/day). Thirty-two patients (45%) required one or more concomitant ITP medications during the first six months of treatment with eltrombopag. Thirty-nine patients (55%) never required concomitant medications. Median platelet counts and proportion of patients achieving the target platelet count of at least 30×10^9/l and 100×10^9/l significantly increased during the first six months of treatment (p value < .0001). Additionally, eltrombopag has been proved effective in the absence of concomitant therapies. The most common Adverse Events were headache (7%) and thrombocytosis (6%).

Conclusions: Our study highlighted the crucial role of eltrombopag as second line treatment in children with chronic ITP.

PB1333 | Relationship between Platelet Function and Glycome Composition in Immune Thrombocytopenia

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Background: One way platelets are cleared in immune thrombocytopenia (ITP) is by desialylation of glycans. We hypothesize that loss of sialic acid might also modify platelet function in ITP patients.

Aims: So we aimed to evaluate the pattern of platelet glycosylation in ITP patients and its involvement in platelet function to contribute to the growing body of knowledge about platelet glycosylation and its role in ITP.

Methods: This was an observational, prospective and transversal study approved by La Paz University Hospital Ethics Committee. Forty two patients with chronic primary ITP and forty eight healthy controls were included after signing the informed consent.

Platelet activation markers in platelet rich plasma and platelet glycosylation in washed platelets were determined by flow cytometry. Table 1 shows lectins tested and their sugar-binding specificity.

Data were analyzed with GraphPad Prism 5.0 software.

Results: Platelets from ITP patients were basally activated, as shown by the exposure of P-selectin and CD63 on their surface, but their response to agonist activation (100 μM TRAP and 10 μM ADP) was reduced (Figure 1A).

Platelets from ITP patients exposed less sialic acid in correspondence to an enhanced binding of lectins to non-sialylated residues (Figure 1B). Levels of α1,6-Fucose, a glycan residue which could directly regulate antibody-dependent cellular cytotoxicity, and of α-Mannose, which could be recognized by the mannose binding lectin and activate complement pathway, were increased in platelets from ITP patients. In accordance, sialic acid loss and consequent platelet surface exposure of other glycan residues were inversely related to platelet count and ability to be activated (Table 1).

TABLE 1

Lectins tested	Abbr.	Sugar specificity	Lectin binding vs Platelet count (Spearman Rho; p)	Lectin binding vs TRAP-induced PAC1 binding (Spearman Rho; p)
Aleuria aurantia	AA	α1,6-Fucose	-0.3946; 0.0002	-0.2297; 0.0392
Concanavalin A	CA	α-Mannose	-0.3964; 0.0002	-0.2567; 0.0191
Dolichos biflorus agglutinin	DB	GalNAcα1-3GalNAc	-0.6110; <0.0001	-0.4172; <0.0001
Datura stramonium	DS	GlcNAc	-0.4091; 0.0001	-0.2581; 0.0185
Ricinus communis agglutinin	RCA	β-Gal	-0.3707; 0.026	-0.298; 0.0160
Wheat Germ Agglutinin	WGA	β-GlcNac	-0.2868; 0.0177	-0.3737; 0.0007

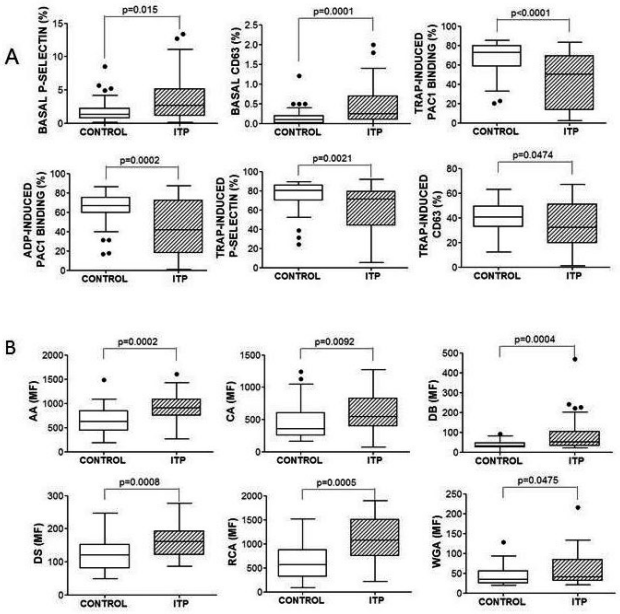

FIGURE 1

Conclusions: Surface of platelets from ITP patients suffered changes in their carbohydrate composition due to the loss of sialic acid that might induce platelet clearence/destruction through several mechanisms.

This work was supported by grants from FIS-FONDOS FEDER (PI19/00772) and Platelet Disorder Support Association.

PB1334 | Can we Predict the Outcome of Splenectomy for Primary Immune Thrombocytopenia? Conclusion Based on Systematic Review and Meta-Analysis

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Background: Splenectomy may lead to a good response in 60-80% of adults with immune thrombocytopenia (ITP). However, available literature does not seem to contain any precise indications concerning possible factors predicting the response to splenectomy.

Aims: To perform systematic review and meta-analysis in an attempt to identify predictive parameters for good therapy response (GTR) on splenectomy in adult ITP patients.

Methods: MEDLINE, Web of Science, EMBASE, Cochrane trials and reviews were searched from 1966 to 2018 for studies where splenectomised ITP patients were followed to determine predictive parameters for GTR. The primary analysis included all papers. During secondary analyses papers were divided in groups according to used response criteria (first used International Working Group (IWG) criteria; second defined GTR as platelet count (PC) > 50x10^9/L). Both groups were subdivided according to the tested type of response: initial, sustained remission and relapse. Random effects method was used to summarise outcomes. The effect estimates were expressed as odds ratio (OR) or standardised mean difference (SMD) with 95% confidence interval. Strength of evidence was assessed by New-Castle Otawa protocol.

Results: The literature search identified 8451 articles; 35 of them were included in meta-analysis (IWG group included 16 articles and second group 19 articles). Meta-analysis did not reveal any significant predictor for successful splenectomy, in both primary and secondary analysis (p > 0.05). Age, sex, the time between diagnosis and surgery, response to steroids, response to IVGs, preoperative PC, antiplatelet antibody, spleen weight, accessory spleen, number of therapy lines, and platelet kinetic were not significantly correlated with response to splenectomy. Low quality of evidence was found for all outcomes.

Conclusions: Our study did not identify any parameter clearly predictive of GTR after splenectomy. Further studies based on appropriate use of standardised criteria for patient recruitment and outcomes are needed to better define splenectomy effect persistence in long term.

PB1335 | Achieving Clinically Relevant Platelet Count Response Thresholds with Avatrombopag (AVA) in Immune Thrombocytopenia (ITP)

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Background: Thrombopoietin receptor agonists (TPO-RAs) are safe and effective treatments for refractory chronic ITP. Unlike other TPO-RAs, AVA does not require a fasting state nor weekly visits for in-office administration. A platelet count ≥ 50,000/μL is often the target endpoint designated in ITP clinical trials; however in real-world practice a PC of 30,000/μL or above is often thought adequate to achieve safety and reduce the risk of serious bleeding.

Aims: The objective of these analyses was to evaluate the ability of AVA to achieve a platelet count ≥ 30,000/μL in ITP patients.

Methods: A randomized, double-blind, Phase 3 study enrolled 32 AVA and 17 placebo-treated patients with chronic ITP. The primary endpoint was the cumulative number of weeks in which the platelet count was ≥ 50,000/μL during the 6-month treatment period in the absence of rescue therapy. Post-hoc efficacy outcomes were evaluated to assess achieving a platelet count ≥ 30,000/μL.

Results: Treatment with AVA resulted in more patients achieving a platelet count ≥ 30,000/μL at least once or twice during the 6 months as compared with placebo (Table 1). The median cumulative number of weeks with a platelet count ≥ 30,000/μL without requiring rescue treatment was 21.1 for AVA-treated patients and 0.0 for placebo. The median consecutive number of weeks maintaining

TABLE 1

Avatrombopag Responders Achieving a Platelet Count ≥30,000/μL		
Outcome	AVA N=32	PBO N=17
At least once during the study, %	90.6%	35.3%
At least twice during the study, %	87.5%	11.8%
Median cumulative # of weeks	21.1	0.0
Median consecutive # of weeks	11.1	0.0
For at least 4 consecutive weeks, %	65.6%	5.9%
Durable response, %*	64.0%	0.0%

AVA=Avatrombopag; PBO=Placebo; * Percentages are based on the number of subjects who had evaluable platelet counts during the last 8 weeks of the study in each treatment group.

a platelet count ≥ 30,000/μL was 11.1 for AVA and 0.0 for placebo. 64.0% of AVA vs. 0.0% of placebo-treated patients experienced a durable platelet response, achieving a platelet count ≥ 30,000/μL for 6 of the final 8 weeks of the study.

Conclusions: When compared with placebo, treatment with AVA demonstrated robust and durable efficacy in reaching a platelet count of ≥ 30,000/μL, a key threshold for clinicians as they evaluate the effectiveness of ITP therapy and make therapeutic management decisions.

PB1336 | Efficacy and Safety of Romiplostim in Children with Chronic and Persistent Immune Thrombocytopenic Purpura

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Background: Romiplostim is a thrombopoietin receptor agonist (TPO-RA) which has recently been approved for treatment of immune thrombocytopenic purpura (ITP) in children.

Aims: We tried to investigate the efficacy and safety of romiplostim in a heterogeneous group of children with persistent and chronic ITP and acquired aplastic anemia.

Methods: We enrolled 14 patients with the following diagnoses: chronic ITP (n = 10), persistent ITP (n = 3) and acquired aplastic anemia (n = 1). Romiplostim dose was adjusted according to the issued treatment guideline (1). The patients were followed for 26 weeks.

TABLE 1 platelet response to romiplostim injection in the study population

diagnosis		Minimum	Maximum	Mean	Std. Deviation
chronic ITP (n=10)	No response	4.50	87.50	35.2700	28.28624
	Partial response	.00	47.90	27.8000	14.85089
	Complete response	.00	76.20	36.9300	25.26816
	Overall response	12.50	95.20	64.7000	28.25011
persistent ITP (n=3)	No response	21.80	43.50	35.4000	11.84947
	Partial response	13.00	30.40	20.5333	8.93159
	Complete response	40.90	47.80	44.0667	3.48473
	Response	56.50	78.20	64.6000	11.84947
Aplastic anemia (n=1)	No response	57.90	57.90	57.9000	.
	Partial response	42.10	42.10	42.1000	.
	Complete response	.00	.00	.0000	.
	Response	42.10	42.10	42.1000	.

Data are shown as percentages.

Partial response: platelet count 50-100×10^9 μ/L; Complete response: platelet count more than 100×10^9 μ/L; Overall response: platelet count more than 50×10^9 μ/L

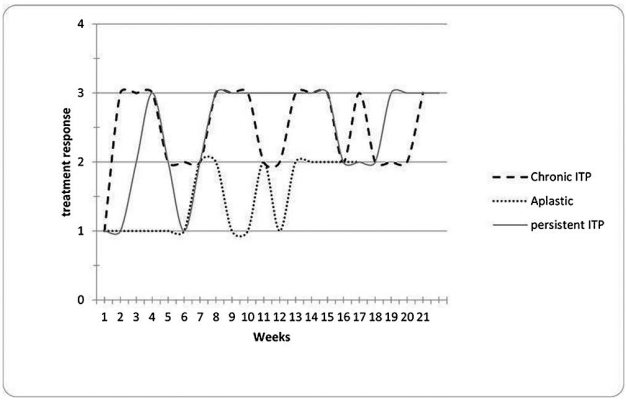

FIGURE 1 The trend of platelet response to romiplostim in the 3 diagnosis category. Treatment response has been categorized according to 3 platelet c

Results: Mean baseline platelet count was 26714 ± 15774 (range 6000-49000). The mean treatment duration was 22.2 ± 5.5 weeks (range 8-26 weeks). Totally, 63% ± 24.7% (range 12.5-95.2%) of the patients showed partial or complete response (Table 1). The overall response was comparable in the 3 treatment category (P = 0.71). Figure-1 demonstrates the trend of treatment response in the 3 diagnosis categories during the first 21 weeks of the treatment course. There was no severe adverse effect during the intervention period. One patient reported headache and body pain in the first week of treatment which was treated with oral analgesic.

Conclusions: We observed that about 65% of patients with chronic or persistent ITP responded to romiplostim which is very close to 73% response rate reported by the Pediatric ITP Consortium of North America (ICON2) study (2). Although romiplostim has not been approved for treatment of severe aplastic anemia, we observed that our case showed partial response in 42% of observations. A larger longitudinal study is advised to elucidate the durability of response and safety of this drug in patients with ITP and aplastic anemia.

PB1337 | Outcome of Splenectomy in Adult Refractory Immune Thrombocytopenic Purpura - A Descriptive Analysis

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Background: Splenectomy is indicated for second line therapy in refractory immune thrombocytopenic purpura (ITP). Risk of infection, thrombosis, pulmonary hypertension and immediate post splenectomy complication may have contributed to the decreased in splenectomy rates.

Aims: To describe the outcome of splenectomy in patients with refractory ITP in a tertiary centre.

Methods: Data was obtained from patients' medical record and database at Ampang Hospital, Malaysia. Adult patients with refractory ITP who underwent splenectomy between January 2009 until December 2019 were included. Demographics, clinical

characteristics and treatment outcomes were collected and analyzed using descriptive statistics.

Results: Approximately 2.5% (n = 12) from total adult ITP cases (n = 487) underwent splenectomy within the study period. Mean age at diagnosis was 29.7 ± 17.0 years old. Mean platelet count was 13.4 ± 11.5 (range 3 - 44) x 10^9/L with 41.7% had intracranial bleeding at presentation. Most of the cases failed at least two line medical therapy; eight cases (66.7%) were treated with Rituximab and half of them received Eltrombopag prior splenectomy. Sixty seven percent (n = 8) achieved complete remission and 16.7% (n = 2) had partial response after splenectomy with median follow up of 44 months (range 1-108). Among the responders, one case relapsed after 24 months post splenectomy. All of the cases had laparoscopic splenectomy except one who needed open splenectomy due to intraoperative bleeding. Four patients had acute complication (2 cases had infection, 2 cases had thrombocytosis) after surgery.

Conclusions: Although surgical risks are important, splenectomy provides long-term benefit for patients who did not achieved durable remissions with glucocorticoid treatment. Our series suggest that splenectomy offers disease remission in 80% of cases with no mortality reported, although there were five patients who had non-fatal complications.

PB1338 | Romiplostim Treatment in Patients with Refractory Inmune Thrombocytopenia Purpura: Experience in a Single Center

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Background: Immune thrombocytopenia purpura (ITP) is an acquired autoimmune disease. Its presentation, characteristics and clinical course are very variable. The pathogenesis is based on accelerated platelet destruction mediated by autoantibodies. There is no definitive clinical test or characteristic to establish its diagnosis. Classic treatments (corticosteroids, the use of gamma globulins and splenectomy) are aimed at modulating the immune system. There are currently new treatments aimed at increasing platelet production such as Eltrombopag and Romiplostim.

Aims: Describe our experience in the use of Romiplostim in patients with ITP refractory to the first line of treatment.

Methods: Data were collected from patients with ITP treated with Romiplostim in our center during 2018 and 2019. In total, 8 patients were treated, of which two were children. All adult patients had received prior treatment with Eltrombopag being refractory to it. The children had only received prior treatment with corticosteroids and immunoglobulins.

Patient data are shown in table 1.

Results: 75% (6) of patients achieved complete remission (CR) with Romiplostim. The two refractory patients were elderly patients with multiple diseases. Of the patients who were refractory to Eltrombopag, 66.6% (4) obtained CR. In addition, in 37.5% (3) of the patients it was possible to suspend Romiplostim after CR. None of the patients suffered side effects.

TABLE 1

Gender	Age	Previous treatment with Eltrombopag	Platelet count after Romiplostim
Male	87	Yes	Less than 10x10e9/L
Female	76	Yes	Normal
Female	27	Yes	Normal
Male	62	Yes	Normal
Male	84	Yes	Less than 10x10e9/L
Male	8	No	Normal
Female	43	Yes	Normal
Male	5	No	Normal

Conclusions: In our experience, Romiplostim is a good alternative in patients in whom the first lines of treatment, including Eltrombopag, have not been effective. In addition the drug has a good safety profile, not founding in our patients side effects, including thrombosis.

PB1339 | Treatment of Immune Thrombocytopenia with Eltombopag in Children: Single Center Experience

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Background: The efficacy of eltrombopag in patients with immune thrombocytopenia has been demonstrated but long-term side effects are not clear yet.

Aims: The aim of this study is to present our results of treatments with eltrombopag.

Methods: The medical records of 12 patients who were treated with eltombopag for immune thrombocytopenia were analyzed retrospectively.

Results: Of 12 patients 8 were female, 4 were male. Eleven patients were diagnosed with immune thrombocytopenia, one patient with Evans syndrome. They were aged between 2.7-15 years when eltrombopag was initiated. The time from diagnosis of immune thrombocytopenia to start of eltrombopag was between 1 month and 12 years. All patients were treated with intravenous immunoglobulin, steroids, anti-D globulin previously and 2 patients were splenectomized.

Platelet counts were between 8-27 x 10^9 / L when eltrombopag was initiated. The starting dose was 50 mg/day for patients age ≥ 6 years, 25 mg/day for patients 6 > years. One patient discontinued in the first week of treatment because of gastrointestinal intolerance. One patient discontinued at the end of the first month because she didn't respond but after 6 months it was started again and this time she achieved a platelet response (>40000 mm3). In 2 patients platelet

counts remained < 30 x10 ^{9}/ L at 75 mg/day but there was reduction in their bleeding symptoms. Eight patients achieved a platelet response (≥50 x10 ^{9}/ L) but they couldn't sustain their response when eltrombopag was discontinued.

Conclusions: Although eltrombopag seems effective and safe in children we need more studies for long-term side-effects in children.

PB1340 | Clinical and Laboratory Factors Affecting Chronicity in Children Diagnosed with Immune Thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is one of the most common hematologic disorders in childhood. Approximately 20-25% of patients with newly diagnosed ITP become chronic.

Aims: We aimed to investigate the risk factors that may affect chronicity of ITP.

Methods: A total of 75 pediatric patients with ITP who were admitted to the Pediatric Hematology Department of Pamukkale University Faculty of Medicine Hospital between January 2013 and July 2018 were included in the study. The patients' characteristics such as clinical features, laboratory results, treatment requirements and type of treatment were retrospectively evaluated using the hospital′s information system.

Results: 46 (79.3%) of the 58 patients with ITP were acute and 12 (20.7%) of those were chronic. The mean age of the patients with acute ITP were 4 ± 3.84 years, while the mean age of the patient with chronic ITP were 9.6 ± 4.6 years. Statistical analysis revealed that children with a diagnosis age greater than 10 years had a significantly higher rate of chronicity. The dominant season in acute cases was spring with 34.8%, while in chronic cases it was summer with 50%. Acute cases were significantly higher in the spring season. 26 of 46 patients with acute ITP (56.5%) and 3 of 12 patients with chronic ITP (25%) were treated with IVIG firstly.

Conclusions: We determined that in newly diagnosed patients with ITP who are older 10 years of age and no history of infection may have high risk of chronicity, while the patients diagnosed in the spring and administered IVIG may have less chronicity.

PB1341 | Eltrombopag Suppresses Excessive Inflammation in Hepatitis B-Related Immunologic Thrombocytopenia through Inhibition of Toll-Like Receptor 4 Signaling Pathway

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Background: Thrombocytopenia is common among patients with hepatitis B virus (HBV) infection, limiting the anti-viral therapy. Eltrombopag (EP), a thrombopoietin receptor (TPO-R) agonist and potent iron chelator, is being evaluated in clinical trials for treatment of patients with hepatitis B-related immunologic thrombocytopenia (HBV-ITP). Pro-inflammatory S100A8/9 as danger-associated molecular patterns activate Toll-like receptor 4 (TLR4). Because monocytes have a critical role in development of both ITP and chronic hepatitis, we explored the contribution of monocyte subsets in control of inflammatory immunity in patients with HBV-ITP. How the activity of monocytes is regulated by EP to avoid excessive inflammatory response is not fully understood.

Aims: This study aims to assess the inhibitory effect of EP on TLR4 signaling, which contributes to diminishment of inflammation in monocytes, and elucidates the underlying mechanisms of EP as an iron chelator for the treatment of patients with HBV-ITP.

Methods: S100A8/9 levels in the plasma of HBV-ITP patients were analyzed by ELISA. EP inhibition of S100A8/9-induced TLR4 pathway activation and pro-inflammatory cytokine expression in monocytes sorted from human PBMCs were evaluated by Western Blot, qPCR, flow cytometry and ELISA. TLR4 signaling was also measured in murine bone marrow-derived monocytes after LPS and S100A8/9 stimulation. Reactive oxygen species (ROS) generation was observed by flow cytometry.

Results: S100A8/9 in the plasma of HBV-ITP patients showed a marked increase compared with healthy controls (Fig 1. a), and EP inhibited TLR4-triggered inflammation in human monocytes (Fig 1. b-d). Iron deprivation caused decreased expression of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) and repression of TLR4 pathway (p-ERK, p-JNK, p-STAT1) in monocytes from both human and mice (Fig. e-g). Moreover, our study demonstrated a reduction of ROS production after exogenous administration of EP (Fig 1. h).

Conclusions: These results reveal the iron chelation of EP in the treatment of HBV-ITP patients, and labile iron is a critical mediator of excessive inflammation and oxidative stress.

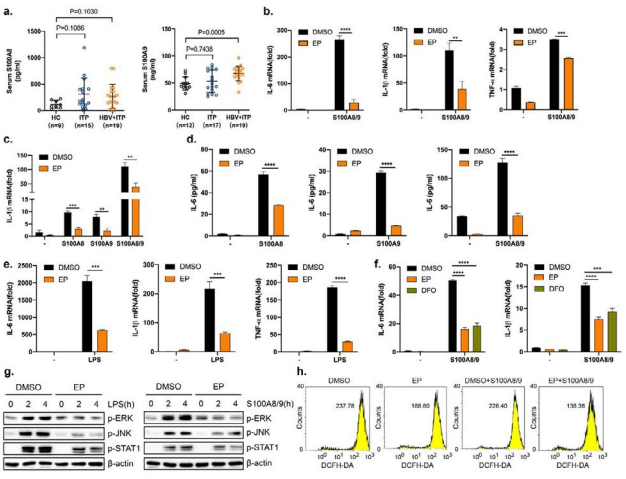

FIGURE 1 EP suppresses excessive inflammation in HBV-ITP through inhibition of TLR4 signaling pathway

PB1343 | Sustained Remission after Withdrawal of Thrombopoietin Receptor-Agonists in Immune Thrombocytopenia

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Background: Thrombopoietin receptor-agonists (TPO-RAs), eltrombopag and romiplostim, are effective in immune thrombocytopenia (ITP), resulting in 70-80% response rates if TPO-RAs are administered continuously. However, recent studies suggest that some patients can sustain their platelet response when treatment is withdrawn.

Aims: How many patients could maintain remission after withdrawal of thrombopoietin receptor-agonists in immune thrombocytopenia.

Methods: We retrospectively evaluated 39 adult primary ITP patients (24 women and 15 men, median age; 71 years) treated with TPO-RAs (28 with eltrombopag and 11 with romiplostim).

Results: Among the 27 patients who achieved complete remission (platelet count > $10x10^4$/mL), TPO-RAs was withdrawn in 19 patients. Of these 19 patients, 11 patients showed sustained complete remission after TPO-RAs withdrawal (6 with eltrombopag and 5 with romiplostim), with a median follow-up of 17 (range; 2-67) months. Age, gender, duration of ITP, previous therapies, platelet count, immature platelet fraction, serum TPO and the positive rates of anti-platelet glycoprotein antibodies before starting TPO-RAs did not significantly differ between the patient groups with and without sustained response after withdrawal of TPO-RAs. Doses of TPO-RAs, duration of TPO-RAs treatment, duration of complete remission and platelet count before TPO-RAs withdrawal did not also significantly differ between the two groups.

Conclusions: Eventually, no factors predicting sustained response after TPO-RAs withdrawal were identified. Nonetheless this study provides observational evidence that 28.2% of adult ITP patients treated with TPO-RAs had sustained complete remission after withdrawal of TPO-RAs, which may be a strategy for cure of ITP.

PB1344 | Risk Stratification for Intracranial Hemorrhage in Adults with Immune Thrombocytopenia: A Retrospective Multicenter Study

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Background: Intracranial hemorrhage (ICH) is a devastating complication of primary immune thrombocytopenia (ITP). Refined risk stratification is required for individualized prophylactic management. Advanced age has been recognized as a potential risk factor, yet there is limited information on ICH in adults under the age of 60, and no predictive tools are available in clinical practice.

Aims: To further identify the potential predictors for ICH and to explore strategies for risk stratification in adults under the age of 60 with primary ITP.

Methods: A nationwide survey was conducted to retrospectively identify ICH in adults with primary ITP from 2005 to 2019. For

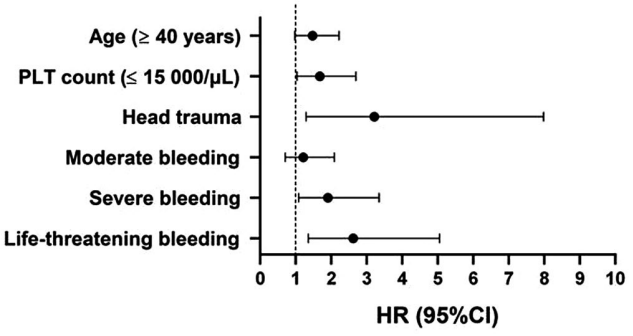

FIGURE 1 Multivariate analysis for ICH in primary ITP

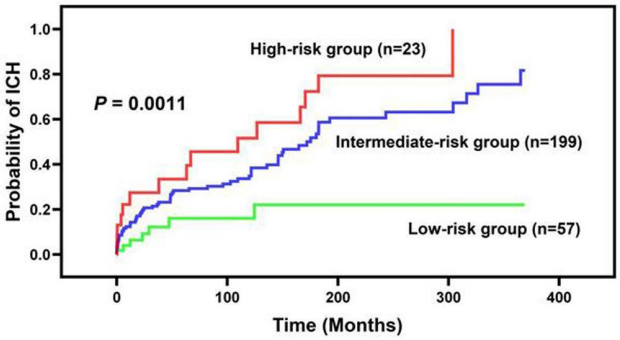

FIGURE 2 Risk stratification according to the predictive model

each case, two controls were provided by the same center and were matched by the time of ITP diagnosis and follow-up time. Both the cases and controls were under 60 years of age. Imaging test was required to confirm the diagnosis of ICH. Multivariate analysis was conducted using conditional logistic regression model.

Results: A total of 93 patients with primary ITP who developed ICH from February 2005 to August 2019 were identified by 27 centers in China. The median duration from ITP diagnosis to ICH onset was approximately 3 years (1098 days). Multivariate analysis identified head trauma, severe thrombocytopenia at ITP diagnosis (≤ 15 000/μL) and history of severe or life-threatening bleeding as independent risk factors (Figure 1). Advanced age (≥ 40 years) was also associated with ICH (multivariate *P* value = 0.063). The patients were further stratified into a low-risk group (without risk factors), an intermediate-risk group (1-2 risk factors) and a high-risk group (≥ 3 risk factors). The Kaplan-Meier estimations of ICH incidence were clearly different among the risk groups (Figure 2).

Conclusions: This study identified head trauma, severe thrombocytopenia at ITP diagnosis and previous severe or life-threatening bleeding as independent predictors for ICH in adults aged under 60 with primary ITP.

PB1345 | Factors Influencing Bleeding Severity in Adult Patients with Primary Immune Thrombocytopenia

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Background: Immune thrombocytopenia (ITP) is characterized by low platelet counts and associated with an increased bleeding risk. Still, bleeding severity in ITP patients differs individually and is not only determined by the platelet count.

Aims: To investigate bleeding severity and bleeding manifestations in a cohort of adult patients with primary ITP.

Methods: Patients with primary ITP were included in two haematological centers after written informed consent (EC 1843/2016). Bleeding severity was assessed with the ISTH ITP BAT (BS).

Results: Eighty-one patients (65.4% female) were included in the study (Tables 1 and 2). The median BS [IQR] was 1 [0-3], with the highest score in the category skin. The most common bleeding manifestations were petechiae (25.3%), bleeding from small wounds (24.1%), and menorrhagia in women (23.5% of women). The median BS [IQR] was higher in patients with a platelet count ≤ $50x10^9$ versus those with > $50x10^9$ (2 [1-7] and 1 [0-2]), in both, the categories "skin" (1 [0-3] and 0 [0-1]) and mucosal bleeding (1 [0-2] and 0 [0-0]). There was no difference in the BS according to sex, blood group O, chronic ITP, splenectomy status, or current ITP treatment. Of note, patients with bleeding symptoms at ITP onset had a higher BS compared to those without (2 [0-4] and 0 [0-1]). In a multivariable linear regression analysis, duration of disease (ß 0.009, 95%CI 0.001-0.016), and the immature platelet fraction (IPF, ß 0.185, 95%CI 0.043-0.327) were independent predictors of the BS, whereas the platelet count, sex, age, BMI and blood group O were not associated with bleeding severity in our cohort of primary ITP patients.

Conclusions: Bleeding severity in our cohort of ITP patients was generally low and predicted by IPF and the disease duration of ITP. This might indicate that ITP caused by high platelet destruction is more prone to bleeding.

TABLE 1 Patients' clinical characteristics

	n	n1	%
Female	81	53	65.4
Acute ITP	72	12	16.7
Chronic ITP	72	51	70.8
Current ITP treatment	78	32	41.0
Previous ITP treatment	78	56	71.8
Splenectomy	78	13	16.7
ITP BAT BS > 0	79	49	62.0
Bleeding at ITP onset	78	51	65.4
Blood group O	75	24	32.0

TABLE 2 Patients' clinical and laboratory characteristics. Data given in median [25-75 percentile

Age, years	40	30-55
BMI, kg/m2	25.4	22.9-29.7
Disease duration, months	59	10-128
Number of previous ITP treatments	2	1-3
ITP BAT BS total	1	0-3
ITP BAT BS Skin	1	0-2
ITP BAT BS Mucosa	0	0-1
ITP BAT BS Organ	0	0-0
Platelet count, x109/L	57.5	28.5-117.5
IPF, %	13.3	7.1-17.2

PB1346 | CD4+ T Cell Expression of IL-10 Compared to IL-17 is Lower in Patients with Immune Thrombocytopenia (ITP) Who Do Not Respond Clinically to High Dose Corticosteroid

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Background: High dose corticosteroids are the recommended first line treatment for adults with ITP, but side effects are common and responses heterogeneous with about 20-30% not responding at tolerable doses. However, currently, there is no way to predict response pre-treatment.

We previously demonstrated that $CD4^+$ T Cells from refractory ITP patients expressed a lower ratio of IL10:IL17 when treated ex vivo with glucocorticoids compared to responders (Blood (2017):130 (Supplement 1);2312).

Aims: To prospectively validate this finding in a cohort of newly diagnosed ITP patients receiving first line corticosteroids.

Methods: Patients with ITP recruited to the FLIGHT multicentre RCT (ISRCTN95606674) were offered inclusion into the laboratory sub-study. Following consent, peripheral blood was taken at randomisation. Here we present data from patients randomised to the corticosteroid only group (not receiving IVIG within 2 weeks of treatment initiation). $CD4^+$ T cells were isolated and examined by multiparameter flow cytometry pre-culture, and after 96 h culture with activation via the T cell receptor +/- dexamethasone. After 2 weeks of corticosteroid, clinical response was classified as complete response (CR):pl > $100x10^9$/L, partial response (PR):pl30-100 $x10^9$/L and no response (NR):pl < 30 $x10^9$/L.

Results: Blood samples were processed from 47 ITP patients in the first line corticosteroid only group who did not receive IVIG (27 male, 20 female, median age = 57y, range 17-87y, 24CR, 10PR, 13NR).

$CD4^+$ T cells from NR patients had reduced IL-10:IL-17 expression ratio than CR patients pre-culture (p = 0.0069; Figure 1A) and when cultured/activated in the presence of dexamethasone (p = 0.0359; Figure 1B). No significant changes were seen between response groups in the other cytokines examined (IL-4, IL-22, IFN-γ, TNF, GM-CSF).

Conclusions: These data support that $CD4^+$ T cells are important mediators of clinical response to corticosteroid treatment, and the cytokine signature of $CD4^+$ T cells may form the basis of a laboratory biomarker to help predict clinical response to corticosteroids for patients with ITP.

TABLE 1 Patient demographics

	Total (n=)	Male (n=)	Female (n=)	Median Age and range (years)
CR	24	12	12	58 (19-87)
PR	10	6	4	60 (31-75)
NR	13	9	4	52 (17-75)
Total	47	27	20	57 (17-87)

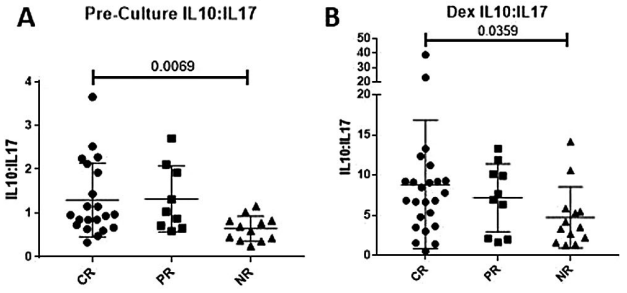

FIGURE 1 Ratio of IL-10 + to IL-17 + CD4 + T cells isolated from newly diagnosed ITP patients pre-culture (A) or after activation + dexamethasone (B)

PB1347 | Clinical Practise of SMOG System for Assessing Severity of Hemorrhagic Manifestations of Immume Thrombocytopenia (ITP) in Children

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Background: In clinical practice it is necessary to use reliable and convenient methods of assessing the severity of hemorrhagic manifestations in children with ITP in order to optimize therapy.

Aims: Analysis of own experience of using SMOG system for evaluation of severity of hemorrhagic syndrome in ITP in children.

Methods: A study on 50 ITP patients from hematology department of the City Children's Hospital 1 in Saint-Petersburg was conducted. The diagnosis was based on expert recommendations of the International Working Group (IWG) on ITP (2009). The severity of hemorrhagic syndrome was quantified according to SMOG parameters developed by experts of the IWG on ITP (2013).

Results: The severity of hemorrhagic syndrome was assessed on the first day of hospitalization. Skin hemorrhagic syndrome (petechiae, ecchymosis, subcutaneous hematomas, bleeding from minor wounds): the maximum S3 rate was noted in 26 (52%) patients, moderate S2 in 15 (30%), minimal S1 in 7 (14%) and absence S0 in 2 (4%) patients. Visible mucosae hemorrhage (epistaxis, gum bleeding, oral cavity, bleeding arter teeth extraction, subconjunctival hemorrhage): maximum M3 expression in 5 (10%) children, moderate M2 in 16 (32%), minimum M1 in 7 (14%), absence in 22 (44%) patients. Organ localization of bleeding was observed in 3 (6%) patients (O1) included hematemesis (patient reported episode) and microhematuria in 2 children (microscopic). Intracranial hemorrhage has not been detected. Clinically significant bleeding was diagnosed 31 (62%) patients, they were immediately given first-line therapy. Progression of hemorrhagic syndrome (11 patients) in the following days required

to start therapy. In 8 patients there was a dynamic observation on the principle "watch and wait."

Conclusions: The study on 50 patients proved that the SMOG system allows the evaluation of the severity of hemorrhagic syndrome to be unified and personalised. Simplicity, usability and adaptability to any phase of disease allow to use SMOG in routine clinical practice.

PB1348 | Platelet Count Variation in a Cohort of Patients with Chronic HCV-Related Liver Disease Treated with Direct-Acting Antivirals

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Background: Patients with HCV-related liver disease usually display thrombocytopenia. Direct Acting Antivirals decrease mortality and clinical complications in these patients. There are no data on the long-term effect of Direct Acting Antivirals on platelet count.

Aims: To evaluate the effects of Direct Acting Antivirals on platelet count of patients with chronic HCV-related liver disease.

Methods: 352 patients (60% men, median age 65 years, IQR 55 - 74) treated with Direct Acting Antivirals between January 2015 and December 2016 were included in this study (Table 1). Platelet count was monitored at the end of treatment and at 1, 2, 3 years follow-up. Data were expressed as means±standard deviation (SD) or medians with interquartile ranges (IQR) as appropriate and compared using tests for analysis of variance.

Results: Platelet count increased significantly at the end of treatment (Table 2) and remained higher than the baseline up to the 3 years of follow-up. The percent increase in platelet count was independent of the baseline platelet count, as it was similar in patients subdiveded in 4 categories of platelet count: normal platelet count (>150x109/L), mild thrombocytopenia (100-149x109/L), moderate thrombocytopenia (50-99x109/L), severe thrombocytopenia (0-49x109/L). In patients with moderate and severe thrombocytopenias the platelet count kept increasing during the 3 years of follow-up (Table 2, $p < 0.05$). At 3 years, 75% of patients with severe thrombocytopenia, 68% of patients with moderate thrombocytopenia and 70% of patients with mild thrombocytopenia switched to a higher platelet count category.

Conclusions: Therapy with Direct Acting Antivirals was associated with an increase in platelet count, which persisted for 3 years.

PB1349 | Response to Avatrombopag (AVA) in Chronic Immune Thrombocytopenia: Alternative Efficacy Measures

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Background: Immune thrombocytopenia (ITP) is an acquired bleeding disorder caused by immune-mediated platelet destruction and insufficient platelet production. AVA is an oral thrombopoietin receptor agonist (TPO-RA) approved for the treatment of chronic ITP when a patient has had insufficient response to a prior therapy. A better understanding of the efficacy profile for AVA will help clinicians make treatment decisions for their patients.

Aims: The objective of these analyses was to evaluate the efficacy of AVA treatment in ITP patients using alternative measurements that may be clinically meaningful.

Methods: A 6-month, multicenter, randomized, double-blind, Phase 3 study enrolled 32 AVA and 17 placebo-treated patients with ITP. The primary endpoint was the median number of cumulative weeks of platelet count (PC) response (achieving a PC ≥ 50,000/μL) over the course of the study without rescue medication. Patients receiving any rescue medication during the study were deemed to be non-responders for the remainder of the study. These post-hoc analyses evaluate the ability to achieve a PC of ≥ 50,000/μL once or twice during the course of the study and consecutive weeks of PC ≥ 50,000/μL.

Results: 87.5% of AVA versus 5.9% of placebo-treated patients attained a PC ≥ 50,000/μL at least once during the study. 81.3% of AVA-treated patients were able to achieve this PC response level at least twice in comparison to 0.0% with placebo. 53.3% of patients had a PC ≥ 50,000/μL for at least 4 consecutive weeks, as opposed to 0.0% with placebo. The mean number of weeks of continuous platelet response was 6.5 for AVA versus 0.1 for placebo-treated patients.

Conclusions: AVA effectively increases PCs in patients with ITP and the majority of treated patients achieved a sustained platelet response. Results of this analysis may be more meaningful for HCPs who understand that treatment of chronic ITP requires constant monitoring and dose modifications to manage PCs.

TABLE 1

Avatrombopag Responders Achieving a Platelet Count ≥50,000/μL

Outcome	AVA (N=32)	PBO (N=17)
At least once during the study, %	87.5%	5.9%
At least twice during the study, %	81.3%	0.0%
Consecutive # of weeks, Mean (SD)	6.5 (6.2)	0.1 (0.49)
For at least 4 consecutive weeks, %	53.1%	0.0%

AVA=Avatrombopag; PBO=Placebo

PB1350 | Pharmacokinetic/Phamacodynamic (PK/PD) Modeling Providing Guidance for Selecting Avatrombopag (AVA) Dose when Switching from Eltrombopag in Chronic Immune Thrombocytopenia (ITP)

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Background: Clinical management of ITP patients can be complex. Changing therapy is based on a variety of factors including efficacy, safety and compliance. AVA is approved for chronic ITP patients with insufficient response to previous treatments. AVA, taken with food, may be viewed as a viable switch option for patients who respond to eltrombopag (ELT) yet struggle with the requisite food restrictions. However, no dose switching guidance exists.
Aims: PK/PD simulations were conducted to inform clinicians on how to initiate a switch from ELT to AVA.
Methods: Population PK and PK/PD models were developed for AVA from 12 studies in healthy subjects and 4 studies in ITP patients. Similar models were previously conducted to simulate ELT concentration-time and platelet-time profiles (Gibiansky 2011, Hayes 2011).
The simulations used fixed daily dosing and included only responders to ELT. Switch simulations included daily dosing with ELT of 25 mg, 50 mg, or 75 mg with a switch to various AVA doses including 20 mg dosing once daily (QD) and three times a week (TIW); AVA dosing started 24 hours after the last ELT dose. These simulations identified regimens for AVA after switching from ELT to maximize platelet counts in the target range (50 to 200 × 10^9/L) and minimize those with decreases below 30 × 10^9/L.
Results: For the ELT to AVA switch, the simulations included 92 non-Asian ITP patients, which were replicated 100 times. Platelet-time profiles suggested AVA was more potent than ELT for a given dose amount per day. The data support initiating AVA 24 h post the last ELT dose. Modeled platelet response data will be presented at the meeting. Based on the model, Table 1 shows the recommended switching dose.
Conclusions: This modeling data provides useful dosing guidelines for AVA to maintain platelet counts in the target range for ITP patients when switching from ELT to AVA.

TABLE 1

PK/PD Model Suggested Dose Switching		
TPO-RA Dose	Start of AVA Dosing	AVA Dose
ELT 25 mg QD	24 hours after last ELT dose	20 mg QD or TIW
ELT 50 mg QD	24 hours after last ELT dose	20 mg QD or TIW
ELT 75 mg QD	24 hours after last ELT dose	20 mg QD

PK/PD = Pharmacokinetic/Phamacodynamic, ELT= eltrombopag; AVA=avatrombopag, QD=per day; TIW= three times per week

PB1351 | The Prevalence, Risk Factors, and Bleeding Tendency of Thrombocytopenia in Patients with Hepatitis C Virus and Hepatitis B Virus Infection

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Background: The prevalence and clinical characteristics of hepatitis C virus-associated immune thrombocytopenia (HCV-ITP) has been documented. However, there is paucity of study about hepatitis B virus associated thrombocytopenia (HBV-TP) and associated risk.
Aims: We conducted this study to clarify the risk factors for thrombocytopenia and the bleeding tendency in HCV-ITP and HBV-TP.
Methods: We retrospectively collected medical records of 2522 HCV and 2405 HBV patients to calculate the incidences of moderate and severe thrombocytopenia (platelet less than 100 × 10^9/L and

Figure 1

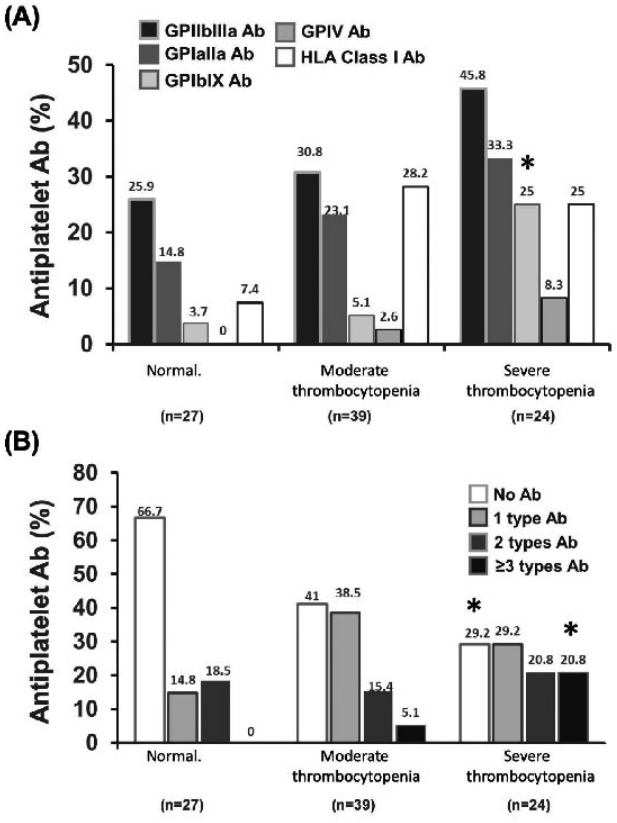

FIGURE 1

50 × 10^9/L) and associated major bleeding. Cox proportional hazard model was used to analyze risk factors for thrombocytopenia. We prospectively enrolled 63 HCV-ITP, 11 HBV-TP and 27 HCV control. We detected specific antiplatelet antibodies to glycoproteins by enzyme-linked immunosorbent assay and analyzed their impacts on thrombocytopenia. This study was approved by the Institutional Review Board in Chang Gung Memorial Hospital and performed according to Helsinki Declaration.

Results: The prevalence of thrombocytopenia less than 100 × 10^9/L were 14.51% and 8.78% in HCV and HBV cohorts, respectively. The HCV to HBV incidence rate ratio and hazard ratio of that were 3.108 and 1.737, respectively. The initial thrombocytopenia was most effective in HCV-ITP. The antiplatelet antibodies correlated to severity of HCV-ITP (Figure 1) and initial thrombocytopenia. Post-interferon therapy was protective for moderate HCV-ITP. But post-therapy and splenomegaly lose their effects in severe HCV-ITP. In contrast, post-nucleoside analogue therapy was consistent with cirrhosis and was the most effective in moderate HBV-TP. The effect of splenomegaly enhanced in severe HBV-TP. The incidence of gastrointestinal bleeding significantly increased in severe thrombocytopenia.

Conclusions: HCV is higher risk for thrombocytopenia than HBV. The clinical risk factors were different from viral hepatitis and severity of thrombocytopenia. The viral hepatitis is a bleeding risk and early intervention when platelet less than 50 × 10^9/L is recommended.

PB1352 | Pooled Safety Analysis of Avatrombopag (AVA) from Clinical Trials

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Background: Immune thrombocytopenia (ITP) is an acquired bleeding disorder that is often treated with thrombopoietin receptor agonists (TPO-RAs). Avatrombopag is an oral TPO-RA approved in patients with chronic ITP who have failed other treatments and those with chronic liver disease (CLD) prior to undergoing a scheduled procedure.

Aims: To evaluate the integrated safety profile of AVA across indications to inform clinicians as they make treatment decisions.

Methods: Group 1 is an integrated analysis of the pooled safety data from 4 ITP studies: two Phase 3, including a head to head study with eltrombopag (ELT), and two Phase 2. Group 2 included data from 9 (five Phase 2 and four Phase 3) studies in subjects with thrombocytopenia related to ITP or CLD, including hepatitis. Extent of exposure and safety profiles were evaluated.

Results: A total of 161 subjects, (128 subjects treated with AVA, 22 with placebo, and 11 with ELT) were evaluated in Group 1. 99.2% of subjects were treated with AVA for at least 7 days and 63.3% continued treatment for at least 180 days with an average duration of exposure of 206.4 days vs 73.5 for ELT and 54.9 with placebo. The incidence of overall treatment emergent adverse events (TEAEs) and grade 3 or 4 TEAEs was greater with AVA and ELT versus placebo but was comparable when adjusting for exposure (Table 1). Group 2 comprised 841 subjects, including 587 treated with AVA. Across the 587 subjects, the mean Duration of Exposure was 70.2 days, and the Total Number of Subject-Days of exposure was 41,183 days. 72% of AVA and 58% of placebo subjects experienced a TEAE.

Conclusions: When adjusted for exposure the rate of TEAEs was comparable between AVA and placebo. This analysis supports the safety profile of avatrombopag in the treatment of thrombocytopenia.

TABLE 1

Group 1 ITP Population			
Category	**AVA (N=128)**	**ELT (N=11)**	**PBO (N=22)**
Mean Days of Exposure (SD)	206.4 (135.6)	73.5 (70.5)	54.9 (47.2)
TEAE, %	97.7%	100%	63.6%
Exposure Adjusted	1.7	5.0	4.2
≥Grade 3 TEAE, %	35.2%	27.3%	0%
Exposure Adjusted	0.6	1.4	0.0

Exposure adjusted = Incidence per subject-year, SD = Standard Deviation, TEAE = Treatment Emergent Adverse Event, AVA = Avatrombopag, ELT = Eltrombopag, PBO = Placebo

PB1353 | Stability of Crushed Avatrombopag Tablets Demonstrated in Multiple Food Vehicles

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Background: Avatrombopag (AVA) is an oral thrombopoietin receptor agonist that is FDA approved for treatment of chronic immune thrombocytopenia (ITP) in adults with insufficient response to previous therapy. AVA is administered as a single tablet with food and does not interact with divalent cations. Stability data on crushed AVA is needed for patients unable to ingest whole tablets.

Aims: To evaluate the stability of crushed AVA mixed with food.

TABLE 1

Stability of Crushed Avatrombopag							
Vehicle	Metric	Control	Prep 1	Prep 2	Prep 3	Mean	% RSD
Yogurt	% LC Drug Recovery of	99.1	102.1	101.5	99.9	101.2	1.1
	% Total Impurities	0.05	0.06	0.05	0.05	0.05	---
Pudding	% LC Drug Recovery	99.1	98.3	91.6	97.8	95.9	3.9
	% Total Impurities	0.05	0.07	0.06	0.06	0.06	---

Yogurt=Dannon®; Pudding=Snack Pack®; LC=Label Claim; %RSD=Percent Relative Standard Deviation

Methods: AVA stability was evaluated crushed in either whole milk yogurt or chocolate pudding. Five 20 mg AVA film-coated tablets per studied sample were crushed to a fine powder, mixed with the food vehicle in triplicate and allowed to sit at ambient temperature conditions for 30 minutes. High performance liquid chromatography was performed, and percent drug recovery was calculated. Regulatory guidance for the use of liquids and/or soft foods as vehicles for drug product administration considers drug recovery within 5% of control to be acceptable.

Results: The mean results for the percent label claim of drug product recovered from each food tested were within 5% of control, thus meeting the regulatory guidance (Table 1). Each crushed preparation had equivalent impurity results to the control whole tablet, indicating negligible degradation. The percent label claim results for the samples exposed to yogurt were all within 3% of the control (mean = 102.1% drug recovery relative to control; relative standard deviation 1.1). The percent label claim results for the pudding samples had higher variability due to the viscosity of the pudding (mean = 96.8% drug recovery relative to control, relative standard deviation 3.9).

Conclusions: These results confirm that whole milk yogurt and chocolate pudding are effective vehicles for the delivery of crushed avatrombopag for up to 30 minutes at ambient temperature after mixing. This mode of delivery could be considered in patients with ITP who cannot ingest whole tablets.

PB1354 | The Significance of Platelet Factor 4 (Pf4 Antibody) in Diabetic and Non-Diabetic Population during Heparin Therapy In Pakistan

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Background: Heparin induced thrombocytopenia (HIT) is a life threatening complicationand requires highly priortize managment It is suggested by decrease in the number of platelet count during or shortly after heparin therapy by any route in the absence of other medical conditions responsible for thrombocytopenia.

Aims: To evaluate the significance of platelet factor 4 (PF4 antibody) in diabetic and non-diabetic population during heparin therapy at LUMHS Jamshoro Pakistan.

Methods: The cross sectional comparative study of one year was conducted at LUMHS Jamshoro. All the (diabetic and non diabetic Patients) already on heparin therapy for 1-7 days duration, due to any clinical disorder, of 18-70 years of age. The patients were observed for changes in the number of platelet count the day after the heparin therapy by advising (CBC) performed Sysmex XN1000 I japan. HBA1C were performed ARKRAY ADAMS A1C lite HA 8380V. Platelet Factor 4 antibodies were detected on ID PaGIA Heparin/PF4 antibody test. The data was analyzed in SPSS, the frequency and percentage was calculated for catagorical variables mean ± SD was calculated for numerical variables.

Results: Total 173 individuals were included in this study which consists of diabetic and non-diabetic population,The diabetic and non diabetic population documented as 80 (46.2%) and 93 (53.8%). The majority of the patients (71.6%) were in 30-59 year age group followed by age group 40-59 years). The male and female population were reported as 100 (57.8%)and73 (42.2%). The PF4 antibodies were identified in 63 (36.4%) individuals. The PF4 antibodies in context to age of the population was statistically significant (p-value 0.00) while the PF4 antibodies in context to thrombocytopenia was statistically significant (p-value 0.00). The PF4 antibodies in context to gender of the population were statistically significant (p-value 0.00).

Conclusions: Diabetes mellitus is associated with a higher likelihood of developing heparin induced antibodies and an increased combined incidence of arterial complications.

PB1355 | Eltrombopag Therapy in Pediatric Acute and Chronic Immune Thrombocytopenia

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Background: Eltrombopag is a thrombopoietin receptor agonist which is a new agent in the treatment of pediatric immune thrombocytopenia (IT).

Aims: In this retrospective report, efficacy and safety of eltrombopag in pediatric patients with acute and chronic IT were presented.

Methods: Eleven children with IT were treated with eltrombopag between October 2017 and January 2020. Patients who had at

TABLE 1 Clinical and laboratory features and respose to eltrombopag treatment in patients with acute and chronic immune thrombocytopenia

	Acute IT (n = 5)	Chronic IT (n = 6)
Age at diagnosis (years)	12.3 (5.1-12.9)	5.45 (1.5-12.5)
Median duration from diagnosis to eltrombopag therapy (month)	5 (1-5)	32.5 (13-81)
Median platelet count at initiation of eltrombopag (x109/L)	11.1 (6.0-24.6)	12.5 (5.3-25.1)
Median platelet count after 4 weeks of treatment (x109/L)	299.6 (32.0-374.0)	115.5 (7.9-461.0)
# non-responders	1	1
# responders	4	5
# patients with adverse effects	1 (depression)	2 (hemolytic uremic syndrome, hepatotoxicity)

least 4 weeks of eltrombopag treatment and follow-up data were included in the study. Bleeding symptoms and laboratory findings at diagnosis, treatment response and side effects were analysed. Platelet count > 50 × 10^9/L was defined as response.

Results: There were 5 patients with acute and 6 with chronic IT (F/M = 8/3). At presentation 7 patients had subcutaneous bleeding, 2 patients had menorrhagia, 1 patient had oral mucosa bleeding, and 1 patient had epistaxis. All patients received intravenous high-dose methyl prednisolon (30 mg/kg/day for three days) and intravenous immunoglobulin treatment before eltrombopag therapy and they were evaluated as non-responders. The initial eltrombopag treatment dose was 25 mg in 4 patients and 50 mg in 7 patients. Treatment dose was escalated to 75 mg in 4 patients. Treatment response was achieved in 9 patients (81.8%), whereas 2 patients were non-responders. Platelet count higher than 50 × 10^9/L was achieved in the 2nd, 3rd and 4th week of treatment in 2, 6 and 1 patient, respectively. Adverse events developed in 3 (27.2%) patients; in 2 of them eltrombopag withdrawal was required despite treatment response. These drug-related adverse events were elevation in aspartate aminotransferase, alanine aminotransferase, hyperbilirubinemia and hemolytic uremic syndrome. Treatment was discontinued between 3 and 9 months in 5 responders and response was stable. Two patients are still on treatment.

Conclusions: Eltrombopag is an effective treatment in selected pediatric patients with acute and chronic IT. Patients should be monitored closely for potential adverse events.

PB1356 | Immune Thrombocytopenia Treatment with Fostamatinib, a Spleen Tyrosine Kinase Inhibitor: Reducing the Risk of Thrombosis

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Background: Immune thrombocytopenia (ITP) is a bleeding disorder linked with an increased incidence of thromboembolic events (TEEs).[1] Additional TEE risk factors include age > 60 years, male sex, comorbidities, and certain ITP treatments (splenectomy and thrombopoietin receptor agonists [TPO-RAs]).[1,2]

Fostamatinib is an oral spleen tyrosine kinase (SYK) inhibitor that thwarts platelet destruction by blocking SYK signaling in macrophages. In an acute stroke model, SYK inhibition/depletion protected mice from aortic thrombosis and diminished brain infarction size without increasing hemorrhage risk.[3] SYK is required for GPVI and CLEC-2 receptor signaling in mouse and human platelets. Both receptors are involved in platelet activation and TEEs but are

TABLE 1 Incidence of TEEs in ITP registration and extension trials of TPO-RAs

Study Reference	No. of patients with events/ No. of patients	Incidence Rate	No. of Events	Type of TEE
TPO-RA Bussel 2009 Lancet. Phase 3 PC study	0/76	0%	0	None
TPO-RA Bussel 2009 Blood. Phase 3 single arm extension study	7/142	4.9%	12	Myocardial infarction, portal vein thrombosis, deep vein thrombosis (DVT), coronary artery occlusion, transverse sinus thrombosis, septic thrombophlebitis, transient ischemic attack
TPO-RA Cheng 2011 Lancet. Phase 3 PC study	3/135	2%	3	Pulmonary embolism, DVT, thrombophlebitis
TPO-RA Gernsheimer 2010 J Thromb Haemost. Phase 3 PC study and Extension study	2/84 (phase 3 PC) and 4/101 (extension)	2.4% (phase 3 PC) and 4% (extension)	2 (phase 3 PC) and 8 (extension)	Myocardial infarction, pulmonary embolism, venous thrombosis, coronary artery occlusion, cerebral ischemic attack
TPO-RA Janssens 2016 Ann Hem. Phase 4 open-label study	15/169	9%	21	DVT, pulmonary embolism, thrombosis, thrombophlebitis, cerebrovascular accident
TPO-RA Jurczak 2018 Br J Haematol. Phase 3 PC and extension studies	4/47	8.5%	4	DVT, asymptomatic pulmonary embolism and a cerebrovascular event
TPO-RA Kuter 2008 Lancet. Phase 3 PC study and Kuter 2013 Br J Hem Extension study	2/83 (phase 3 PC) and 19/291 (extension)	2.4% (phase 3 PC) and 6.5% (extension)	2 (phase 3 PC) and 25 (extension)	Popliteal artery thrombosis and cerebrovascular incident. Myocardial infarctions, neurological events and venous TEE events
TPO-RA Wong 2017 Blood. Phase 3 extension study	19/302	6%	24	Pulmonary embolism, DVT, myocardial infarctions, CNS ischemic events

nonessential for normal hemostasis in mice.[4,5] R406 (active metabolite of fostamatinib) inhibits GPVI and CLEC-2 signaling in human platelets in vitro.[6]

Aims: Review the incidence of TEEs in registration/extension trials for ITP.

Methods: We conducted a standardized MEDRA query (SMQ) search for TEEs in registration/extension ITP trials of fostamatinib and reviewed published reports of TEEs in registration/extension trials of TPO-RAs.

Results: 146 ITP patients were exposed to fostamatinib (163 patient-years). Patients with risk factors for TEEs included 37 (26%) > 65 years, 51 (35%) splenectomized, and 58 (40%) males. In fostamatinib phase 3 placebo-controlled (PC) and extension studies, 1 (0.7%) patient had a TEE (transient ischemic attack in a patient with pre-existing atherosclerosis, which resolved spontaneously). The incidence of TEEs for TPO-RA studies was up to 9% (Table 1).

Conclusions: These data suggest that SYK inhibition with fostamatinib, despite increasing the platelet counts, may decrease the risk of TEEs in ITP patients relative to what has been observed in TPO-RA studies

1. Rodeghiero, Am J Hematol 2016;**91**:39-45.
2. Ekstrand, Thromb Res 2019;**178**:124-131.
3. van Eeuwijk, Arterioscler Thromb Vasc Biol 2016;**36**:1247-53.
4. Andrews, J Blood Med 2014;5:59-68.
5. Payne, Blood. 2017;**129**(14):2013-2020.
6. Spalton, J Thromb Haemost 2009;**7**:1192-9.

PB1357 | Long Term Complications after Splenectomy in Chronic pITP Patients: A Retrospective Case Control Study

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Background: Splenectomy has always been considered a very efficacious surgical second line therapy for chronic primary Immune Thrombocytopenia (pITP), although bleedings, infections and thromboses can occur as short- and long-term complications. In light of the introduction of rituximab and TPO-receptor agonists, clinicians have reduced the use of splenectomy.

Aims: To evaluate splenectomy long-term complications in a large pITP cohort and to investigate their impact on this therapeutic choice in this era.

Methods: Patients were followed in our Center between 1955 and 2018. Data related to demographics, treatments, infections, thromboses and neoplasms were collected in a Microsoft Excel File and then processed with SPSS software.

Results: We enrolled 164 splenectomized patients and 1503 controls. Patients characteristics are showed in table 1. Among controls, 896 underwent at least 1 therapy line, 607 were only observed. Infections prevalence was significantly higher in splenectomized patients vs the whole control cohort (29.87% vs 10.84%; p 0.001) an the treated and observed subgroups (29.87% vs 12.95% or vs 7.74%; p 0.001) (table 2). Prevalence of thromboses was significantly higher in splenectomized cases compared to observed ones (5.4% vs 1.97%, p 0.015), while was not significant when compared to the whole control cohort or to treated patients (p 0.154 and p 0.412, respectively). Rate of neoplasms was significantly higher in splenectomized patients compared to observed cases (7.32% vs 2.96%, p 0.010) while was not significative compared to the whole control cohort or with treated patients (p 0.194 and 0.654, respectively).

Conclusions: Splenectomy presents a higher risk of infections. The rate of thromboses and neoplasms is not statistically different comparing splenectomized patients with treated ones, suggesting a role for medical therapies in such complications. Splenectomy still has a role in the therapeutic course of chronic ITP patients, but it is necessary a very good infections prophylaxis and monitoring.

TABLE 1

	Splenectomized (n.164)	Non splenectomized (n. 1503)
Sex	M = 45; F = 119	M = 534; F = 969
Median age at diagnosis, years	24 (2 - 64)	43 (0.5 - 93)
Median time between diagnosis and splenectomy, months	26.5 (0 - 312)	NA
Therapies	Before splenectomy 0 lines = 3 (1.83%); 1 line = 86 (52.44%); 2 lines = 46 (28.01); 3 lines = 20 (12.2%); more than 3 lines = 7 (4.3%); missed data = 2 (1.22%).	yes = 896; no = 607. 1 line = 586 (65.40%); 2 lines = 199 (22.20%); 3 lines = 74 (8.20%); more than 3 lines = 40 (4.20%)
	Post - splenectomy (57 relapsed patients) 0 lines = 3 (5.26%); 1 line = 29 (50.9%); 2 lines = 11 (19.3%); 3 lines = 8 (14.04%); more than 3 lines = 6 (10.5%).	

TABLE 2

	Infections	No infections	Total
Splenectomized	49 (29.87%)	115 (70.13%)	164
Treated, not splenectomized	116 (12.95%)	780 (87.05%)	896
Observed	47 (7.74%)	560 (92.26%)	607

PB1358 | Fostamatinib as Second-Line Therapy for ITP and in Earlier Stage ITP Patients

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Background: Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder characterized by platelet destruction. Autoantibodies bind to platelet antigens leading to platelet phagocytosis by Fcγ receptor bearing macrophages that requires spleen tyrosine kinase (SYK) signaling. Fostamatinib, an oral SYK inhibitor, was approved for the treatment of ITP based on phase 3 studies in adults with persistent and chronic ITP. As autoimmune diseases develop chronicity, they can become harder to manage due to longitudinal epitope spreading, increased autoantibody titer, and associated complement and T cell involvement. Early treatment of autoantibody-mediated diseases like ITP with drugs targeting corresponding pathways, such as Fcγ receptor driven phagocytosis, could produce better outcomes.

TABLE 1 Baseline Characteristics

Baseline Characteristic	Fostamatinib as second-line therapy (n = 32)	Fostamatinib exposure population (N = 145)
Age, median (range) in years	50 (20-88)	53 (20-88)
Duration of ITP, median (range) in years	2.7 (<1-50)	8.4 (<1 -53)
ITP classification (%) Persistent (<1 year)	5 (16%)	10 (7%)
ITP classification (%) Chronic (≥1 year)	27 (84%)	135 (93%)
Baseline platelet count, median (/μL)	21,500	16,000

All patients provided written informed consent prior to study and the study protocol was reviewed and approved by an independent ethics committee.

TABLE 2 Common adverse events for the 32 second-line therapy patients

Most Common AEs	Mild	Moderate	Severe	Total
Hypertension	6 (19%)	4 (13%)	0	10 (31%)
Diarrhea	3 (9%)	5 (16%)	0	8 (25%)
Upper respiratory tract infection	2 (6%)	3 (9%)	0	5 (16%)
Nausea	4 (13%)	0	0	4 (13%)
Vomiting	3 (9%)	1 (3%)	0	4 (13%)
Petechiae	3 (9%)	1 (3%)	0	4 (13%)
Headache	1 (3%)	3 (9%)	0	4 (13%)
Elevated alanine aminotransferase	3 (9%)	1 (3%)	0	4 (13%)
Elevated aspartate aminotransferase	3 (9%)	1 (3%)	0	4 (13%)

Aims: We conducted a post hoc analysis of the phase 3 studies to evaluate the response of patients who received fostamatinib as second-line therapy and those with disease < 2 years.

Methods: 145 adults with ITP received fostamatinib 100 mg BID which was increased to 150 mg BID after 4 weeks if platelets were < 50,000/μL. A platelet response was ≥ 1 platelet count ≥ 50,000/μL at any visit without rescue. Second-line therapy patients previously received steroids +/- immunoglobulins.

Results: Fostamatinib was second-line therapy for 32 patients (baseline characteristics in Table 1). A platelet response occurred in 25/32 (78%) patients compared with 47% of later line therapy patients. Adverse events (Table 2) were consistent with that of the overall study population.

A platelet response occurred in 9/10 (90%) patients with persistent ITP (< 1 year) and 11/19 (57%) patients with 1 to < 2 years ITP. The whole phase 3 population had a 54% platelet response rate.

Conclusions: Fostamatinib use as second-line therapy or in earlier stage disease for ITP may be more effective than as later-line therapy or in later stage disease.

PB1359 | Glibenclamide-Induced Thrombocytopenia: A Case Reort

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Background: Drug-induced thrombocytopenia is a common side-effect. Up to 25% of thrombocytopenia in critically patients are drug-induced.

Aims: The aim of this case is to recall the potentiel adverse effects of drugs in elderly patients.

Methods: We report a Glibenclamide-induced thrombocytopenia in an elderly woman.

Results: A 75-year-old woman was admitted for headache. She had mellitus diabetes, high blood pressure and coronary insufficiency. She was treated with Glibenclamide, Metformine, Aspirin, Molsidomine and Captopril. Physical examination showed global cardiac failure. She had no features of bleeding. Laboratory tests showed thrombocytopenia with a thrombocyte count at 42000/mm3, hemoglobin levels of 14 g/dL and leucocytes at 7910/mm3. Renal and liver fonction were within normal range. Abdominal ultrasound was normal. Hepatitis B and C and human immunodeficiency virus serologies were negative. Glibenclamide was withdrawn. Three days after the withdrawal of Glibenclamide, platelets count increased with normal levels at 174000/mm3.

Conclusions: The etiological analysis of Glibenclamide-induced thrombocytopenia is not obvious. Different mechanisms were uncertain including auto-immune disease, bone marrow failure and impaired platelet production. Severe bleeding can occur. Therefore, platelet count should be monitored when patient are treated with Glibenclamide.

PB1360 | Randomized Study for the Treatment of Primary Immune Thrombocytopenic Purpura (pITP) in Newly Diagnosed Untreated Adult Patients. Comparison of Standard Dose Prednisone versus High-dose Dexamethasone. Preliminary Results. GIMEMA Protocol ITP0207

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Background: Corticosteroids are the mostly used first-line treatment in adult patients (pts) with pITP, but the best initial approach to this disease is still matter of debate.

Aims: To evaluate therapy intensification with HD-DXM in newly diagnosed untreated adult pITP pts for response improvement at 6 months after initial response, in comparison with standard PDN doses.

Methods: Open label, randomized, multicentre phase III study: ARM A, PDN 1 mg/Kg/d p.o. for 4 consecutive weeks, then tapering within 14 days; ARM B, DXM, 40 mg/d p.o. for 4 consecutive days, every 14 days, for 3 consecutive courses. Initial response: ARM A, at day 42 from PDN start; ARM B, at day 46 from HD-DXM start. Final response: at day 180 from initial response for both arms. Response criteria: complete response (CR), platelet (plt) count ≥ 100x10^9/L; partial response, (PR), plt count > 50 < 100x10^9/L.; minimal response (MR), plt count > 20 < 50x10^9/L; no response (NR), plt count ≤ 20x10^9/L or > 20x10^9/L and < 50x10^9/L plus bleeding. Patients: untreated adult pITP pts (>18 < 80y), plt count < 20x10^9/L or > 20x10^9/L and < 50x10^9/L plus bleeding.

Results: Results are shown in table 1 and table 2. Initial response: difference between the 2 ARMS concerning overall responses is statistically significant (p = 0.0284). Final response: difference between ARM A and ARM B was not statistically significant (P = 0.8907). Persistent CR+PR+MR at 12 months from initial response: ARM A 80.65%; ARM B 58.33% (P = 0.0496). Considering the 49 responding pts, at 48 mos, 80.71% (95%CI: 68.65-94.89) alive in response, difference between the two ARMS was not statistically significant: ARM A 89.84% (95%CI: 77.34-100) vs. ARM B 70.41% (95%CI: 51.22-96.79) (p = 0.1069).

TABLE 1

Total number of pts	113
M/F	43/70
Median age (years)	45 (18.2-79.6)
Median plt count (x 10^9/L)	8.5 (1-48)
Median follow-up (months) (IQR)	46 (16.9-80.4)

TABLE 2

	ARM A	ARM B
Enrolled patients	59	54
Evaluable patients for initial response	56	49
Initial response CR+PR+MR	44/56 (78.57%)	46/49 (93.88%)
NR	12/56 (21.43%)	3/49 (6.12%)
Evaluable patients for final response	43	39
Final response CR+PR+MR	26/43 (60.47%)	23/39 (58.98%)
NR	12/43 (27.91%)	3/39 (7.69%)
Loss of response	5/43 (11.62%)	13/39 (33.33%)

Conclusions: Initial response is significantly better in ARM B, but this result is not confirmed at day 180 (p = 0.8907) and during follow-up. HD-DXM, indeed, seems to be able to induce a prompt initial response, reducing bleeding risk.

PB1361 | TOutcomes of Patients with Worsening Acquired Thrombotic Thrombocytopenic Purpura (TTP) Despite Daily Therapeutic Plasma Exchange (TPE) in the Phase 3 HERCULES Trial

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Background: TTP is a rare, life-threatening thrombotic microangiopathy. While daily TPE is the mainstay of therapy, some patients are refractory to treatment and acute mortality remains 10%-20% despite treatment (Scully, *Br J Haematol*. 2012). In the Phase 3 HERCULES trial, 3 placebo-treated patients met the definition of refractoriness versus none in the caplacizumab group (Scully, *NEJM*. 2019). There was a subgroup of patients with a complicated disease course despite daily TPE, without meeting the definition of refractoriness.

Aims: Describe outcomes of patients with suboptimal responses to TPE.

Methods: In this post hoc analysis of the HERCULES intent-to-treat population (caplacizumab: N = 72; placebo: N = 73), we identified patients with a suboptimal response to daily TPE, defined by decreasing platelet counts and increasing lactate dehydrogenase (LDH) after initial but nonsustained partial improvement of platelet count and LDH during daily TPE. Baseline disease characteristics (previous TTP episodes, platelet count, cardiac troponin, LDH, serum creatinine, disease severity) and treatment outcomes (time to platelet count response, time to stop daily TPE, death, exacerbation) were descriptively summarized.

Results: No patient in the caplacizumab group and 8 patients (11%) in the placebo group demonstrated a suboptimal response to TPE. Baseline disease characteristics were variable (Table).

Their median time to achieve platelet count response was 10.88 days and median time to stop daily TPE 13.5 days (ranges: see Table), compared with 2.88 and 7.00 days, respectively in the overall HERCULES placebo group. One patient had worsening TTP that led to coma and death. Subsequent exacerbations were reported in 5/8 patients (63%).

Conclusions: These results show the unpredictability of suboptimal responses to TPE, which can be fatal, and which were not observed in the caplacizumab group. This highlights the importance of the

TABLE Characterization of patients with a suboptimal response to TPE[a]

Patient (with TTP history)	Baseline Platelet count (10^9/L)	Baseline cTnI (µg/mL)	Baseline LDH(U/L)	Baseline Serum creatinine (µmol/L)	Baseline Disease severity[b]	Outcome Time to platelet count response (days)	Outcome Time to stop of daily TPE (days)	Outcome TTP-related death	Outcome Exacerbation
A-recurrent	9	7.025	1113	82	Very severe	22.37	23	No	No
B-initial	15	0.022	520	106	Less severe	11.97	13	No	Yes
C-recurrent	23	4.435	3343	211	Very severe	5.71	8	No	Yes
D-initial	14	0.213	338	145	Very severe	16.58	20	No	Yes
E-recurrent	82	<0.010	253	145	Less severe	6.75	9	No	No
F-recurrent	12	0.018	346	92	Less severe	18.79	21	No	Yes
G-initial	21	1.020	359	137	Very severe	6.07[c]	7	Yes	No
H-initial	30	NA	NA	NA	Less severe	9.79	14	No	Yes

cTnI, cardiac troponin I; LDH, lactate dehydrogenase; NA, not available; TE, thromboembolic event; TPE, tissue plasma exchange; ULN, upper limit of normal.[a]Suboptimal response to TPE defined as worsening platelet counts and LDH levels (after initial improvement) despite ongoing TPE.[b]Disease severity at baseline was defined as follows: (i) A French severity score ≥ 3, or (ii) severe neurological involvement (ie, coma, seizures, or focal deficit), or (iii) cardiac involvement (cTnI > 2.5 × ULN). The French severity score is a discrete risk score that ranges from 0 to 4 (with severity scores ≥ 3 indicating very severe disease) and involves evaluation of three parameters: Cerebral involvement: yes = 1, no = 0; lactate dehydrogenase: >10 × ULN = 1, ≤10 × ULN = 0; age: >60 years = 2, >40 and ≤ 60 years = 1, ≤40 years = 0.[c]Represents censored observation.

fast and sustained protective response observed with caplacizumab treatment in patients with aTTP.

PB1363 | Response Rate and Response Duration after Discontinuation of Treatment with Thrombopoietin Receptor Agonists (TPO-RAS) in Patients Affected by Primary Immune Thrombocytopenia (pITP): Retrospective Study. Preliminary Results. GIMEMA Protocol ITP0714

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Background: Eltrombopag (El) and Romiplostim (Rom) (TPO-RAs) represent second/third-line agents for pITP therapy with high success rate, but long-term treatment is generally required, because platelet (plt) level return to baseline within 2 weeks after discontinuation. However, literature data indicate the possibility of maintaining response after stop of both drugs.

Aims: To evaluate sustained response rate after discontinuation of TPO-RAs, in persistent or chronic adult pITP patients (pts) > 18y who failed one or more therapy lines, including splenectomy.

Methods: Retrospective, observational, multicentre study. Definitions: Response (R): plt count ≥ 30x10^9/L and at least 2-fold increase baseline count and no bleeding; Complete Response (CR): plt count ≥ 100x10^9/L without bleeding, both in absence of concomitant medications and rescue therapy in the preceding 4 weeks; Sustained R (SR) and Sustained CR (SCR): responses assessed as the first plt count, at more than 4 weeks after discontinuation of TPO-RAs, that satisfy the response definitions.

Results: Characteristics of patients are reported in table 1 and table 2. Enrolment period 2015-2016. Responses evaluation in 139 pts: 69 pts (46.64%) achieved a CR, 41 a R (29.50%), 29 (20.86%) were unresponsive. Ninety pts suspended TPO-RAs (Rom 58, El 32), 34/90 (37.8%) in CR or R. Out of 31 evaluable pts, 29 obtained SR or SCR, (Rom 22/24; El 7/7) for a median time duration of 135.4 weeks (range 4.7-317.0).

TABLE 1

Eligible patients	147
Participating GIMEMA Centers	19
F/M	93/54
Median age, years	58 (17-83)
Median plt count at therapy start (x 10^9/L)	16 (1-134)
Romiplostim	86/147 (58.50%)
Eltrombopag	61/147 (41.50%)
Concomitant therapies (IvIg, Corticosteroids, other)	
Yes	96
No	51

TABLE 2

Median number of previous therapies	4 (1-8)
Splenectomised patients	50/147 (34.01%)
Reasons of TPO-RAs start:	
no response to splenectomy	8.70%
relapse after splenectomy	27.54%
contraindication to splenectomy and failure of one or more therapy lines	30.43%
refusal of splenectomy and failure of one or more therapy lines	23.19%
no eligibility to splenectomy	10.14%

Conclusions: 29/147 (19.72%) pts treated with TPO-RAs, reached a SR or SCR maintained for a median time of 2.1 years, in agreement with published data. Our results highlight that TPO-RAs therapy can be successfully suspended in a subset of pITP pts.

PB1364 | Comparison of Various Diagnostic Methods in Assessing Platelet Count in Patients with Immunotrombocytopenia

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Background: Immune thrombocytopenia (ITP) is a disease caused by the production of anti-platelet autoantibodies that destroys platelets. The basic method assessing PLT is peripheral blood morphology with EDTA or with citrate. The older is counting PLT under the microscope (FONIO), newer and more accurate is fluorescence method. The method still difficult to access but raising great hopes is the evaluation of PLT by flow cytometry.

Aims: The purpose of this study is to compare PLT using the various avaliable diagnostic methods.

Methods: PLT were assessed by five methods in adult patients with ITP (peripheral blood with EDTA and citrate, FONIO, fluorescence and immunofluorescence).

Results: 66 patients with newly diagnosed, previously untreated ITP were enrolled in the study. The median age at diagnosis was 56 years (+/-19) and 56% were men. The mean PLT in the EDTA method was 74 G/l (SD +/-43), with citrate 73 G/l (SD +/-46), fluorescent 76 G/l (SD +/-44), FONIO 103 G/l (SD +/-64), immunofluorescent 97 G/l (SD +/-65). EDTA method was compared with FONIO, citrate, fluorescent and immunofluorescence. The significant difference in PLT was observed in the results comparing the immunofluorescence method (53% +/-123%), followed by the FONIO method (51% +/-91%). No difference was observed when comparing the standard method with the fluorescence method and citrate morphology. PLT results obtained from the immunofluorescence method differed from the fluorescent method by 40% +/-78%. The obtained results were correlated with standard morphological platelet parameters (PDW, MPV, P-LCR), white and red blood cell counts, immature platelet fraction and mean fluorescence intensity.

Conclusions: Based on the results, the most reflective method of PLT is the immunofluorescence. Unfortunately, this expensive and labor-intensive method, should be reserved for selected patients. For more accurate knowledge larger group with various types of thrombocytopenia should be evaluated.

PB1365 | A Long Term Survival Chronic DIC with ITP Treated with Low Dose Steroid

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Background: It is common that unfractionated heparin (UFH) is subcutaneously used for chronic DIC cases such as abdominal aortic aneurism (AAA).

Aims: We report a long term survival chronic DIC with ITP treated with low dose steroid.

Methods: Case presentation: A ninety-six-old male who had a past history of asymptomatic cerebral infarction admitted to our hospital because of his marked low level of platelet of $14x10^9$/L. Laboratory data showed APTT of 30.1sec, PT-INR of 1.05, Fbg of 193.5 mg/dL, FDP of 37.3 μg/mL, DD of 16.7 μg/mL, PAIgG of 51.4 ng/10^7Plt, and Helicobacter pylori antigen of stool was negative. BM findings showed Mgk of 75/μL and mild dysplasia of 3 series of hematopoiesis. Clinical course: This case was diagnosed as chronic DIC complicated with ITP and administration of 20 mg of prednisolone (PSL) was started. One week later platelet count increased to $87x10^9$/L, and then gradually could tapered to 5 mg of PSL. More than 3 years later, platelet count was stable with $100\text{-}120x10^9$/L and no bleeding was observed in spite of sustained hypercoagulable laboratory data. Because of patient's super advanced age, he received no surgical treatment at all.

Results: Chronic DIC based on AAA often shows bleeding tendency and UFH is often subcutaneously used for it. Our case was diagnosed as DIC complicated ITP and was started with oral low dose PSL, which improved platelet count and bleeding after passing more than three years. It was thought that ITP treatment with PSL let platelet life extended and participated in the improvement of the platelet counts.

Conclusions: There is the need to search concurrence of ITP in the chronic DIC, and the case that low dose PSL is effective for a long term exist although there is no effect of PSL for DIC in general.

PB1366 | Artificial Neural Network for Prediction of Hemorrhagic Severity in Patients with Immune Thrombocytopenia Purpura

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Background: Predict the severity of new bleeding in patients affected by Immune Thrombocytopenia (ITP) is a challenge. There is no consensus on severity of new bleeding in these patients. Therefore, artificial neural networks (ANN) - relating clinical factors with consequences of ITP - may have great potential to assist in treatment.

Aims: To obtain an ANN model capable of predicting the severity of new bleeds based on blood count and patient history.

Methods: Data from 631 *C consultations* and 631 *C + 1 consultations* - of 118 patients with ITP were collected between 2007 and 2018. Twenty six clinical and laboratorial parameters were considered: sex, age, comorbidities (hypertension, diabetes, pregnancy, neoplasia, chemotherapy or radiotherapy, infection and/or fever, lupus, dyslipidemia, stroke and/or acute myocardial infarction, venous thromboembolism, others), hepatitis B, hepatitis C, HIV, ANF, anticardiolipin, lupus anticoagulant, hemoglobin, erythrocytes, leukocytes, platelets, mean platelet volume, bleeding presence and bleeding severity (between 1 and 5). All these variables considered in *C consultation* were used as ANN inputs. The bleeding severity in *C + 1 consultation* were used as ANN output. The data were randomly divided into train (70%), test (15%), and validation (15%) data. ANNs with the number of neurons from 5 to 30 were tested, trained with the Levenberg-Marquardt method, and minimizing the mean square error (MSE) between the clinical parameter and the calculated.

Results: The best result was obtained with a two hidden layers ANN, with 25 neurons in both layers. The correlation was 0.8854 (train),

1.0000 (test) and 0.9998 (validation). This model was able to predict severity of new bleeding in 99.21% of cases with a relative error less than 1%.

Conclusions: The results obtained show that ANN have great potential to assist in the treatment of ITP, and must be refined and validate in other group of patients to be used in clinical practice.

PB1367 | Possible Impact of Polymorphism of GSTM1 and GSTT1 Genes on Bleeding Tendency in Immune Thrombocytopenia

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Background: Immune thrombocytopenic purpura (ITP) is an autoimmune disorder. Besides platelets specific auto-antibodies the T-cells imbalance may contribute in occurrence of this disease. This imbalance may be influenced by polymorphism of glutathione S transferases as observed in other autoimmune diseases.

Aims: Current study was planned to evaluate the impact of polymorphisms of glutathione S transferases on T-cells disparity in ITP patients and its association with disease severity.

Methods: Seventy six, confirmed ITP patients and 76 healthy controls were enrolled in this study following declaration of Helsinki. Peripheral blood was collected and complete blood profile parameters were recorded using XN 1000. The helper (Th) and cytotoxic (Tc) cells were enumerated by flow-cytometric analysis on BD FACS Calibur. GSTM1 and GSTT1 genes polymorphism were detected by multiplex PCR with beta-globin gene as internal control. The significant difference between newly diagnosed/persistent ITP or chronic ITP and healthy control groups was determined by F-test using SPSS ver. 21.

Results: Significantly low hemoglobin levels, platelets count with high immature platelet fraction were detected in newly diagnosed/persistent and chronic/refractory ITP patients ($p = < 0.05$). GSTM1 deletion was the most frequent genotype observed (45.45%). The patients with normal GST genotypes had an average Th/Tc ratio of 1.4 with less frequent bleeding symptoms while those with homozygous deletion of both GSTM1 and GSTT1 had a significantly low Th/Tc ratio of 0.8 with frequently observed gum bleeding.

Conclusions: Higher Th counts in patients with normal GST genotype may have protective effect as expressed by lower bleeding tendency as compared to patients with dual GSTM1 and GSTT1 null deletion. The dual deletion may have possible detrimental effect on immune function and antioxidant status suggesting a potential benefit of presence of functional GSTM1 and GSTT1 enzymes in ITP patients.

PB1369 | A Subpopulation of ITP Patients Resembling Glanzmann Thrombasthenia Phenotype Revealed by Flow Cytometry Analysis of Platelet Intracellular Signalling

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Background: Immune thrombocytopenia (ITP) is an autoimmune condition characterized by isolated low platelet count, primarily induced by the loss of immune tolerance to the platelet glycoproteins. Different auto-antibodies may affect platelet function in addition to the platelet count. Distinguishing between the different ITP causes would facilitate the appropriate therapy selection.

Aims: Comparison of platelet calcium signalling, integrin activation and thrombus formation between ITP patients, Glanzmann thrombasthenia patients and healthy donors.

Methods: Whole hirudinated blood of healthy pediatric donors (CNT; n = 11) or pediatric patients with immune thrombocytopenia (ITP; n = 36) and Glanzmann thrombastenia (GT; n = 8) within 3 hours of collection was used in the experiments. Continues flow cytometry was used for characterization of calcium signalling and fibrinogen binding in quiescent platelets or in response to ADP, collagen-related peptide or TRAP. All numbers are given for ADP stimulation. Calcium levels are given in nM, fibrinogen binding is given in percentage of total integrin activation. Parallel-plate flow chambers were used for observation of thrombus formation. Numerical and statistical analysis was performed in Python 3.7.

Results: All ITP patients had increased basal platelet calcium concentrations (15 ± 5 (ITP) vs 8 ± 5 (CNT)). Clustering analysis of the flow cytometry revealed two subpopulations of ITP patients: the high fibrinogen binding (HFB) group with comparable to CNT fibrinogen binding and higher calcium mobilization (154 ± 27 (HFB) vs 92 ± 22 (CNT)), and the low fibrinogen binding (LFB) group with lower fibrinogen binding (8 ± 5 (LFB) vs 16 ± 3 (CNT)). GT platelets had calcium mobilization (81 ± 23), fibrinogen-binding (5.1 ± 0.3) and thrombus growth comparable to LFB. Two computational models of platelet intracellular signalling leading to integrin activation for HFB and LFB were developed.

Conclusions: The group of ITP patients with GT-like phenotype was revealed. This group is characterized by low integrin activation, high quiescent platelet calcium and thrombus growth impairment. The systems-biology analysis suggested weakened integrin activation for this population.

PB1370 | First Report of Rituximab Dependent Anti-platelet Antibodies as Cause of Acute Thrombocytopenia

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Background: Rituximab induced acute thrombocytopenia (RIAT) is a rare side effect characterised by severe thrombocytopenia within hours of first episode drug exposure. The mechanism remains unclear. We hypothesise that RIAT is immune-mediated secondary to drug-dependent auto-anti-platelet antibodies.
Aims: To clarify the mechanism of rituximab induced acute thrombocytopenia.
Methods: Serum was analysed from three patients with severe first dose rituximab infusion reaction who developed thrombocytopenia within 24 hours post infusion. Serum samples taken post rituximab exposure or control serum (1:25) were incubated with washed donor platelets in the absence and in the presence of 600 μg/ml of rituximab and analysed for anti-platelet antibody binding by flow cytometry using anti-human IgG-AF647. Control serum was pre-exposure serum when available or normal serum. Patients provided written consent and the study was approved by the local ethics committee.
Results: All patients had marginal zone lymphoma with bone marrow infiltration and splenomegaly. Thrombocytopenia nadir ranged from 5-60 x 10^9/L. Rituximab-dependent antibody binding on platelets was not seen with control serum. In the post-rituximab exposure serum samples, antibody binding was detected only in the presence of added rituximab.
Conclusions: Rituximab-dependent anti-platelet antibodies were identified in three patients with acute thrombocytopenia following first dose of rituximab. The rapidity of onset suggests peripheral destruction and the testing is consistent with immune mediated platelet clearance due to drug-dependent recognition of platelet antigen. To our knowledge, this is the first demonstration of rituximab dependent anti-platelet anti-bodies and suggests that the cause of the rapid thrombocytopenia in these cases was immune mediated.

PB1372 | Pattern of Central Nervous System Involvement an Emerging Allied Complications of Dengue Hemorrhagic Fever. A literature Review

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Background: Dengue an arbovirus disease has become an episodic epidemic in Pakistan since 2005.The Neurological manifestations < 1% 1 however this can be misleading as actual incidence of CNS manifestations has not yet been reported from developing countries.
Aims: This study was conducted to review the number of patients presented with different neurological manifestation of dengue in Pakistan.
Methods: This retrospective literature review was structured by compiling data of dengue infected patients presenting with different neurological complaints i.e, headache, confusion, seizures, intracranial bleeds,stoke and hematoma (CT/MRI diagnosed) in organized questinare. Data available from ICU, general surgery and neurosurgery department of government and private tertiary healthcare centers were compiled from 2011 to 2019.All these patients were multiple transfused with RCC,FFP and platelet concentrates.
Results: We divided our study population (1582 patients) into 2 groups,first group consisted of 1458 (92%) patients that were managed conservatively. Platelets counts of these patients ranged from 12 to 52,000 cmm though we did not find any positive correlation between platelets count and CNS presentation (p value > .05). 36 patients (2.4%) died from group 1. While in second group 73 (4.6%) patients only 12 (16%) had emergency neurosurgery while 61 (83%) patients mostly elderly and children (significant p > .05) died as specialized treatment is expensive and not readily available at all hospitals that dealt with dengue epidemics in Pakistan.
Conclusions: Through experience dealing with dengue hemorrhagic fever has improved survival but the mortality still high in neurological affected patients as evident from data of epidemics. New national health policy for prevention as well as medical insurance is required according to changing world anticipated epidemics in coming years.

PB1374 | Characteristics and Outcome of Patients with Thrombocytopenia Secondary to Acute Cytomegalovirus Infection: Case Series and Literature Review

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Background: *Reports on cytomegalovirus (CMV)*-related thrombocytopenia in immunocompetent patients are scarce, possibly because CMV infection in healthy adults is usually asymptomatic. Here, we describe 5 new cases of CMV-related thrombocytopenia and review cases reported in the literature.
Aims: To describe characteristics and outcome of immunocompetent patients with acute CMV-related thrombocytopenia.
Methods: Clinical data were retrieved from the patients' files and merged with data reported in previous publications.
Results: Thirty-one immunocompetent patients (five new cases and 28 previously described) were identified. Their median age was 33 years (range 18-81), 71% were men, 77% presented with

TABLE 1 1st-line treatment and response

1st-line treatment	number of patients (%)	response rate n (%)
Steroid-containing regimen	27 (93)	9 (33)
Steroids only	19 (66)	5 (17)
Steroids + IVIG	4 (14)	3 (75)
Steroids + anti-CMV	1 (3)	1 (100)
IVIG only	2 (7)	1 (50)

CMV-related symptoms (e.g., malaise, fever), 48% had an enlarged spleen, 95% had atypical lymphocytes in peripheral blood, and 68% had an elevated transaminase level. Bone marrow examinations revealed increased or normal numbers of megakaryocytes in 96% of cases. Two patients had spontaneous resolution and 29 (94%) patients underwent treatment. The response rate to steroid-containing regimens (n = 27) and steroid monotherapy (n = 17) as 1st-line therapy was 34% and 17%, respectively. The response rate in polymerase chain reaction-proven cases was 2/15 (13%). The response rate to intravenous immunoglobulins (IVIG) was 29%. Five patients were treated with thrombopoietin (TPO) receptor agonists, with a 60% response rate. Ten patients were treated with ant-CMV therapy with a 80% response rate (table 1).

Conclusions: CMV should be considered in immunocompetent patients presenting with thrombocytopenia. Response rates to steroids and IVIG are lower than reported for immune-mediated thrombocytopenia unrelated to CMV infection. Anti-CMV therapy and TPO receptor agonists have better response rates.

PB1375 | Evaluation of Thrombocytopenia and Fetomaternal Outcome in Pregnancy at a Tertiary Care Hospital

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Background: Thrombocytopenia, a common hematologic abnormality during pregnancy, is associated with high incidence of preterm deliveries, fetal distress and intrauterine growth restriction. Moderate to severe maternal thrombocytopenia points to a higher degree of severity of the primary disease, which increases perinatal complications. Pregnant women with thrombocytopenia have a higher risk of bleeding during or after childbirth.

Aims: To determine the etiology and feto-maternal outcome of thrombocytopenia among pregnant women.

Methods: This prospective study was conducted at Department of Pathology together with the Department of Gynecology& Obstetrics, LUMHS Jamshoro from January to October 2019. 52 pregnant women, who were diagnosed with thrombocytopenia, were included in the study. All results were analyzed on statistical software SPSS version 20.

Results: Among the study subjects, 5.76% had mild thrombocytopenia while 75%, 7.79% and 11.5% were moderate, severe and very thrombocytopenic respectively. The mean maternal platelet count was 70879/μL with a range of 2000/μL to 102000/μL, whereas the mean neonatal platelet count was 159896 μL with a range of 72000/μL to 210000/μL. The most common cause of thrombocytopenia was gestational thrombocytopenia (50%) followed by nutritional deficiencies (21.15%) and ITP (15.4%).The common symptoms were petechie (21.15%) and bleeding seen in (13.46%) of the patients. The total incidence of fetal death was 5 (9.62%) and the causes identified were HELLP, malaria, in 1 (20%) patient each, abruptio placenta 2 (40%) while the no cause was identified in the remaining 1 patient. The maternal mortality too was 11.5% and the causes identified were HELLP, malaria, DIC and PPH.

Conclusions: In pregnancy with thrombocytopenia, gestational thrombocytopenia is the commonest benign condition which does not alter the obstetrical management. Still a vigilant monitoring should be kept on maternal platelet count in antenatal period to prevent unfavorable outcome in serious conditions that may require specific and urgent management.

PB1376 | Investigations of the Impact of Sialidase-mediated Changes on the Life Span of Human Platelets Using a Humanized *in vivo* Mouse Model

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Background: Immune thrombocytopenia (ITP) is a bleeding disorder due to multiple alterations of the immune system leading to low platelet count and bleeding. Thus, these data suggests that targeting desialylation might be potential approach to modulate bleeding tendency.

Aims: To preclinically verify the efficacy of sialidase inhibition on the clinical outcome in ITP, we aimed to establish a humanized mouse model to study and investigate antibody -mediated desialylation of human PLTs, with a focus on bleeding.

Methods: An antibody against murine CD42b was injected into NOD/SCID mice to depleted murine PLTs. After 24 hours, the % of circulating murine PLTs was determined by flow cytometry (FC) and human PLTs were injected in the tail vain with or without pre-incubation with a sialidase. The survival of human PLTs was then determined by FC. The site of PLT destruction was investigated by collecting cells from explanted lung, liver and spleen. Human PLTs in these organs were detected using a novel FC method.

Results: A drastic reduction of the murine PLTs in the mouse circulation (>95%) was observed after injection of CD42b antibody without affecting animal survival. Next, the human PLTs were injected in the

mouse tail vein. To investigate the impact of desialylation on PLT clearance, human PLTs were injected upon incubation with sialidase. Interestingly, a fast reduction of the PLT survival compared to control was observed (% human PLT survival after 2 hours, mean±standard error mean (SEM): 28 ± 5%, p value 0.019). Desialylated PLTs were mainly found in the liver. The bleeding time in the humanized mouse model was tested after murine PLTs depletion. In particular, a significant reduction of the bleeding time was observed after injection of human PLTs upon depletion.

Conclusions: Our finding suggests that the use of a humanized mouse model is a useful experimental approaches to investigate PLT-related bleeding diseases.

PB1377 | Antimicrobial Prophylaxis in Patients with Immune Thrombocytopenia Treated with Rituximab: A Retrospective Multicenter Analysis

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Background: In patients (PTs) affected by haematological neoplasms, therapy with anti-CD20 (Rituximab-RTX) increases the risk of viral and fungal infections. Antimicrobial prophylaxis (AP) is adopted in these PTs as well as in those with rheumatological diseases; evidences are lacking with reference to AP during RTX for relapsed/refractory immune thrombocytopenia (ITP).

Aims: We here report on a multicenter retrospective analysis of AP in patients with ITP treated with RTX.

Methods: Charts of adult ITP-PTs, treated with RTX from January 2008 to June 2018 were retrospectively reviewed, after IRB approval, for the following: demographic data, comorbidities, complete blood count (CBC), serum protein electrophoresis (SPEP),AP (type, duration, side effects), data on any infection with reference to onset, treatment and outcome. Data were analyzed with the software *Open source R*.

Results: Overall, we analyzed 53 patients (PTs) with ITP, treated with RTX in second (N=43) or third line (N=10). RTX was weekly administered at the dosage of 375mg/m^2 for 4 weeks in all cases. Overall, 47% (25/53) PTs received AP (Table 1). Reported infections were: Herpes Zoster Virus (N=2), pneumonia (N=1), cutaneous opportunistic infection (N=1), simultaneous infection by Herpes Simplex Virus and Candida Auris (N=1). At multivariate analysis, previous exposure to steroids for longer than 6 months and diabetes as a comorbidity were significantly associated with AP. Of note, the absolute neutrophil count (ANC) was statistically reduced (p=0.03), before and after a median of 32 days (range 21-90) after the last the RTX. One patient showed ANC < 500/mmc, not confirmed at the 6 months FU after RTX.

Conclusions: AP was administered in almost half (47%) of ITP-PTs. Prolonged corticosteroid exposure and/or diabetes, were judged as the most appropriate indications to AP. Subjects not under AP experienced more infections *vs* those treated with AP. Notably, a significant reduction of ANC after RTX was observed.

TABLE 1 Characteristics of patients

Patients (n°)	53
Age years,mean (range)	53 (18-86)
M-F (n°)	26 - 27
Follow-up after RTX, median (range), months	13 (6-62)
ANC, mean + SD (range) • Pre-RTX	6.4+3.2 (1.2-18)
• Post-RTX	5.2 +2.8 (0.5-14)
Comorbidities (n° pt) •Diabetes mellitus •Hypertension •Rheumatological diseases •Endocrine diseases •COPD	10. 22. 7. 8. 2.

PB1378 | Corticosteroid Reduction or Discontinuation after Initiation of Avatrombopag Treatment in Patients with Chronic Immune Thrombocytopenia (ITP)

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Background: ITP is an immune-mediated disease resulting in platelet destruction and diminished platelet production. Corticosteroids are often used as first line treatment for ITP. New societal guidelines and an international consensus statement (Neunert, 2019 and Provan, 2019) suggest limiting the utilization of corticosteroids in ITP patients due to untoward side effects and safety concerns. Ideally, second line treatment options in ITP would allow for dose reduction or discontinuation of steroids. Avatrombopag (AVA) is an oral thrombopoietin-receptor agonist approved for adult patients with chronic ITP who have failed a previous treatment.

Aims: The objective of these analyses was to characterize the reduction in baseline corticosteroid use in patients who received AVA for the treatment of chronic ITP.

Methods: A 6-month, multicenter, randomized, double-blind, phase 3 study enrolled 32 AVA and 17 placebo-treated patients with chronic ITP. Eligible patients could enter a 96-week open-label extension period following the core study. Stable doses of corticosteroids were allowed at baseline and continued use was

TABLE 1

Corticosteroid Reduction or Discontinuation with Avatrombopag Treatment		
	Avatrombopag (N=32)	Placebo (N=17)
Baseline corticosteroid, n	14	7
Core Study, n		
Reduction in dose	3	0
Discontinuation	2	0
Extension, n	Avatrombopag	Placebo → Avatrombopag
Reduction in dose	1	4
Discontinuation	0	0
Total	6/14 (42.9%)	4/7 (57.1%)

permitted. During weeks 6 to 18 of the core study and after the first 6 weeks of the extension, the investigator was allowed to decrease the dose and/or discontinue the use of corticosteroids in conjunction with study drug titration if needed. The proportion of patients able to reduce corticosteroid use without the need for rescue treatment during either the core or extension phase of the study was evaluated.

Results: At baseline, 14 AVA and 7 placebo-treated patients used baseline concomitant corticosteroid medication. Six patients originally randomized to AVA (42.9%) were able to either reduce or discontinue baseline concomitant corticosteroid. During the extension, patients who received placebo during the core study were switched to AVA, and 4 additional patients were subsequently able to reduce corticosteroid dose (57.1%).

Conclusions: Avatrombopag use allowed for the reduction or discontinuation of corticosteroids in ITP patients.

PB1379 | Monoclonal Antibody Immobilization of Megakaryocyte Antigens (MAIMA) Assay: A Novel Tool for Detecting Human Platelet Antigens

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Background: The monoclonal antibody immobilization of platelets (MAIPA) assay, developed by Kiefel et al., is the standard assay for the detection of auto-/alloantibodies in patients with thrombocytopenia. Labile glycoprotein that dissociates from older platelets require fresh platelets, especially to detect antibodies against antigens of the HPA-3 and HPA-15 systems. We established a potentially novel diagnostic tool based on the use of hematopoietic stem cell-derived megakaryocytes (MKs) instead of donor-derived platelets.

Aims: To develop a diagnostic tool, based on megakaryocytes, as alternative to the standard MAIPA assay for providing fresh megakaryocytes with consistent antibody expression.

Methods: CD34+ cells were magnetically isolated from buffy coat and differentiated for 28 days in vitro into MKs. We performed a flow cytometry analyses to characterize the MK cell lines (CD41, CD42a expression and DNA content). The alloantigens of MKs and platelets were determined using a MAIPA derived assay whit WHO standard sera containing antibodies directed against HPA-1a, -3a and -5b.

Results: Lower number of MKs was needed for MAIMA in comparison to the number of PLTs used in MAIPA ($100x10^3$ vs. $20x10^6$, respectively). Most importantly, the monoclonal antibody immobilization of megakaryocyte antigens (MAIMA) assay showed similar OD amplitudes compared to MAIPA when WHO reference reagents were used: Anti-HPA-1a (optical density [OD]: 1.35 vs 1.16), Anti-HPA-1a (minimum potency OD: 0.437 vs 0.543), Anti-HPA-3a (OD: 0.349 vs 0.284) and Anti-HPA-5b (OD: 0.134 vs 0.335), for PLT and MKs, respectively.

Conclusions: Our preliminary results demonstrate that the MAIMA assay has similar performance characteristics compared with MAIPA suggesting it as a suitable donor independent assay for laboratory investigations of alloimmunisation.

PB1381 | Prevalence and Clinical Significance of Abnormal T-cell Large Granular Lymphocytes in Patients with Immune Thrombocytopenia

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Background: T-cell Large Granular Lymphocyte (T-LGL) abnormalities have been associated with certain autoimmune diseases. The prevalence of abnormal T-LGL and the association with immune thrombocytopenia (ITP) is uncertain.

Aims: To determine the prevalence of abnormal T-LGL in patients with ITP and thrombocytopenic controls and to describe the association of abnormal T-LGL with ITP disease severity.

Methods: Retrospective study of peripheral blood or bone marrow flow cytometry (FC) of patients from the McMaster ITP Registry (2010 - 2019). Samples that presented increased CD3+/CD56+ cells or CD4:CD8 ratio< 0.5 or if specifically requested by the physician were tested for CD3+/CD16+/CD57+ T-LGL cells and total cells counts were reported. T-cell receptor (TCR) gene rearrangement by PCR was done if T-LGL counts>$2x10^9$/L or at the physician's discretion. Abnormal T-LGL was defined as cell counts >$0.3x10^9$/L or evidence of TCR clonality.

Results: FC results were available for 132 patients with ITP and 66 patients with non-immune thrombocytopenia (controls). From the ITP group, 12 (9%) patients presented abnormal T-LGL and 8 of them (66%) were tested for TCR clonality, all with a positive result. For the control group, 7 (10%) patients presented abnormal T-LGL and 2 of them were tested for TCR clonality, both with a positive result. ITP patients with abnormal T-LGL had lower nadir platelet counts than ITP with normal T-LGL (20 vs. 63 $x10^9$/L). ITP patients with abnormal T-LGL received a median of 7 ITP treatments, whereas ITP patients with normal LGL received a median of 2 ITP treatments. Four severe ITP patients with abnormal T-LGL were treated with methotrexate.

Of those, 1 had a reduction in T-LGL and none had an improvement in platelet counts levels.

Conclusions: Abnormal T-LGL occurred in 9% of patients with ITP and was associated with more severe ITP. T-LGL directed treatment with methotrexate did not improve platelet counts levels.

PB1382 | Management of Steroid Refractory Acute Immune Thrombocytopenia Purpura - A Single Center Experience from India

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Background: Optimal management of the patients with acute immune thrombocytopenia refractory to steroid therapy is not known. Immunosuppressive/immunomodulator drugs (azathioprine, MMF, rituximab, dapsone etc.) or even splenectomy have been used in past. Now a days Thrombopoietin (TPO) receptor agonists are being used in difficult to treat population. Here we report our experience of treating these patients in real world resource constraint setting from India

Aims: To evaluate treatment and outcome to steroid refractory severe immune thrombocytopenia patients.

Methods: Retrospective review of medical records of patients with ITP who did not respond to steroid after one week of therapy or who showed only transient response was done from January 2017 to December 2019. Outcome data was analyzed for 2nd line and subsequent treatment

Results: A total of 30 patients (male 14: female 16) were identified. Dapsone was our preferred second line of therapy in early part of the study period as it is easily available and inexpensive, was given to 11 patients and lead to a response in 6 (54%) patients. Eltrombopag was given to 16 patients with 12 (75%) response. Romiplostim was used in only 4 patients (along with azathioprine) as a generic version has become available lately (innovator drug is not yet available in India) and all responded. Azathioprine alone was used in 6 patients and 2 (33%) responded. Four patients did not respond to any of the second line therapy

Conclusions: Outcome to steroid refractory ITP patients have improved with the availability of TPO agonists (romiplostim and eltrombopag). About 80% patients will respond but relapse invariably happen on drug discontinuation. Older therapies like dapsone or azathioprine still had a role to play in a resource constraint setting, can be combined with TPO agonists and lasting responses can be seen in a subset of patients.

ANTIPLATELET THERAPY

PB1384 | Platelet Function Assay PFA-200 for Monitoring of Antiplatelet Drug Effect

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Background: The need for monitoring of the effect of antiplatelet drug and for prediction of hemorrhage in pre-surgical/perioperative patients lead to development of different platelet function testing (PFT) assays.

Aims: To compare light transmission aggregometry (LTA) and PFA-200 system in monitoring of the antiplatelet treatment.

Methods: A total of 68 patients taking antiplatelet drugs for at least 6 weeks (45 patients with aspirin 100mg/day and 23 with clopidogrel 75mg/day) were tested with LTA and PFA-200 system in parallel. LTA assay measures from 0% to 100% platelet residual activity (RA). PFA-200 measures the prolongation of the closure time (CT) in seconds (s) (Col/EPI test > 150s = positive effect of aspirin; < 150s = no effect. PFA P2Y test > 106s = positive effect of clopidogrel; < 106s = no effect). Platelet count and hematocrit were within the normal range. Patients did not take other drugs that could impact the PFT result.

Results: LTA results for aspirin showed satisfactory drug effect in 77.7% (5%-30% RA), borderline effect in 17.7% (50-70% RA) and no effect in 4.4% of the patients. LTA results for clopidogrel showed satisfactory effect in 74% (5%-30% RA), borderline effect in 21.7% (50-70% RA) and no effect in 4.3% of the patients. According to the PFA-200 results, positive effect of aspirin
(190s-300s) and of clopidogrel (160s-300s) was observed in 77.7% and in 69.5% of the patients respectively; no effect was observed in 22.2% and in 30.4% of the patients respectively. In patients with borderline LTA result, no antiplatelet effect was registered by the PFA-200.

Conclusions: PFA-200 seems to be more suitable as a point-of-care tool for assessment of perioperative bleeding risk caused by a severe platelet dysfunction. LTA is laboratory standard to diagnose platelet function disorders and to monitor the effect of antiplatelet drugs.

PB1387 | Long-term Variation of Platelet Reactivity in High-Risk Patients on Antiplatelet Therapy after Percutaneous Coronary Intervention

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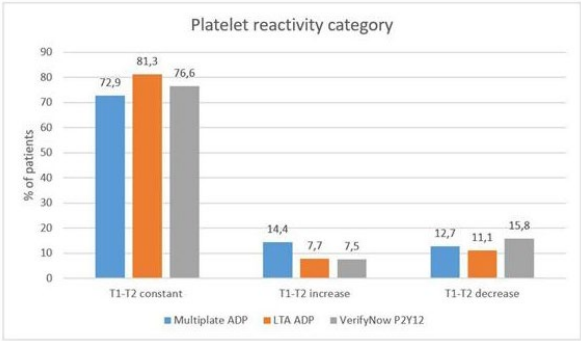
Background: Long-term stability of platelet reactivity has been prospectively demonstrated in small, non-high-risk populations on dual antiplatelet therapy (DAPT) with clopidogrel and aspirin.

Aims: Aim of our study was 1) to investigate whether platelet function testing (PFT) performed 1 month after percutaneous coronary intervention (PCI) remains stable for the total treatment duration with DAPT in a specifically high-risk, vulnerable patient group, and 2) to compare stability in 3 different, simultaneously measured PFTs.

Methods: In this prospective study 123 patients were included with ≥3 risk factors on DAPT consisting of clopidogrel (n=95), prasugrel (n=22) or ticagrelor (n=6) in combination with aspirin (n=106) and/or anticoagulants (n=19). Platelet reactivity was measured after a median of 45 [36-57] (T1) and 198 [184-215] (T2) days post-PCI, using three different PFTs; Multiplate ADP, light transmission aggregometry (LTA ADP) and VerifyNow P2Y12. According to consensus-based therapeutic windows (*JACC 2013*), patients were classified in categories of high, optimal or low on-treatment platelet reactivity (HTPR, OTPR and LTPR, respectively) at both time points.

Results: As shown in table 1, median platelet reactivity at T1 and T2 was stable when measured with Multiplate and LTA, whereas VerifyNow demonstrated a small decrease from 150.0 [69.0-207.3] to 140.5 [57.5-200.5] PRU (p=0.03). Figure 1 shows that 72.9-81.3% of patients were classified in the same platelet reactivity category at both time points, while their classification increased in 7.4-14.4% (e.g. from LTPR to OTPR) and decreased in 11.1-15.8% (e.g. from HTPR to OTRP).

Conclusions: In this high-risk population, platelet reactivity measured by Multiplate and LTA is stable from 45 to 198 days post-PCI, contrary to VerifyNow, which demonstrated a significant, but probably clinically irrelevant, decrease of 9.5 PRU (therapeutic window: 85-208 PRU). Thus, PFTs performed 1 month post-PCI could possibly assist in risk assessment of high-risk, vulnerable patients using DAPT, without the need for repetitive measurements.



T1-T2 constant indicating HTPR-HTPR, OTPR-OTRP or LTPR-LTPR. T1-T2 increase indicating LTPR-OTPR, LTPR-HTPR or OTPR-HTPR. T1-T2 decrease indicating HTPR-OTRP, HTRP-LTRP or OTRP-LTPR.
Abbreviations: LTRP, low on-treatment platelet reactivity; OTPR, optimal on-treatment platelet reactivity; HTPR, high on-treatment platelet reactivity; LTA ADP, light transmission aggregometry adenosine diphosphate

FIGURE 1 Distribution of patients switching between platelet reactivity categories between both time points as measured with 3 PFTs Click to previe

PB1388 | Synthesis of Antiplatelet *Ortho*-Carbonyl Hydroquinones: A Spiro Derivative is Selective against Collagen-Stimulated Platelets

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Background: Cardiovascular diseases are the leading cause of death in the world. Platelets have a major role in cardiovascular events as they bind to the damaged endothelium activating and forming thrombi.

Aims: The goal of this research was to evaluate the antiplatelet effect of a series of *ortho*-carbonyl hydroquinone derivatives performing cytotoxicity and platelet function studies.

Methods: Synthesis of acylhydroquinones. The already reported methodology of obtention of acyl hydroquinones was improved using microwave irradiation.

TABLE 1 Pairwise comparison of median residual platelet reactivity between T1 and T2 in three platelet function tests

Platelet function test	Platelet reactivity T1, median [IQR]	Platelet reactivity T2, median [IQR]	Pairwise comparison Wilcoxon Signed Rank test, p
Multiplate ADP (6.4 μmol/L) (AU)	47.0 [34.5-66.0]	47.5 [31.0-70.5]	0.34
LTA ADP (20 μmol/L) (% max. aggr.)	39.0 [25.5-48.6]	41.0 [27.0-49.0]	0.41
VerifyNow P2Y12 (20 μmol/L) (PRU)	150 [69.0-207.3]	140.5 [57.5-200.5]	0.03

Preparation of human platelets. Platelets were obtained from the human blood of healthy volunteers (two weeks drug-free) with written informed consent.
Cytotoxic activity. Platelets were analyzed with lactate dehydrogenase (LDH) cytotoxicity assay kit.
Platelet aggregation. Platelet aggregation was evaluated using a lumi-aggregometer.
Flow cytometry. Phosphatidylserine externalization, and platelet secretion (intracellular and extracellular ATP, and dense granules) and activation were evaluated by flow cytometry.
Mitochondrial bioenergetics. Platelet mitochondrial function was evaluated by mitochondrial membrane potential, NAD(P)H and calcium levels, and reactive oxygen species.
Results: Compounds **3** and **8** inhibit agonist-induced platelet aggregation in a non-competitive manner with IC_{50} values of 1.77±2.09 μM (collagen) and 11.88±4.59 μM (TRAP- 6) respectively and show no cytotoxicity. Studies evaluating the mechanism of action of these compounds were carried out showing that compounds **3** and **8** do not affect intracellular calcium levels and mitochondrial bioenergetics. Consistently, they reduce the expression of P-selectin, activation of GPIIb/IIIa, and release of adenosine triphosphate and CD63 from platelet granules.
Conclusions: In this work, we present a structure-activity relationship study on the inhibition of platelet activation by an *ortho*-carbonyl substituted hydroquinone series. Based on these findings, we identify compounds with an inhibitory effect on platelet activation. Notably, spiro derivatives of hydroquinones have high specificity toward platelet activation dependent on collagen induced-signaling. These findings may be used for further development of more effective drugs in platelet-related thrombosis diseases.

PB1389 | The Effect of Aspirin in Tumor Growth of Obese Mice Involving Inhibition of Metabolism

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Background: Aspirin is a widely used antiplatelet drug. Beyond its cardiovascular and cerebrovascular effects, epidemiological, clinical, and experimental evidence has shown that aspirin plays certain roles with regards to anticancer.
Aims: This study was conducted to identify any metabolic alterations in obese mice and to also investigate the anticancer effects of aspirin on obesity-driven cancer malignancy, by taking advantage of the syngeneic tumor model.
Methods: Using a syngeneic tumor model involving Lewis Lung Carcinoma (LLC) cells and C57BL/6 mice fed with a high fat diet, obesity was found to be associated with dysregulated glucose and glutamine metabolism, inflammation, along with platelet activation and the promotion of tumor growth.
Results: Tumor-bearing lowered glucose levels while moderately increasing inflammation, platelet activation, and glutamine levels. The antiplatelet drug aspirin, mitigated tumor growth in obese mice, paralleled by a decrease in systemic glucose, insulin, inflammation, platelet activation, glutamine and tumor expression of cell proliferation, aerobic glycolysis, glutaminolysis, platelets, and leukocyte molecules.
Conclusions: The anticancer effects of the antiplatelet drug aspirin are multifactorial. Glucose and glutamine metabolism are proposed targets for the anticancer effects of aspirin.

PB1390 | Desmopressin for Reversal of Antiplatelet Drugs in Stroke due to Haemorrhage (DASH): Interim Report on Recruitment from a Phase II Double Blind Randomised Controlled Trial

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Background: Intracerebral haemorrhage causes approximately 3 million deaths worldwide per year. Pre-stroke antiplatelet drug use is associated with increased risk of death or disability.
Aims: To assess the feasibility of administering desmopressin or placebo to patients with antiplatelet associated intracerebral haemorrhage.
Methods: In the ongoing DASH trial, we aim to include 50 patients with spontaneous antiplatelet drug associated intracerebral haemorrhage, within 24 hours of symptom onset (figure 1). This time window was initially set at 12 hours but was increased on 01 December 2019 to maximise recruitment. Patients with capacity are approached for informed consent. A personal or professional legal nominee is approached for consent for patients who lack capacity (approved by East Midlands - Nottingham 2 research ethics committee [18/EM/0184]). Patients are randomised (1:1) to receive intravenous desmopressin 20 micrograms or matching placebo. Feasibility

TABLE 1 Characteristics of participants in the DASH trial interim report

Number of patients randomised*N*	20
Age, years*Mean (standard deviation)*	76.9 (10.3)
Sex*Male (%)*	11 (55.0)
Pre-stroke modified Rankin Scale*Median [interquartile range]*	0 [0, 3]
Glasgow Coma Scale*Median [interquartile range]*	15 [13, 15]
Systolic blood pressure (mmHg)*Mean (standard deviation)*	160.7 (23.7)
Aspirin*N (%)*	14 (70.0)
Clopidogrel*N (%)*	9 (45.0)
History of Ischaemic stroke or transient ischaemic attack*N (%)*	5 (25.0)
History of ischaemic heart disease*N (%)*	10 (50.0)

outcomes include proportion of patients approached being randomised, number of patients receiving allocated treatment, rate of recruitment, and adherence to treatment and follow up. Secondary outcomes include change in intracerebral haemorrhage volume at 24 hours; early mortality < 28 days, death or dependency at day 90, and serious adverse events up to day 90.

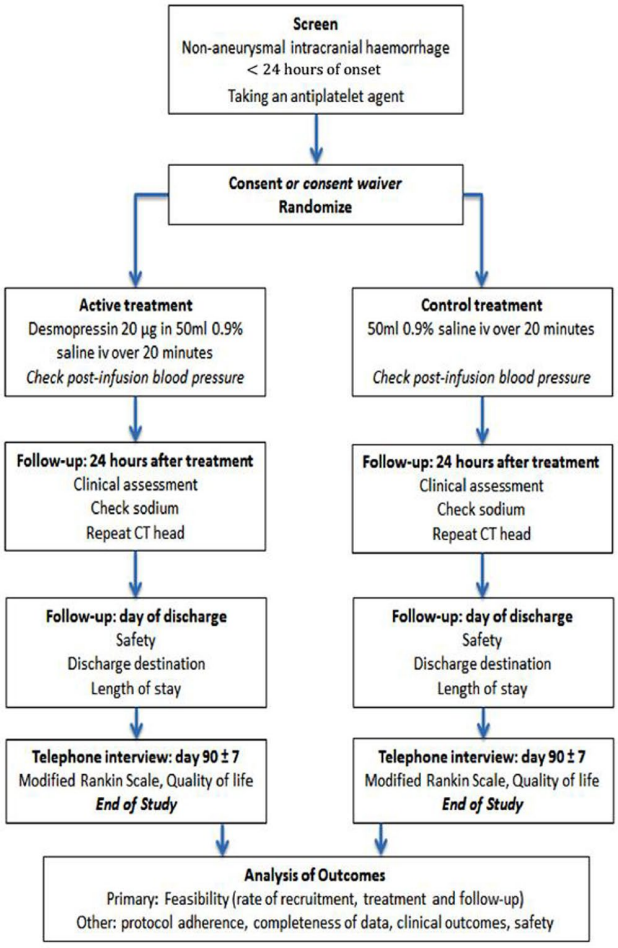

FIGURE 1 Trial summary

Results: 276 potential patients with intracerebral haemorrhage have been screened between 15 February 2019 and 28 January 2020 at 9 UK acute stroke centres. 20 patients (out of a planned 50 patients) have been recruited so far (table 1). Median time from symptom onset to administration of desmopressin or placebo was 5.1 hours (range 1.4 to 17.7 hours). The most common reasons patients were excluded were not being on an antiplatelet drug (133/256; 52%); presentation after the inclusion time window (38/256; 15%) or presentation at times when research staff were not available (29/256; 11%).

Conclusions: This is an ongoing feasibility trial, which will inform the design of a definitive trial.

Website: http://dash-1.ac.uk

PB1391 | Lysine-Containing Peptides as Promising Antiplatelet Drugs

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Background: The prothrombotic state of the organism is observed in a number of diseases, including metabolic syndrome (MS). One of the main mechanisms of hypercoagulation in MS is an increase in platelet activity, the formation of large hyperactive platelets, and an increase in platelet aggregation (PA). Reducing pathologically elevated PA plays an important role in decreasing the prothrombotic organism status. For these purposes, direct-acting drugs are used in clinical practice. We have previously shown that regulatory lysine-containing peptides have anticoagulant and antiplatelet properties in healthy rats.

Aims: To study the influence of new proline-containing peptides with the addition of the arginine and lysine to their structure on ADP-induced PA (ADP-PA) by development of MS in rats.

Methods: The experiments were carried out on Wistar rats according to ethical principles of the Helsinki Declaration. Experimental MS was induced by feeding the rats a fat-rich high-caloric diet (HCD) during 6 weeks. MS-rats were intranasally administrated peptides KKRRPGP, RKKRPGP and KRKPGP or saline (control) for 7 days by continuation of HCD. Blood for measuring of ADP-PA was collected from v.jugular 20 h and 7 days after the last peptides injection.

Results: The our results showed that the control MS-rats had increased ADP-PA (by 43% relative to norma). RKKRPGP and KRKPGP injection to MS-rats reduced ADP-PA by 30% and 22% compared with control MS-rats, KKRRPGP didn't change ADP-PA 20 h after peptide administration. After 7 days of peptides withdrawal the antiplatelet effect was preserved in KRKPGP, but it was not observed in KRKPGP. KKRRPGP reduces ADP-PA by 14% during this period. So, RKKRPGP administration leads to the maximum reduction in ADP-PA for a long period of time.

Conclusions: Thus lysine-containing peptides exhibit antiplatelet activity in rats with MS, reducing enhanced PA. Lysine-containing peptides can be used as promising antiplatelet drugs in prothrombotic conditions of the organism.

PB1392 | Efficacy of Antiplatelet Therapy in Patients with Acute Ischemic Stroke Treated with Acetylsalicylic Acid

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Background: Effective antiplatelet treatment is essential in secondary prevention of atherosclerotic stroke. As generally recommended, acetylsalicylic acid (ASA) in dose 100-325 mg daily is the main drug administered after thrombolysis to patients with acute ischemic stroke.
Aims: Systematic monitoring of onset ASA effect during the first four days after the loading dose 500 mg ASA intravenously and subsequent daily maintenance dose 100 mg ASA orally.
Methods: Evaluated a group of 23 patients admitted with acute ischemic stroke to the neurological department.
Blood samples for platelet function monitoring were drawn in four consecutive days. The first initial sample was drawn 24 hours after thrombolysis, immediately before the loading dose 500 mg ASA.
The second sample was taken 24 hours later and 3rd and 4th sample was taken after the maintenance doses. The samples were analyzed by multiple electrode aggregometry on Multiplate® Analyser (Roche) using ASPItest. Cut-off value for effective antiplatelet therapy was determined
≤30 U.
Results: The initial 500 mg loading dose of ASA induces effective antiplatelet therapy in 79% of patients 24 hours after drug administration. Three days later ASPItest values decreased in 100% of patients but only 52% accomplished effective antiplatelet therapy. 35% of patients did not achieved effective therapy but their values were close to cut-off (30-37 U). Results of remaining 3 patients (13%) were in the range 42-66 U.
Conclusions: We can demonstrate high efficacy of the loading dose 500 mg ASA intravenously 24 hours after thrombolysis in patients with acute ischemic stroke.
According to our analysis, 35% of patients would benefit from an ASA maintenance dose higher than 100 mg daily.
Supported by: MH CZ - DRO (University Hospital Pilsen - FNPl, 00669806):

PB1393 | Detection of Clopidogrel Resistance: Comparison of Light Transmission Aggregometry and INNOVANCE® PFA P2Y Cartridge and Correlation with CYP2C19 Variants

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Background: Platelet function tests allow identification of poor responders to the antiaggregant clopidogrel, who are exposed to a higher risk for cardiovascular events. Light transmission aggregometry (LTA) is regarded as the golden standard method, however, with several drawbacks that can be overcome by the INNOVANCE PFA P2Y test.
Aims: We evaluated the inter-assay variability between both tests to detect clopidogrel resistance.
Methods: Results of 106 patients on clopidogrel treatment were compared between LTA Chronolog 700 platelet aggregometry (Chronolog) and PFA P2Y performed on INNOVANCE® PFA-200 (Siemens Healthcare). Responsiveness to clopidogrel was defined as a peak aggregation < 50% and/or disaggregation >20% after adding 2.5 μM and/or 5 μM ADP for LTA and a closure time (CT) of >113 seconds for PFA P2Y. All patients classified as clopidogrel non-responders by LTA *were* genotyped for *CYP2C19*.
Results: For PFA P2Y, a clopidogrel effect was observed in 72% of the samples compared to 85% using LTA. In total, 18/106 (17%) discrepancies were reported between both methods. In 16/18 (89%) of discrepant cases, a normal CT was obtained with PFA P2Y, classifying a higher number of patients as clopidogrel resistant. In 2/18 (11%) cases, a prolonged CT was found with PFA P2Y, thus potentially missing clopidogrel resistance. One of these patients was a heterozygote carrier of the *CYP2C19*2* variant allele (intermediate clopidogrel metabolizer). Overall, our results showed a moderate comparability of PFA P2Y to LTA.
Conclusions: PFA P2Y is insufficiently sensitive to detect a clopidogrel effect compared to Chronolog 700 LTA and may additionally miss clopidogrel non-responding *CYP2C19*2* carriers.

PB1394 | Potent platelet inhibition results in improved long-term clinical outcome independent from clinical variables

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Background: Dual antiplatelet therapy with P2Y12- inhibitors and aspirin is a cornerstone treatment in patients with ACS, who

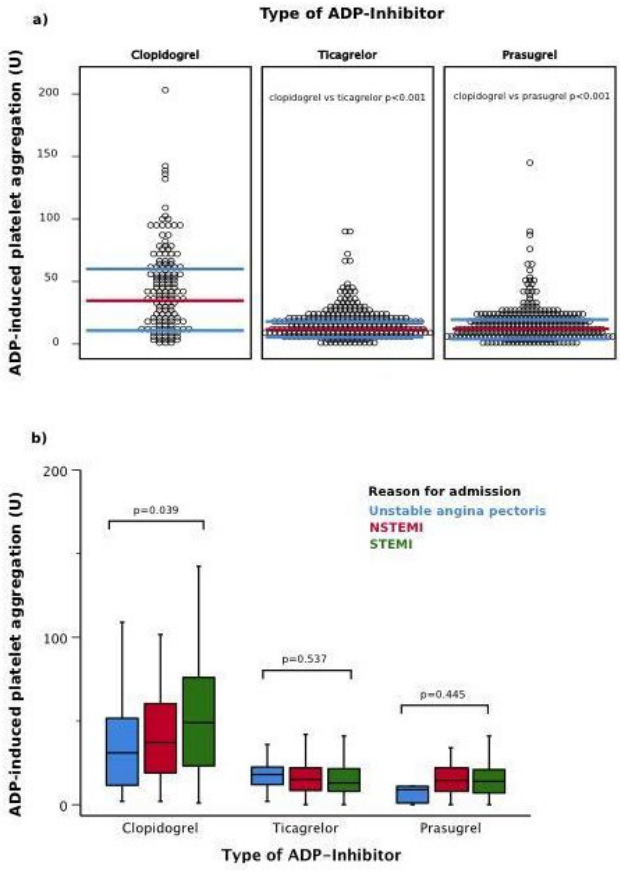

FIGURE 1 ADP-induced platelet aggregation assessed by MEA in relation to A) the type of ADP-inhibitor; and b) diagnosis at hospitalization

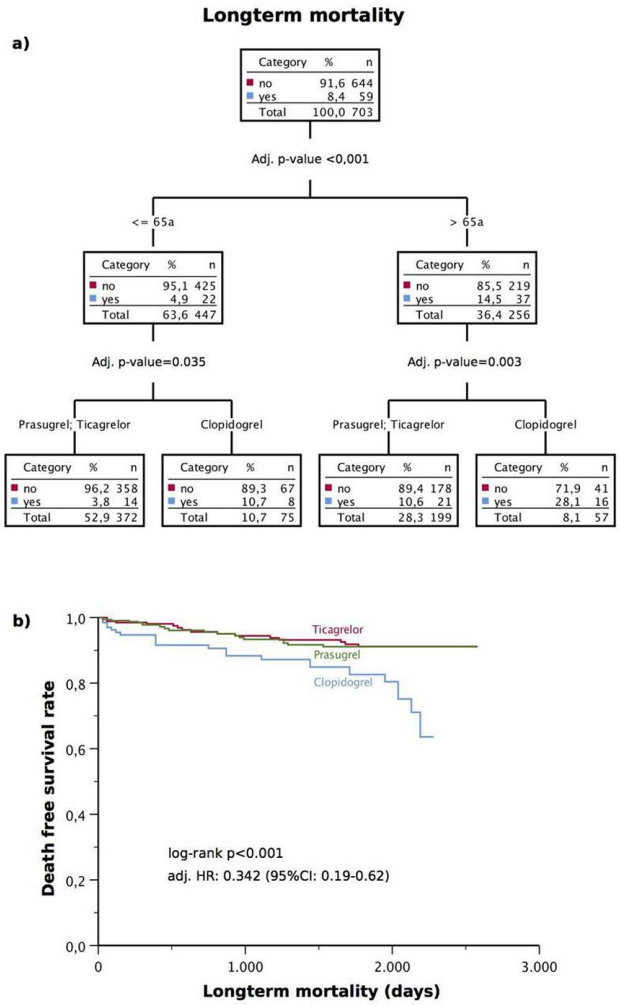

FIGURE 2 a) Chi-squared automatic interaction detection (CHAID) for predictors of long-term mortality b) Kaplan-Meier survival analysis for long-term mortality]

underwent percutaneous coronary intervention. Differences between clopidogrel and novel P2Y12-inhibitors (e.g. ticagrelor and prasugrel) are well-known.

Aims: To investigate whether dual antiplatelet therapy with potent P2Y12- inhibitors as compared to clopidogrel is associated with long-term clinical benefit.

Methods: In this prospective observational study we enrolled 708 patients with acute coronary syndrome (ACS) treated with clopidogrel (n=137), ticagrelor (n=260) or prasugrel (n=311). Major adverse cardiac events (MACE over 1 year) and long-term mortality (over 5 years) were assessed. Multiple Electrode Aggregometry (MEA) was used to define ADP (adenosine diphosphate) - and AA (arachidonic acid) - induced platelet aggregation.

Results: Type of ADP-inhibitor emerged as independent predictor of long-term mortality and MACE: patients treated with clopidogrel were at higher hazard to die within 5 years (adjusted HR=0.350; 95%CI: 0.20-0.61) or experience a MACE (adjusted HR=0.345; 95%CI: 0.20-0.59) as compared to the potent drugs prasugrel or ticagrelor. The effect of prasugrel and ticagrelor was homogenous independently from clinical risk factors. In contrast, clopidogrel effect decreased with increasing severity of ACS: patients with STEMI had 37% and with NSTEM 25% higher platelet aggregation as compared to patients with instable angina (p=0.039). Patients diagnosed with diabetes achieved less potent ADP and AA- induced platelet inhibition when treated with clopidogrel, compared to those without diabetes (p=0.045; p=0.030, respectively).

Conclusions: In the setting of ACS, treatment with ticagrelor or prasugrel is associated with an improved long-term mortality and short-term MACE as compared to clopidogrel.

PB1395 | Comparison of Platelet Function Testing in Patients Undergoing Neuroradiological Flow Diversion Stents

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Background: Patients scheduled for intracranial flow diversion shunts (Pipeline embolization device,PED) are at risk for

thromboembolic events (TEE) and in-stent thrombosis (ST) with potential catastrophic consequences and are routinely prescribed aspirin and ant-P2Y12 drugs. Ensuring the presence of inhibition of the P2Y12 receptors is of important but the choice of test remains enigmatic.

Aims: To compare the results of platelet function testing in patients taking anti-P2Y12 drugs prior to PED using whole blood impedance (WBI), light transmission aggregometry (LTA) and the VerifyNow device (VND)

Methods: Citrated whole blood samples were collected from patients prior to PED. All samples were studied within 3 hours. WBI and LTA were performed using diluted whole blood and Platelet rich plasma, respectively, at 5 mM ADP. Inhibition was defined as < 5 Ohms for WBI, less than 40% aggregation for LTA and less than 195 PRUs in the VND. Data were assessed for concordance (all tests in agreement) or discordance (difference in interpretation between tests).

Results: 99 patients had 113 tests performed, the overwhelming majority taking 75 mg clopidogrel daily. Concordance was observed in 77 /113 (70%) of which 57/77 (75%) showed inhibition and 20/77 (25%) showed resistance. All patients showing resistance had their anti-P2Y12 therapy altered and were retested. 36/113 (30%) patients showed discordant results between tests and decisions regarding therapy and retesting individualized. Correlation between test results were low despite statistical significant (WBI v VND, $r = 0.49$, $p < 0.01$; WBI v LTA, $r = 0.5$, $p < 0.01$; LTA v VND, $r = 0.49$, $p < 0.01$). All patients underwent PED procedures and there were no TEEs or ST.

Conclusions: Testing platelet function for P2Y12 resistance in the case of PED placement identifies resistant patients but using a single test may not be the optimal strategy

PB1397 | Ticagrelor Mechanism of Action on Gram-Positive Bacteria

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Background: Ticagrelor is an antiplatelet agent with both *in vitro* and *in vivo* bactericidal properties against gram-positive bacteria. The *in vitro* bactericidal activity of ticagrelor is direct, *i.e.* not mediated by platelets. Ticagrelor is the only antiplatelet drug with such dual action, which might make it superior to other available drugs through its ability to protect cardiovascular patients against thrombosis and potentially against gram-positive bacterial infections, *e.g.* infective endocarditis. While the mechanism of action of ticagrelor on platelets has been extensively studied, nothing is known about its direct action on gram-positive bacteria.

Aims: Our study aimed at unveiling the mechanism of action of ticagrelor against gram-positive bacteria.

Methods: A multistep mutant generation of *Staphylococcus epidermidis* was undertaken to isolate ticagrelor resistant clones. Whole Genome Sequencing (WGS) and comparative genomics analysis was performed on 10 clones and on wild type (wt) bacteria.

Membrane phospholipids of wt and mutant bacteria were analyzed by thin layer chromatography. Calcein-encapsulated unilamellar staphylococcus like-liposomes were prepared using the extrusion and ultracentrifugation method. Calcein release assay was performed to assess membrane bilayer disruption. Confocal microscopy was used to visualize ticagrelor effect on *Bacillus subtilis*, a gram-postive bacteria model.

Results: Three ticagrelor-resistant clones showed a non-synonymous mutation in *cdsA*, an essential gene for cell membrane phospholipid biosynthesis. Comparative lipid profiles of wt and resistant clones revealed increased cardiolipin content in the resistant clones compared to wt bacteria. In a calcein release assay ticagrelor was able to disrupt unilamellar staphylococcus like-liposomes. Confocal microscopy images of *Bacillus subtilis* treated with sub-inhibitory concentrations of ticagrelor showed a disruption of cytoplasmic membrane integrity already after 15 minutes of treatment.

Conclusions: Ticagrelor exerts bactericidal activity against gram-positive bacteria by disrupting their phospholipid membrane. As such, ticagrelor might also act as a sensitizing agent, by increasing the activity of other antibiotics.

PB1398 | Evaluation of Dual Antiplatelet Therapy Combining Aspirin and Clopidogrel for Intracranial Stenting Procedures: A Retrospective Single Center Study

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Background: Dual antiplatelet therapy (DAPT) with acethyl-salicic acid (ASA) and clopidogrel has been recommended in patients with cerebral aneurysms who are going to be submitted to endovascular procedures, to prevent thromboembolic events.

Resistance to APT is not rare. It may be assessed using point-of-care assays, allowing a change of dose or type of antiplatelet agents in order to achieve the desired levels.

Aims: To evaluate DAPT response in patients with cerebral aneurysms before intracranial stenting.

Methods: We retrospectively analyzed, from January 2015 to June 2019, DAPT response in 22 patients (18 females, median age 50.8 years-old) with cerebral aneurysms before intracranial stenting. Thrombotic or bleeding complications associated to the procedures were also evaluated.

To evaluate DAPT response, PFA-200 System (Col/EPI and Col/P2Y12 cartridges) was used in all patients, impedance aggregometry (ASPI and ADP tests) was used in 10 patients. Results were compared to decide which test should be used.

Results: AAS resistance was found in 3 (13.6%) patients and clopidogrel resistance in 11 (50%) patients. PFA and impedance aggregometry gave similar results when used concomitantly. Three hemorrhagic complications were observed within 24 h after intervention in patients without resistance to DAPT: 1 massive intracranial hemorrhage leading to death, 2 extracranial bleedings efficiently controlled. One patient resistant to clopidogrel had an ischemic stroke leading to mild neurological impairment.

In clopidogrel resistant group, 6 patients received higher doses of clopidogrel and clopidogrel was replaced with ticagrelor in 5 patients.

Conclusions: DAPT is used to prevent thromboembolic complications. In our small study, a high prevalence of clopidogrel resistance was found. Ticagrelor is a reversible P2Y12 inhibitor with predictable activity and rare low responders. Replacing clopidogrel with ticagrelor in all patients without DAPT response evaluation could be the strategy to follow.

PB1401 | The Effect of Plasma Fibrinogen Level on Predictive Value of VerifyNow P2Y12 Assay Results in Patients Undergoing Percutaneous Coronary Interventions

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Background: Observational studies demonstrated that high on-treatment platelet reactivity is associated with an increased risk for adverse cardiovascular events in patients (pts) after percutaneous coronary intervention (PCI). Earlier, in experiments *in vitro*, we have shown the inverse association of platelet reactivity (PRU) measured by the VerifyNow P2Y12 assay with plasma fibrinogen (Fg) levels and suggested a formula for correcting of Fg effect [Biochemistry (Moscow) 2016; 81: 439-44].

Aims: This study sought to examine whether adjusting for plasma Fg level improves the predictive value of PRU for ischemic events following PCI.

TABLE 1 Platelet reactivity and ischemic outcomes after PCI

	Without adverse events, n=276	Thrombotic events, n=18	Composite end point, n=41
Fibrinogen, g/l	3.39 [2.99-3.83]	3.58 [3.10-4.0], p=0.197	3.60 [3.19-3.90], p=0.090
PRU	175 [124-206]	192 [150-208], p=0.088	196 [149-223], p=0.061
Fg-PRU	182 [131-230]	217 [180-249], p=0.039	210 [172-252], p=0.010

Methods: A total of 317 pts (61±10 years) with stable coronary artery disease on dual antiplatelet therapy after elective PCI were enrolled. Platelet reactivity was measured using VerifyNow P2Y12 cartridges (Accumetrics, USA), and Fg (g/l) - by the clotting assay of Clauss. Fibrinogen-adjusted PRU (Fg-PRU) was calculated according to formula: Fg-PRU=PRU (measured) + 54* (patient Fg - 3.2).

Results: At 1-year follow-up thrombotic events occurred in 18 pts and 23 pts received repeat revascularization. Both measured PRU and Fg levels were numerically higher in pts with thrombotic events or composite end point than in those without adverse events, although the differences were not statistically significant (Table). Correcting PRU for plasma Fg significantly increased the magnitude of difference in PRU values between pts with and without adverse events. The ROC analysis showed that Fg-PRU levels significantly discriminated between patients with and without adverse events (AUC: 0.624; 95% CI: 0.534 to 0.715; p=0.01) and Fg-PRU value ≥199 was identified as the optimal cut-off to predict ischemic outcomes with sensitivity of 56.1% and specificity of 60.5%.

Conclusions: Correcting PRU for Fg, which itself is a risk factor for CVD, improves the value of the VerifyNow P2Y12 assay in identification of pts at high risk of ischemic events following PCI.

PB1402 | Discontinuation of Chronic Low Dose Acetylsalicylic Acid Therapy Upon Intensive Care Medicine Department Admission Appears to Increase Mortality

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TABLE 1 Baseline characteristics of patients with and without ICMD ASA therapy

Variable	No ASA n=114	ASA n=118	p
Age, years, median [IQR]	75 [17]	74 [17]	0.459
Gender, male n (%)	60 (52.6)	75 (63.6)	0.092
ICDM mortality n (%)	23 (20.2)	13 (11.0)	0.054
Mortality 90 days, n (%)	30 (26.3)	21 (17.8)	0.125
ICMD LOS, days, median [IQR]	3 [4]	3 [3]	0.169
qSOFA ≥2, n (%)	42 (36.8)	47 (39.8)	0.640
SAPSII score, median [IQR]	39 [21]	37 [18]	0.753
Max Lactate level, median [IQR]	2.05 [2.55]	1.99 [1.57]	0.359
Infection, n (%)	31 (32.5)	38 (32.2)	0.967

TABLE 2 Hazard ratios for mortality within 90 days using Cox proportional hazards regression

	Crude HR			Adjusted HR ζ		
Variable	HR	95% CI	p	HR	95% CI	p
Age	0.984	0.956-1.013	0.280	0.970	0.931-1.011	0.149
Gender, male	1.751	0.862-3.560	0.122	1.237	0.523-2.925	0.628
ASA during ICMD	0.520	0.264-1.027	0.060	0.391	0.170-0.898	0.027
SAPSII score	1.048	1.026-1.071	<0.0005	1.039	1.006-1.073	0.021
Maximum Lactate level	1.065	1.006-1.127	0.029	1.030	0.936-1.132	0.546
Infection	1.072	0.536-2.143	0.845	1.545	0.682-3.498	0.297
Unscheduled surgery	2.494	1.276-4.877	0.008	1.492	0.628-3.546	0.365

ζ The model included age, SAPSII score, maximum lactate level in first 24 hours, emergency department procedures (eg. central venous catheter placement, vasopressor therapy), non-programmed surgery and ICDM ASA therapy as independent variables.

Background: The role of platelets in hemostasis and thrombosis has long been studied. There's growing evidence of their importance in inflammatory and immunological responses to infection.

Critically ill patients with systemic inflammation and sepsis show platelet activation. Inhibition of platelet activation has been postulated to have a beneficial effect in critically ill patients.

Aims: Evaluate the correlation of chronic acetylsalicylic acid (ASA) therapy discontinuation upon intensive care medicine department (ICMD) admission and critically ill patients' mortality.

Methods: Retrospective, descriptive, single center cohort study performed during 2014, on a 65 bed ICMD. Adult patients with unscheduled admission through the ED and a prior record of chronic ASA therapy were included. Clinical variables at ICDM admission were extracted from the medical records. Primary outcomes were ICMD mortality and global mortality 90 days. Survival analysis and adjusted mortality with a cox regression was also calculated with a follow up time of 90 days. The study was approved by the local ethics committee with a waiver of informed consent.

Results: A total of 232 patients were included. Patient baseline characteristics can be seen in table 1. Table 2 shows the crude and Cox adjusted hazards ratio.

Patients that received ASA during ICDM had a lower crude ICMD and global mortality and a significantly lower model adjusted mortality (HR=0.391 p=0.027).

Conclusions: Upon ICMD admission, patients who have been discontinued from their chronic ASA therapy have higher crude and adjusted mortality. Some confounding variables may justify some of this difference in mortality.

Despite this, when admitting to an ICMD, the decision to suspend the patient's chronic therapy must be carefully considered.

PB1403 | Evaluation of Platelet Reactivity in Pediatric Patient on Dual Antiplatelet Therapy after Stent Implantation of Ductus Arteriosus: A Case Report

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Background: Antiplatelet therapy is commonly used in children to prevent thrombotic vascular complications. While numerous clinical trials in adults have performed for assessment of platelet reactivity on dual antiplatelet therapy (DAPT), less is known about the assessment of platelet reactivity on DAPT in children.

Aims: To evaluate platelet aggregation using MEA in pediatric patient after stent implantation of ductus arteriosus to confirm the antithrombotic efficacy.

Methods: We presented a case of 4-month baby with pulmonary valve atresia successfully managed with ductal stenting. A male baby with weight of 5.220 kg received clopidogrel 1 mg/d (0.2 mg/kg/d) and aspirin 30 mg/d (5 mg/kg/d) from 4 months. We performed assessment of antiplatelet therapy with Multiplate impedance aggregometry (MEA) by ADP and ASPI tests.

Results: We found optimal platelet inhibition at 4 months therapy: ADP-test 28 U (optimal inhibition at 15-45 U for adults) on clopidogrel therapy and ASPI- test 21 U (optimal inhibition at 4-30 U for adults) on aspirin therapy. Re-examination after 1 month showed also platelet inhibition in therapeutic range: ADP-test 20 U, ASPI-test 22 U. Follow-up treatment was without thrombotic or bleeding complications.

Conclusions: We evaluated platelet reactivity using MEA, based on our previous experience in adults groups to confirm the efficacy of antiplatelet therapy. Assessment of platelet inhibition is rarely performed in populations of neonates and children. Further validation in trials is necessary to determine the most appropriate threshold values for pediatric populations.

PB1404 | Circulating Myeloid-related Protein (MRP)-8/14 May Contribute to Suboptimal Response to Low Dose Aspirin in Patients with Type 2 Diabetes mellitus

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Background: Enhanced thromboxane (TX)-dependent platelet activation plays a pivotal role in atherothrombosis and type 2 diabetes mellitus (T2DM). Incomplete suppression by low-dose aspirin (ASA) of TX metabolite excretion (urinary 11-dehydro-TXB_2) is predictive of cardiovascular (CV) events in high-risk patients. Accelerated platelet turnover is one of the main determinants of the interindividual variability in ASA response, as reflected by accelerated platelet cyclooxygenase (COX-1) recovery during the 24-h dosing interval. Myeloid-related protein (MRP)-8/14 is a heterodimer secreted on activation of platelets, monocytes, and neutrophils, known to regulate inflammation and to predict CV events. Under hyperglycemic conditions, MRP 8/14 induces thrombopoietin (TPO) production and thrombocytosis.

Aims: To assess whether MRP8/14 may contribute to interindividual variability in response to low dose ASA in T2DM.

Methods: We enrolled 100 patients, in chronic treatment with ASA (100 mg day) for CV prevention. The kinetics of platelet COX-1 recovery was characterized by measuring serum TXB_2 after 10 (the nadir of ASA effect on TXB_2) and 24 hours after a witnessed ASA administration. The population was stratified into tertiles, based on the serum TXB_2 recovery slope. The third tertile indicated an incomplete suppression of COX-1 activity. Plasma MRP 8/14 was measured by enzyme-linked immunosorbent assays (ELISA).

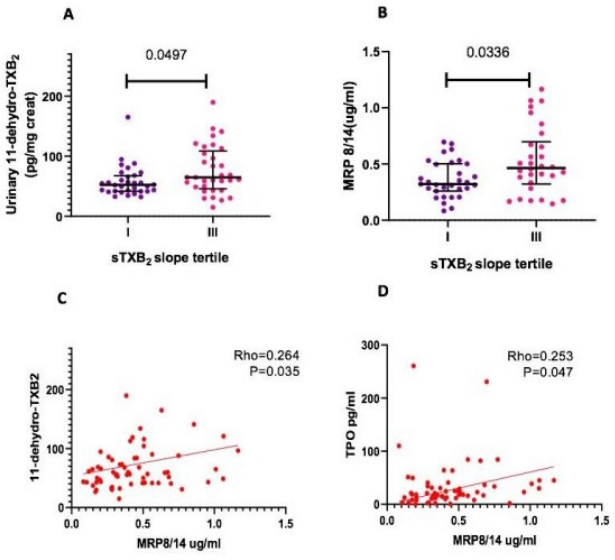

FIGURE 1 Panel A-D

Results: The levels of U-11-dehydro-TXB_2 and MRP 8/14 were higher in the third tertile versus first tertile of the $sTXB_2$ slope (p=0.0497 and p=0.0336) (figure, panel A and B); MRP 8/14 correlated with C-reactive protein (CRP) (rho=0.610, p< 0.001), white blood cells (rho= 0.460, p< 0.001), U-11-dehydro-TXB_2 (rho=0.264, p=0.035) and with thrombopoietin (TPO) (rho= 0.253, p=0.047) (Figure, panel C and D).

Conclusions: Enhanced circulating MRP 8/14 in the setting of low grade inflammation may stimulate TPO production from hepatocyte driving megakaryopoiesis, thus contributing to suboptimal response to ASA and residual TX-dependent platelet activation.

PB1406 | Correlation of VASP Phosphorylation Assay with Light Transmission Aggregometry for Patients Treated with Clopidogrel or Ticagrelor

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Background: High on-treatment platelet reactivity is a potential risk factor for adverse cardiac events in patients taking anti-P2Y12 drugs.

Aims: Correlation of VASP phosphorylation with light transmission aggregometry assays.

Methods: Whole blood samples from 20 individuals with no reported hemostasis disease and 21 patients treated with aspirin and either clopidogrel (n=11) or ticagrelor (n=10) were tested head-to-head by the consensus recommended platelet function tests (PFT) including vasodilator-stimulated phosphoprotein phosphorylation (VASP) and light transmission aggregometry (LTA) upon stimulation with 10 and 20 µM of adenosine diphosphate (ADP). All tests were performed using standardized procedures within 3 hours after sampling. Correlation between assays was tested using Microsoft Excel.

Results: The correlation between the VASP assay and LTA at 10µM ADP was high for samples from clopidogrel treated-patients (0.7-0.8), moderate for ticagrelor (0.5-0.6) and low for controls (< 0.2). When combining clopidogrel and ticagrelor samples together, the correlation raised above 0.8. For a 20µM ADP stimulation, the correlation between the VASP assay and LTA was >0.8 for clopidogrel samples; > 0.5 for ticagrelor and < 0.1 for control samples. Similarly, the correlation was found > 0.8 for samples from clopidogrel- and ticagrelor-treated patients.

Conclusions: Despite that the VASP assay and LTA upon 10 or 20µM ADP stimulation are correlated for patients treated with either clopidogrel or ticagrelor, the agreement among tests is higher (> 0.8) considering results obtained from both clopidogrel and ticagrelor altogether.

PB1407 | Intra-individual Residual Platelet Reactivity upon Antiplatelet Therapy in the Perioperative Period Significantly Differs over Time - What Is the Ideal Moment to Monitor Efficacy of Antiplatelet Therapy in Vascular Patients?

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Background: Antiplatelet therapy is the mainstay of thromboprophylaxis in patients with advanced atherosclerosis eligible for surgical intervention/endarterectomy. Up to 40% of patients are reported to have high on-treatment platelet reactivity, which is associated with an increased thrombosis risk and warrants a switch in antiplatelet therapy. However, little is known about intra-individual platelet responses to antiplatelet therapy over time, nor about the optimal timing of residual platelet reactivity measurements.

Aims: To determine the optimal moment for monitoring efficacy of P2Y12 inhibitor use by measuring residual platelet reactivity over time in individual patients undergoing carotid endarterectomy (CEA).

Methods: In a prospective, single-centre, observational study, we measured residual platelet reactivity of 50 vascular patients with the VerifyNow, VASP and an in-house developed flow cytometry-based platelet activation test prior to induction of anesthesia and 1 day, 5 days and >2.5 months postoperatively. Eligible subjects included adult patients scheduled for CEA without indication for post-operative intensive care admission, and treated with P2Y12 inhibitors. Presence of the *CYP2C19*2* or *CYP2C19*3* loss-of-function alleles or the *CYP2C19*17* gain-of-function allele were identified by *CYP2C19* genetic testing and associated to the measured efficacy of antiplatelet therapy. Informed consent was obtained and the study was approved by the local medical ethics committee.

Results: CEA resulted in a higher platelet response directly post intervention, despite P2Y12 inhibitor intake. First signs of platelet inhibition were observed 5 days postoperatively. Significant inhibition of platelet function was only observed after long-term use, i.e. >2.5 months post-CEA. Loss-of-function due to *CYP2C19*2* or *CYP2C19*3* alleles or gain-of-function due to the *CYP2C19*17* allele were leading in the response of patients to P2Y12 inhibitor use.

Conclusions: Patients undergoing CEA are not protected when P2Y12 inhibitor treatment is started just days prior to surgery, since antiplatelet therapy based on P2Y12 inhibition is only effectively inhibiting platelet reactivity after treatment for weeks.

PB1408 | Assessment of the Hemostatic System by Total Thrombus-formation Analysis System (T-TAS) in Patients with Chronic Coronary Syndromes

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Background: An analysis of the blood coagulation system in patients with cardiovascular diseases, including during antithrombotic and antiplatelet therapy, is an actual clinical task.

Aims: The aim of the study was to quantify the process of thrombus formation under conditions of blood flow by T-TAS technology in patients with chronic coronary syndrome (CCS) using antiplatelet therapy.

Methods: 140 measurements were performed using T-TAS on two type microchip flow-chamber system in a group of 54 patients with CCS treated 100 mg acetylsalicylic acid and 16 healthy controls: collagen-coated microchip PL and collagen+tissue factor-coated microchip AR for a functional assessment of the contribution and activation of platelets and plasma coagulation factors in the process of thrombus formation.

Results: The following dates (Me(Q1-Q3)) were obtained in CCS patients and controls for indicators measured on the AR microchip: AR T10=7.92 (5.32-8.88) sec; AR T80=10.69 (8.69-12.36) sec; AR AUC=1659.3 (1584.1-1795.6) versus AR T10=8.54 (8.0-9.09) sec; AR T80=10.64 (10.0-11.29) sec; AR AUC=1647.5 (1598.6-1696.3) and for PL microchip: PL T10=3.32 (2.40-4.54) sec; PL AUC=126.60 (69.4-233.8) versus PL T10=3.23 (3.16-4.37) sec; PL AUC=256.65 (17.4-301.9), respectively. The significant twofold decrease in PL AUC (p=0.02) should be noted, reflecting a decrease in the overall functional activity of platelets in CCS patients compared with healthy individuals due to the use of antiplatelet agents. However, 10 patients were aspirin-resistant and their T-TAS PL indicators were higher than in the controls and other CCS patients: PL T10=2.4 (1.9-2.7) sec; PL AUC=344.5 (292.8-388.0) versus PL T10=3.23 (3.16-4.37) sec; PL AUC=256.65 (17.4-301.9) and PL T10=4.3 (3.2-5.0) sec; PL AUC=120.1 (88.6-159.1), respectively (p< 0.05).

Conclusions: The value of PL AUC measured by T-TAS is a potential parameter for evaluation of the antiplatelet therapy effectiveness in patients with chronic coronary syndrome.

HIT

PB1409 | Evaluation of Fondaparinux (Arixtra®) at a Large Academic Medical Center

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Background: Fondaparinux is FDA-approved for prophylaxis of venous thromboembolism (VTE) in patients undergoing surgery as well as for the treatment of acute deep vein thrombosis and pulmonary embolism. Off-label, fondaparinux is used as an alternative to argatroban in the treatment of suspected or confirmed heparin-induced thrombocytopenia (HIT).

The Antithrombotic/Hemostatic Stewardship Committee at NYU Langone Health (NYULH) provides guidance for the safe and effective use of fondaparinux, while assuring that an evidenced-based and cost-effective approach for utilization is maintained.

Aims: To evaluate the utilization of fondaparinux at NYULH and to assess guideline adherence, efficacy, safety, and cost avoidance.

Methods: This was a retrospective review of adult patients who received fondaparinux between November 2016 and June 2019 at NYULH. The primary outcome was assessment of fondaparinux utilization based on the dosing guideline. Secondary outcomes included tolerability, safety, and cost-avoidance.

Results: Ninety-eight patients received fondaparinux, with the most frequent indications being suspected HIT (44%), VTE prophylaxis (23%), and continuation of home therapy (14%). Based on the NYULH fondaparinux dosing guideline, 97 (99%) patients were dosed appropriately. One patient (1%) received fondaparinux while on hemodialysis. Thromboembolic events occurred in 3 (3%) patients, major bleeding occurred in 4 (4%) patients and clinically relevant non-major bleeding occurred in 2 (2%) patients. In the 52 patients with suspected or confirmed HIT, the cost-avoidance of utilizing fondaparinux instead of argatroban was approximately $100,000.

Conclusions: The majority of fondaparinux utilization at NYULH is for suspected HIT, and dosing guidelines were followed in most cases. A low thromboembolic event rate with the use of fondaparinux was observed. The major and clinically relevant non-major bleeds experienced in six patients may be attributed to these patients' baseline high bleeding risk. Fondaparinux was a cost-effective alternative to argatroban in patients with suspected or confirmed HIT in this patient population.

PB1412 | Prospective Comparison of Two Specific IgG Immunoassays (HemosIL® AcuStar HIT IgG and HAT 45-G®) for the Biological Diagnosis of Heparin-Induced Thrombocytopenia: A Bayesian Approach

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Background: Accurate Diagnosis of Heparin-induced thrombocytopenia (HIT) is essential to ensure well adapted anticoagulant treatment, and first-line screening assays include rapid immunoassays (IAs) and enzyme-linked immunosorbent assays (ELISAs).

Aims: We evaluated performances of IgG PF4/polyvinylsulfonate (PVS) ELISA (HAT45®, Immucor) and HemosIL® Acustar HIT IgG (chemiluminescent assay) since both immunoassays use the same antigenic target.

Methods: Our prospective cohort included 184 consecutive patients with clinical suspicion of HIT with intermediate (n = 158) or high (n =26) clinical probability of HIT (4T score). 45 other patients with low probability were not further investigated. Definite HIT was confirmed when serotonin release assay (SRA) was positive.

Results: Definite HIT was diagnosed in 29 patients (12.6%). Sensitivity (Ss) and negative predictive value (NPV) of ELISA were excellent (100%), allowing to exclude HIT when the test was negative with post probability < 0.1%.

In contrast, Ss and NPV of Acustar HIT IgG were lower, i.e. 93.1% and 98.6% respectively, and the assay was negative in 2 definite HIT. Therefore, post-test probability remained significant (9.1%) in patients with high pre-test probability of HIT, when the Acustar was negative. Noticeably, a chemiluminescent signal greater than 10

TABLE 1 Performances of Acustar HIT IgG and ELISA PVS/PF4 IgG

		Acustar® IgG HIT		HAT45®IgG		Post test probability Acustar IgG HIT®		Post test probabilityHAT45®IgG	
Clinical probability	Pre-test probability	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
Intermediate (n = 158)	8.9% (14 definite HIT)	n = 138 (1 definite HIT)	n = 20 (13 definite HIT)	n = 130 (no HIT)	n = 28 (14 definite HIT)	0.7%	61%	≤0.01	48.6%
High (n = 26)	57.7% (15 definite HIT)	n = 10 (1 definite HIT)	n = 16 (14 definite HIT)	n = 9 (no HIT)	n = 17 (15 definite HIT)	9.1%	65.6%	≤0.01	93%
		NPV : 98.6% PPV: 75%		NPV : 100% PPV : 64.4%					

IU/ml was highly predictive of definite HIT (PPV 100%), but there was no correlation between quantitative values of Acustar and OD measured in ELISA (r = 0.4).

Conclusions: The AcuStar HIT® IgG, like all rapid immunoassays, is a good tool for excluding HIT only when the pretest clinical probability is intermediate. A strongly positive result is predictive of definite HIT, but additional studies are mandatory to propose an integrated approach using clinical probability and quantitative values obtained with this assay as previously proposed with ELISA (Cuker et al, Sem Thr Hem 2014).

PB1413 | Characterization of Murine Monoclonal Anti-PF4/Polyanion Antibodies, as Potential Useful Tools for Studies on Typical and Autoimmune HIT

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Background: Heparin-induced thrombocytopenia (HIT) is caused in most patients by platelet-activating antibodies (Abs) against platelet factor 4 (PF4) complexed with heparin (H). More rarely, HIT presents as an autoimmune disease, distinguished by platelet activation without heparin, and presence of both anti-PF4/H and anti-PF4 IgG (Nguyen *et al*. 2017). Recently, we developed murine monoclonal Abs with a human Fc, 1E12, 1C12, and 2E1, which mimic atypical HIT Abs, and we evaluated whether they could help us better understand the different HIT presentations.

Aims: To characterize 1E12, 1C12, and 2E1 versus two monoclonal anti-PF4/H IgG 5B9 and KKO, and polyclonal Abs from patients without (group-1), with typical (group-2) and auto-immune (group-3) HIT.

Methods: Interactions of Abs with PF4 and PF4/H were studied by ELISA, single molecule force spectroscopy, isothermal titration calorimetry and dynamic light scattering. Serotonin release assay and heparin-induced platelet activation assay were used to assess platelet activation induced by Abs. The binding sites of monoclonal Abs on PF4 were predicted *in-silico* (MAbTope method).

Results: 1C12, 1E12, and 2E1 displayed higher affinity for PF4/H than 5B9 and KKO, similar to human group-3 Abs compared to other groups. Only 1C12, 1E12, 2E1 and group-3 Abs interacted with native PF4 and formed in solution large complexes with PF4. They were also prompt to cluster PF4, as reflected by their ability to activate platelets without heparin. The predicted binding sites of 1C12, 1E12, and 2E1 on PF4 were close, but different to those of KKO and 5B9. However, Q9, P34 and P37, which are critical PF4 residues for the binding of human HIT Abs, 5B9 and KKO, appeared also involved in the binding of 1C12, 1E12, and 2E1.

Conclusions: The characteristics of 1C12, 1E12, and 2E1, similar to those of autoimmune HIT Abs, make them good tools for studying the pathophysiology of auto-immune HIT.

PB1414 | Autoimmune Heparin-Induced Thrombocytopenia Successfully Treated using Direct Oral Anticoagulant and Intra-Venous Immunoglobulin

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Background: Clinical auto-immune heparin-induced thrombocytopenia (aHIT) defines unusual syndrome such as persistent severe thrombocytopenia, and HIT-related complication including microvascular thrombosis and disseminated intravascular coagulation (DIC).

Aims: We describe the case of an aHIT where direct oral anticoagulant (DOAC) and intra-venous immunoglobulin (IVIg) were successfully used.

Methods: The patient is a 71-year-old man with comorbidities (hypertension, diabetes, coronary artery disease).

Results: He was hospitalized for clinical worsening due to an erysipelas of the lower limb. A prophylactic anticoagulation was started (enoxaparin, LMWH). On D7, the patient presented a left hemiplegia due to a carotid cerebral ischemia. A more than 50% drop in platelet count (PltCt) was observed (nadir: 20x10^9/L) (Fig 1). The 4Ts score was 6 and anti-PF4 antibodies were detected (ZymuTest, HyphenBiomed), supporting the diagnosis of HIT. LMWH was stopped. Despite international guidelines, danaparoid was started at prophylactic-intensity dosing, associated with platelet and

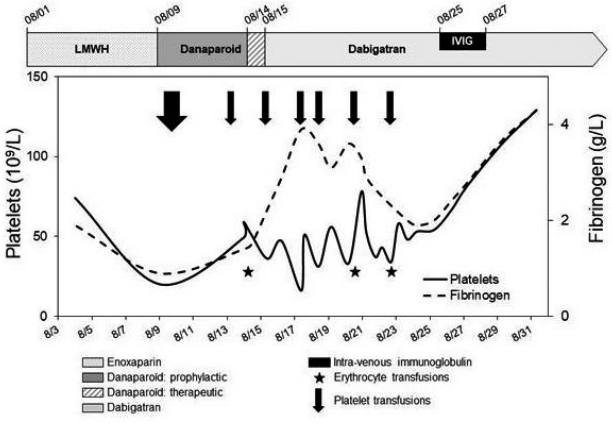

FIGURE 1 Evolution of platelet count and fibrinogen level

fibrinogen transfusions. The patient was discharged and transferred to our hospital. On arrival, petechia and livedo reticularis on lower limb were noticed. PltCt was 49x10^9/L, TP:59%, Fibrinogen:1.4 g/L and D-Dimers> 4000ng/mL, suggesting an overt DIC. Danaparoid was increased to therapeutic dosing but the thrombotic picture worsened. New ischemic brain lesions appeared, while PltCt stayed < 20x10^9/L and D-Dimers>4000ng/mL. Treatment with DOAC (dabigatran) was started because cross-reactivity with danaparoid was initially suspected (and later excluded using functional assay). An acute cholangitis was efficiently treated using antibiotic therapy. Yet, PltCt was < 20x10^9/L. On D5 of dabigatran treatment, a rectal bleeding occurred, requiring erythrocyte transfusion. IVIg (1 g/kg) was proposed, which resulted in a rapid normalization of the PltCt. Due to a calf DVT, treatment with AOD was continued for 3 months without any complication.

Conclusions: This case highlights the usefulness of alternative anticoagulation with IVIg and DOAC in the management of aHITs with confounding causes of severe persistent thrombocytopenia.

PB1415 | Interaction of Unfractionated Heparin and Sulodexide with Functional Anti-Heparin Platelet Factor 4 Antibodies as Investigated in Platelet Aggregation Assays

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Background: Sulodexide is a mammalian tissue derived glycosaminoglycan antithrombotic agent which is clinically used for the management of vascular disorders and is pharmacologically active after both parenteral and oral administration.

Aims: The purpose of this study is to compare the relative effect of sulodexide in mediating heparin induced thrombocytopenia (HIT) antibodies induced aggregation of human platelets in a standardized test system which is developed to compare the effects of heparins and related drugs.

Methods: Active pharmaceutical ingredients (API versions of sulodexide), fast moving heparin and dermatan sulfate were obtained from Alfa Sigma (Bologna, Italy). API versions of UFH were obtained from Medefil, Inc (Glendale Heights, IL, USA). The source of functional HIT antibody represented a frozen pool of aphresis fluid (AF) collected from five clinically confirmed heparin induced thrombocytopenia patients. All results were compiled in terms of percent aggregation of platelets and represented as mean ± standard deviation.

Results: UFH produced a concentration dependent aggregation from 0 - 10 ug/ml reaching a peak aggregation of 60 ± 18.76%. Sulodexide produced a relatively weaker aggregatory response and produced mild aggregation in the same concentration range exhibiting comparable platelet aggregation at 10 ug/ml. The fast moving heparin component of sulodexide produced a concentration dependent response comparable to heparin reaching a maximum aggregation of 66 ± 30.67% at 10 ug/ml. The dermatan sulfate component did not produce any significant aggregation of platelets and no concentration dependence was observed (< 10% aggregation).

Conclusions: These results clearly show that in comparison to heparin, sulodexide produces a relatively weaker response in the functional HIT antibody screening studies. However, the fast moving heparin component of sulodexide produces comparable responses to UFH whereas dermatan sulfate has much weaker aggregation response.

PB1416 | Insights into Autoimmune Heparin-Induced Thrombocytopenia

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Background: Autoimmune heparin-induced thrombocytopenia (aHIT) is increasingly recognized. It is a life-threatening highly prothrombotic condition with prolonged thrombocytopenia. Patient sera cause strong platelet activation even in the absence of heparin (buffer reactivity), which can be inhibited by high concentrations of heparin.

Aims: To assess the prevalence of aHIT among samples referred to a reference laboratory with clinically suspected HIT and further characterization of the aHIT antibodies.

Methods: All patients referred to our laboratory with clinically suspected HIT between 2017 and 2019 were enrolled. Data were retrieved from the laboratory files. For further characterization the HIPA test (washed platelet activation test) was performed. In brief, patient sera were incubated with washed platelets of healthy donors under different conditions: buffer (control) and low dose heparin (reviparin 0.2 aFXaU/mL). Platelet aggregation was visually detected every 5 min. Confirmed aHIT sera were step-wise diluted with AB-serum of a healthy donor and retested.

Results: Of 7,510 referred sera, 5,226 (69.6%) were negative; 1,196 (15.9%) ELISA+ & HIPA negative; and 998 (13.3%) ELISA+ & HIPA+. 86 sera (1.15% of all referred sera; 3.9% of all ELISA+ sera; 8.6% of all HIPA+ sera) showed the typical aHIT pattern. After retesting, 40 sera still showed the typical aHIT pattern. Upon serial dilution, different patterns of platelet activation were observed: 25 sera contained low titer antibodies (Fig. 1a); 8 sera contained high titer

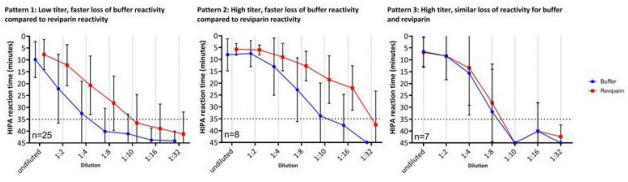

FIGURE 1 Mean HIPA reaction time of serially diluted autoimmune HIT samples

antibodies (Fig. 1b). 7 sera contained only autoantibodies, as heparin did not increase reactivity (Fig. 1c).

Conclusions: The prevalence of aHIT reactivity pattern in sera is higher than currently anticipated, with 3.9% of all ELISA reactive sera and 8.6% of all HIPA positive sera. Most sera contain a mixture of heparin-independent and heparin-dependent platelet activating antibodies. The antibody titers differ considerably, which may impact the efficacy of treatment.

PB1418 | Comparison of Clinical Adverse Events during Empiric Therapy for Patients with Suspected HIT

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Background: Empiric management of suspected heparin induced thrombocytopenia (HIT) is challenging due to variable bleeding or thrombotic risk among patients, and utilization of alternate treatment strategies.Generally, empiric non-heparin anticoagulation is suggested, historically with direct thrombin inhibitors (DTIs). Alternatively, off-label fondaparinux and watchful waiting in select cases have gained traction.Outcomes of patients who ultimately do not have HIT are frequently overlooked. Further research is needed to explore outcomes and identify risk factors among various empiric management strategies.

Aims: Categorize and compare overall, bleeding, and thrombotic AE during empiric HIT management with argatroban, fondaparinux, and watchful waiting.

Methods: A retrospective chart review of patients tested for HIT at an academic hospital from 4/2017-4/2019 was conducted. AE were defined as new, radiologically- confirmed thrombosis or clinically significant bleeding while awaiting confirmatory testing. Subjects within three management groups were compared with logistic regression analysis adjusted for age, sex and pre-treatment bleeding risk.

TABLE 1 Baseline characteristics

Item		Total	Argatroban Therapeutic Dose	Argatroban Reduced Intensity Dose	Fondaparinux Therapeutic Dose	Fondaparinux Reduced Intensity Dose	Watchful Waiting On Heparin	Watchful Waiting Off Heparin	p-value
Total, N (%)		170 (100)	24 (14.1)	23 (13.5)	11 (6.5)	32 (18.8)	20 (11.8)	60 (35.3)	< 0.001
Sex, N (%)	Male	107 (62.9)	14 (8.2)	17 (10.0)	7 (4.1)	22 (12.9)	15 (8.8)	32 (18.8)	0.331
	Female	63 (37.1)	10 (5.9)	6 (3.5)	4 (2.4)	10 (5.9)	5 (2.9)	28 (16.5)	
Age, (Mean ± S.D.)		61.1 ± 16.1	59.4 ± 15.8	65 ± 15.2	53.6 ± 16.1	58.6 ± 13.7	62.2 ± 13.5	62.4 ± 18.4	0.416
IMPROVE bleeding RAM Score, (Mean ± S.D.)		8.7 ± 4.3	7.8 ± 3.8	11.1 ± 3.4	5.6 ± 4.4	7.2 ± 3.9	8.8 ± 2.1	9.4 ± 4.7	0.002
Platelet Count, (Mean ± S.D.)		81.32 ± 41.1	99.5 ± 52.0	72.4 ± 35.7	82.2 ± 11.0	85.7 ± 31.1	85.4 ± 28.8	73.6 ± 45.7	0.216
4Ts Score, (Mean ± SD)		4.8 ± 0.9	5.4 ± 1.2	4.9 ± 1	4.8 ± 0.6	4.7 ± 0.8	4.5 ± 0.7	4.6 ± 0.8	0.060
PF4 - Postive Result, N (%)		61 (35.9)	15 (8.8)	14 (8.2)	4 (2.4)	14 (8.2)	2 (1.2)	12 (7.1)	< 0.001
SRA - Positive Result, N (%)		23 (13.5)	8 (4.7)	6 (3.5)	3 (1.8)	2 (1.2)	0 (0)	4 (2.4)	< 0.001

TABLE 2 Adverse event outcomes

	Total		HIT		Non-HIT	
Adverse Event	Thrombosis	Bleed	Thrombosis	Bleed	Thrombosis	Bleed
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Argatroban therapeutic dose	0 (0.0)	1 (16.7)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
Argatroban reduced intensity dose	0 (0.0)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (40.0)
Fondaparinux therapeutic dose	0 (0.0)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (40.0)
Fondaparinux reduced intensity dose	3 (50.0)	1 (16.7)	2 (50.0)	0 (0.0)	1 (50.0)	1 (20.0)
Watchful waiting on heparin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Watchful waiting off heparin	3 (50.0)	0 (0.0)	2 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)

Results: 170 patients were studied. Table 1 contains a comparison of baseline characteristics. AE occurred in 7.1% of patients. 50% of the AE were thrombotic events and 50% were bleeding
(Table 2). 50% of all AE occurred in patients treated with fondaparinux. Logistic regression analysis revealed fondaparinux was associated with more overall AE than watchful waiting (P=0.03). However, evidence did not suggest argatroban was associated with more overall AE compared to watchful waiting (p=0.46). Intensity of anticoagulation and time to treatment initiation also were not associated with a significant difference in overall AE.

Conclusions: Pre-treatment bleeding risk significantly differed between management groups, suggesting this variable impacts clinical decision-making. Interestingly, with and without adjustment for pre-treatment bleeding risk, empiric treatment with fondaparinux was associated with increased overall AE, while argatroban was not. Findings further suggested no advantage to reduced dosing with either anticoagulant. These findings warrant careful interpretation and challenge current paradigms of empiric HIT management.

PB1419 | Therapeutic Plasma Exchange in the Pre-Operative Management of Acute HIT

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Background: Heparin-induced thrombocytopenia (HIT) is a severe adverse reaction to heparin treatment caused by IgG antibodies that recognize platelet factor 4/heparin complexes (PF4/H) associated with an increased risk of thrombosis. When HIT is suspected, heparin must be stopped immediately and replaced by another anticoagulant such as danaparoid sodium or argatroban. Their use during cardiac surgery is associated to an increased risk of bleeding and/or thrombosis. Therapeutic plasma exchange (TPE) has been proposed to remove HIT antibodies quickly to allow heparin use during surgery.

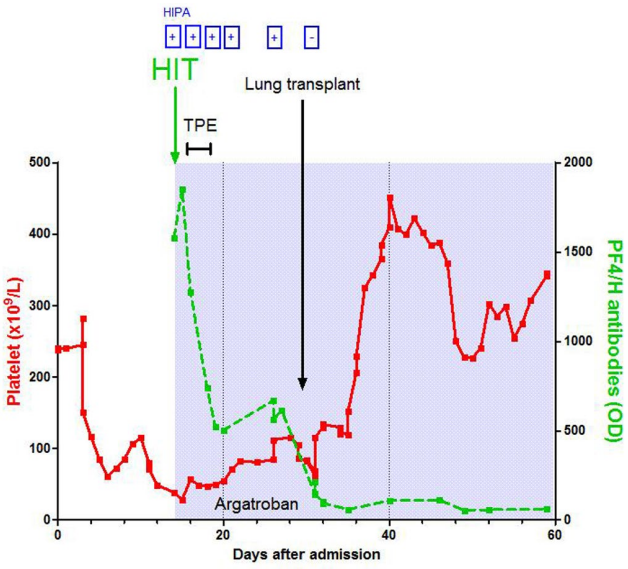

FIGURE 1 IgG-specific anti-PF4/H EIA and HIPA test results in relation to 5 TPE

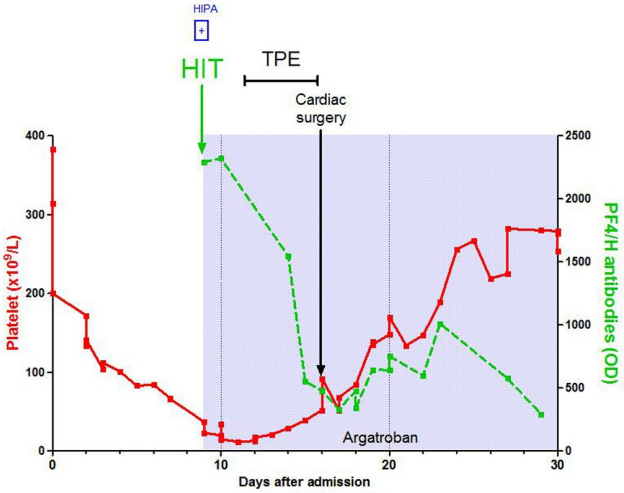

FIGURE 2 IgG-specific anti-PF4/H EIA and HIPA test results in relation to 4 TPE

Aims: We report 2 cases of patients who underwent repeated TPE, achieving negative anti-PF4/H and allowing cardiac surgery with heparin for one of them.

Methods: Testing for HIT antibodies was performed using anti-PF4/heparin IgG-specific EIA (Zymutest HIA IgG, Hyphen Biomed) and heparin-induced platelet activation (HIPA) assay.

Results: A 36-year-old woman received veno-arterial extracorporeal membrane oxygenation (ECMO) support for an acute exacerbation of pulmonary fibrosis. She developed thrombocytopenia on day 10 of anticoagulation with unfractionated heparin (UFH) (nadir 29G/L) and a deep femoral vein thrombosis. EIA was strongly positive (2609 mU.OD), confirmed by HIPA assay. Five TPE were performed to remove anti-HLA, which also achieved negative anti-PF4/H (125 mU.DO) and HIPA on the day of lung transplantation.

A 63-year-old patient was hospitalized for cardiogenic shock complicated with multiple organ failure and required ECMO. He developed thrombocytopenia on day 12 of anticoagulation with UFH (nadir 15G/L) and thrombosis in the ECMO circuit. HIT was confirmed by strongly positive anti-PF4/H (2291 mU.DO) and HIPA. Anticoagulation with argatroban was initiated. Because surgical treatment of interventricular communication was required, 4 TPE sessions were performed and achieved negative anti-PF4/H (481 mU.OD pre-surgery). The patient underwent surgery with usual UFH doses, without thrombotic complication.

Conclusions: These 2 cases confirm that TPE is safe to remove HIT antibodies and allow heparin use during surgery in the acute phase of HIT.

PB1420 | Heparin-Induced Thrombocytopenia in Intensive Care Patients with Extracorporeal Circulation

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Background: Heparin-induced thrombocytopenia (HIT) is a serious drug induced reaction caused by antibodies against platelet factor 4 (PF4)/heparin complexes. Since thrombocytopenia and thrombosis are common findings in intensive care (ICU) patients undergoing extracorporeal circulation (ECC) it is a diagnostic challenge to confirm or rule out HIT based on clinical manifestations alone and the high rate of IgG immunization limits the use of immunoassays in the diagnostic approach.

Aims: Evaluation of the frequency of HIT and HIT testing results in intensive care patients with extracorporeal circulation.

Methods: Data were collected from surgical ICU patients with suspected HIT (n=340), of whom 83 had ECC. Inclusion criteria were thrombocytopenia and or thrombosis under heparin treatment. The diagnosis of HIT was verified using the 4Ts-score, an IgG specific immunoassay and the function assay (HIPA).

Results: Antibodies against PF4/heparin were found in 55/257 (21%) surgical patients and in 27/83 (33%) patients with ECC. 10/55 (18.18%) EIA positive patients with surgery had platelet activating antibodies. One patient had a heparin-independent platelet activation. In contrast, sera from 7/27 EIA positive patients with ECC tested positive in the HIPA test. 3/27 sera showed heparin-dependent and 4/27 (14.8%) sera showed heparin-independent platelet activation. While prevalence of HIT seems to be similar between patients with and without ECC (3.5% vs. 3.6%), higher amount of heparin-independent platelet activation was observed in patients with ECC compared to surgical patients (4.8% vs. 0.38%, respectively). [BT1] 13 sera had an optical density above 2.0. Interestingly 46% of these sera revealed HIPA negative results.

Conclusions: Our study demonstrates the challenges in the current diagnostic approach for HIT in ICU with surgery and ECC. The higher incidence IgG immunization against PF4/heparin complexes impairs the performance of a standardized diagnostic algorithm and the magnitude of the OD of the antibodies is not predictive for HIPA result.

PB1422 | The Utility of a Platelet Count-Based Electronic Medical Record Alert to Facilitate Diagnosis of Heparin-Induced Thrombocytopenia

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Background: Heparin-induced thrombocytopenia (HIT) is a potentially devastating consequence of heparin exposure. Prompt diagnosis with initiation of a non-heparin anticoagulant can prevent life-threatening thrombotic complications. In an institution-wide effort to increase early recognition, an alert in the electronic medical record (EMR) has been sent to providers for the last 15 months when a patient's platelet count dropped by ≥50%.

Aims: Assess the effect of a platelet count-based alert on HIT diagnosis and management.

Methods: Retrospective evaluation of alert firings and subsequent actions at an academic medical center over a one-month period.

Results: An average of 3.3 alerts fired per day. Of 100 total alerts, 21 were considered inappropriate due to lack of heparin exposure, inaccurate platelet count due to clumping, or patient characteristics such hematopoietic stem cell transplant recipient (HSCT) or organ donor (n=10; excluded from further analysis). Characteristics of the remaining 90 alerts are outlined in Table 1. The majority (59%) of alerts fired in patients with low HIT probability with 4Ts scores ≤3. Immunoassays were ordered for 34 patients (38%); 18/34 (53%) had a low probability score (Figure 1). Seven (21%) immunoassays were positive and one patient was confirmed to have HIT with the serotonin release assay. Of the patients tested with the immunoassay, heparin was held in 11 (32%) and a non-heparin anticoagulant started in 4 (12%). Following a negative immunoassay result, heparin prophylaxis was not restarted in 6 and bivalirudin was continued in

TABLE 1 Patient characteristics

Medical service, n (%)	
Cardiovascular surgery	**25 (28%)**
Intensive care unit	19 (21%)
Trauma	17 (19%)
Cardiology	10 (11%)
Internal medicine	9 (10%)
Others	10 (11%)
Platelet count, mean (range)	
Peak prior to drop (x10^3/μL)	304 (67-1077)
At the time of alert (x10^3/μL)	136 (31-528)

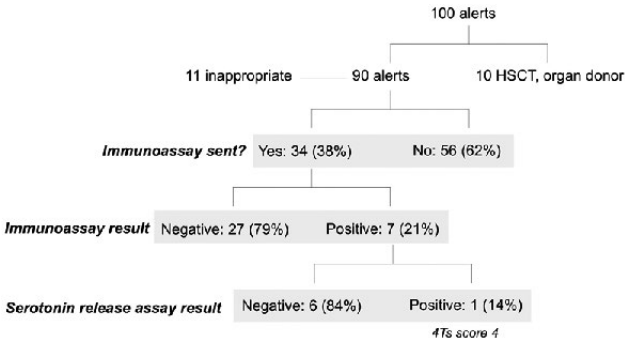

FIGURE 1 Diagram of electronic medical record alerts, selection for testing, and test results

5 for a total of 57 days. The average number of HIT immunoassays ordered in the 2 years prior to the alert creation was 50/month (SD 10); the number has since increased to 63 (SD 9).

Conclusions: An EMR alert based on a drop in platelet count may have increased HIT immunoassay ordering but did not contribute to appropriate testing and use of non-heparin anticoagulants.

PB1423 | Detection of Pathogenic Anti-platelet Factor 4 Antibodies by Impedance Spectroscopy

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Background: Heparin-induced thrombocytopenia (HIT) antibodies develop in ~3% of patients with Heparin (H) administration. HIT is the most frequent immune-mediated adverse drug reaction involving blood cells and could be life-threatening. To detect HIT antibodies, immunological assays are widely used because of their high sensitivity (≥95%) and fast turnover, but only ~50% of these tests show a spectrum of clinical HIT. Pathogenic HIT antibodies can be better identified by functional assays such as the serotonin release assay or Heparin-induced platelet aggregation with an accuracy of over 90%. However, functional assays require fresh human blood and are only available in specialized laboratories/countries. Recently, we found that binding of Platelet Factor 4/Heparin (PF4/H) antibodies are qualitatively different.

Aims: We aim to develop an electrical sensor to better differentiate pathogenic antibodies from non-pathogenic antibodies based on their distinct binding characteristics.

Methods: As a model, we used monoclonal non-pathogenic antibody RTO and pathogenic antibody KKO. PF4/Heparin complexes were immobilized on gold chips and their binding was tracked in Quartz crystal microbalance (QCM) and Electrochemical impedance spectroscopy (EIS). Mass change (Sauerbry equation) and electron transfer rate (Nyquist fitting) were analyzed from QCM and EIS respectively. Binding strengths between antibodies and PF4/H complexes were determined by single-molecule force spectroscopy (SMFS).

Results: Mass change obtained by QCM and electron transfer rate detected by EIS increased when PF4/Heparin complexes were immobilized on the chips and further increased when KKO was added. No significant changes were observed when RTO was added. SMFS results consistently showed that KKO interacted with PF4/H complexes with stronger binding force than RTO.

Conclusions: Pathogenic HIT antibody KKO could be differentiated from the non-pathogenic antibody RTO. Our study provides a potential approach for better detection of pathogenic HIT antibodies from patients.

PB1424 | Evaluation of 4T Probability Scores, Heparin PF4 Antibody, and Serotonin Release Values in End Stage Renal Disease Patients in the Assessment of Heparin Induced Thrombocytopenia

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Background: Diagnosing heparin induced thrombocytopenia (HIT) in patients with end stage renal disease (ESRD) can be difficult as they are frequently exposed to heparin and have multiple etiologies for thrombocytopenia.

Aims: To correlate 4T scores, PF4 ELISA results, and serotonin release assays (SRA) in patients with ESRD.

Methods: A retrospective review of patients with ESRD (CrCl < 15 mL/min or on renal replacement therapy (RRT), with IgG heparin-PF4 ELISA testing between October 2015– September 2019 was performed. Baseline characteristics, route and type of heparin, type of RRT, calculated 4T scores, PF4 and SRA results were collected. False positive PF4s were defined as a positive PF4 with a negative SRA, low 4T score, or lack of treatment for HIT. True positive PF4 results required intermediate to high 4T score, positive SRA, and treatment for HIT. Indeterminate cases were classified based on clinical treatment.

Results: Of 265 patients with ESRD (92% on RRT), 29 patients (10.9%) had a positive PF4. Eleven had confirmed diagnosis of HIT: 10 with 4T ≥4, positive SRA, one with 4T of 6 and PF4 of 1.947 but no SRA; all were treated for HIT. False positive PF4 values occurred in 8 patients (27.5%) with negative SRA, 4T < 4, and not treated for HIT. Of 10 patients with negative SRA but intermediate-high 4T and positive PF4, only 3 were treated for HIT. Fifteen (51.7%) patients with negative SRA were not diagnosed and treated as HIT despite positive PF4 and intermediate-high 4T.

Conclusions: In ESRD patients, PF4 results and 4T scores did not consistently correlate with a clinical diagnosis of HIT. Patients with intermediate-high 4Ts and PF4 > 1.0 had negative SRAs and were

TABLE 1 Key Baseline Characteristics

Type of Heparin Exposure (n=265)	n (%)
Unfractionated heparin only; Low molecular weight heparin only (LMWH); Combination of unfractionated heparin and LMWH; No heparin while inpatient	214 (80.8); 1 (0.4); 25 (9.4); 25 (9.4)
Route of heparin (n=240)	n (%)
Subcutaneous; Intravenous; Flushes; Combination; Other	106 (44.2; 77 (32.1); 6 (2.5; 42 (17.5); 9 (3.7)
End Stage Renal Disease (n=265)	n (%)
CrCl<15 mL/min not on renal replacement therapy; Continuous renal replacement therapy; Hemodialysis; Peritoneal dialysis	21 (7.9); 99 (37.4); 143 (54.0); 2 (0.7)

TABLE 2 Clinical and Laboratory Results for Suspected HIT

IgG ELISA PF4 OD	SRA Negative (n=18)	SRA Positive (n=10)
OD 0.4-0.8 n (%); OD 0.4-1.0 n (%); OD>1.0 n (%)	9 (50); 12 (67); 6 (33)	1 (10); 2 (20); 8 (80)
4T Scores	SRA Negative (n=18)	SRA Positive (n=10)
Low Suspicion (</=3); Intermediate Suspicion (4-5); High Suspicion (>/=6)	8 (44); 6 (33); 4 (22)	1 (10); 6 (60); 3 (30)
PF4 Result by 4T score	PF4 OD 0.4-1.0 (n=12)	PF4 OD > 1.0 (n =6)
Low Suspicion HIT (0-3); Intermediate Suspicion HIT (4-5); High Suspicion HIT (>5)	7 (58.3); 2 (16.7); 3 (25.0)	1 (16.7); 4 (66.7); 1 (16.7)

not treated for HIT with no adverse outcomes. PF4 testing and 4T scores may not be accurate in this population.

PB1425 | Inhibition of 12-LOX with VLX-1005 Has Clinical Utility for Prevention and Intervention of HIT

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Background: Heparin is widely used clinically. Some patients treated with heparin will form an immune complex resulting in activation of the platelet immune receptor FcγRIIa. FcγRIIa engagement results in platelet activation, thrombus formation, and platelet clearance resulting in an increase in thrombosis as well as bleeding due to thrombocytopenia. Traditional antiplatelet drugs have failed to prevent platelet activation due to immune-receptor activation and the only FDA-approved intervention for HIT is the factor IIa inhibitor, argatroban, which carries with it a significant risk for bleeding. Hence, a new target for prevention of HIT is needed for adequate treatment of this rare disease.

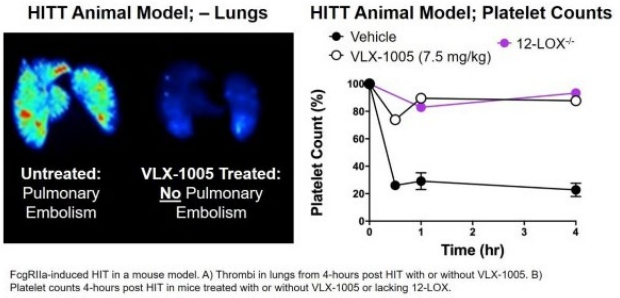

FIGURE 1 VLX-1005 prevents platelet loss and thrombus formation in HIT

Aims: To identify the utility of inhibiting platelet 12-lipoxygenase (12-LOX) as a viable target for prevention and intervention in HIT. Previous work by our lab and others have shown that inhibition of 12-LOX may be a new approach that both prevents thrombosis without increased risk in bleeding.

Methods: The 12-LOX inhibitor, VLX-1005 (previously denoted as ML355), was used to assess bleeding as well as prevention and intervention of platelet activation during HIT in the mouse model.

Results: Mice were treated with or without VLX-1005 followed by induction of HIT. VLX-1005 prevented platelet loss and thrombus formation for all times assessed compared to control animals. Further, to assess the utility of VLX-1005 in patients already diagnosed with potential HIT, mice were treated with anti-CD9 to induce HIT followed after a specified time with or without VLX-1005. VLX-1005-treated mice showed no significant platelets loss and only minimal platelet accumulation in the vascular lung space. Finally, using the mouse tail-bleeding assay, argatroban administration resulted in increased bleeding in the mouse, while VLX-1005 showed no increase in bleeding compared to control.

Conclusions: Targeting 12-LOX with VLX-1005 is a new therapeutic approach for prevention and intervention of HIT with no increased risk in bleeding.

PB1426 | Underlying Causes Greatly Influence the Development of HIT Antibodies and Clinical Outcomes in Patients with Heparin-induced Thrombocytopenia

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Background: Heparin-induced thrombocytopenia (HIT) is caused by heparin administration, which results in the production of anti-platelet factor 4 (PF4)/heparin IgG with platelet-activating properties (HIT antibodies). Previous publications demonstrated that disease severity influences the risk of HIT immune response.

TABLE 1 HIT antibody development and clinical outcomes in HIT patients with different underlying causes

Underlying cause	Trauma such as traffic accident or fall	Cardiovascular surgery with unfractionated heparin anticoagulation	Initiation of hemodialysis with heparin anticoagulation	Others (mainly prevention or treatment of thromboembolic event using heparin)
Number of HIT patients (Total: 184)	10	37	32	105
Patients who developed HIT antibodies with heparin-independent platelet-activating properties	3/10 (30%)	4/37 (10.8%)	1/32 (3.1%)	14/105 (13.3%)
*Optical density value in ELISA for anti-PF4/heparin IgG/A/M	2.478 (1.301-2.967)	2.282 (1.585-2.804)	2.162 (1.267-2.642)	2.517 (1.806-2.857)
*Optical density value in ELISA for anti-PF4/heparin IgG	1.949 (1.689-2.084)	1.990 (1.490-2.236)	1.620 (1.123-2.642)	2.062 (1.458-2.291)
*HIT-associated thromboembolic events [arterial, venous, both]	8/10 (80%) [0%, 80%, 20%]	17/37 (45.9%) [41%, 35%, 23%]	12/32 (37.5%) [8%, 83%, 8%]	69/105 (66%) [20%, 62%, 17%]
*Onset of HIT-associated thromboembolic events (day)	9 (7.5-9.75)	12.5 (9.75-15.25)	13 (11.5-23.5)	11 (8-13)
*Reduction in platelet count (%)	66.8 (56.0-73.6)	72.4 (59.2-83.3)	76.8 (61.8-83.9)	81.8 (71.8-87.7)
*Onset of HIT (first day with more than a 30% reduction in platelet count) [Patients with late HIT onset (> day 14)]	9 (7.25-9.75) [0/12 (0%)]	9 (8-10) [3/37 (8.1%)]	11.5 (8.75-14) [6/32 (18.8%)]	9 (7-11) [4/105 (3.8%)]

*: median (1st-3rd quartile)

Aims: To investigate how the underlying causes that necessitated heparin administration influenced HIT immune responses and clinical outcomes in HIT patients.

Methods: Using a nationwide HIT registry, we obtained data of 835 patients who were clinically suspected of having HIT. Among them, 222 patients were ultimately diagnosed with HIT according to clinical probability scores (4Ts) and the presence of HIT antibodies, which were detected in a washed-platelet activation assay. We excluded patients who developed rapid-onset HIT and those whose HIT antibodies were not assessed within two weeks of HIT onset. Then, we categorized HIT patients into four groups based on their underlying cause: trauma, cardiovascular surgery, hemodialysis, and others.

Results: As shown in Table 1, HIT antibodies with heparin-independent (i.e., strong) platelet-activating properties developed significantly more frequently in trauma patients than in hemodialysis patients, possibly resulting in trauma patients' significantly higher incidence of HIT-associated thromboembolic events. The optical density values in ELISA for anti-PF4/heparin IgG were lower in the hemodialysis group than in other groups. However, platelet count reduction did not differ among the four groups. The onset of HIT-associated thromboembolic event tended to be earlier in the trauma group and the onset of HIT appeared to be later in the hemodialysis group. Interestingly, 18.8% of hemodialysis patients developed HIT more than 14 days after the initiation of heparin.

Conclusions: These results suggest that patients' underlying causes differentially impact HIT immune responses, especially the development of HIT antibodies with strong platelet-activating properties, and thus influence clinical outcomes. This information may make it possible to diagnose and treat HIT patients more appropriately by carefully considering their underlying causes.

PB1427 | Platelet Flow Cytometry in the Diagnosis of Heparin-induced Thrombocytopenia

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Background: Heparin-induced thrombocytopenia (HIT) is a rare medical complication of heparin therapy, when antibodies are formed against the complex "heparin-PF4". They activate and destroy platelets, damage endothelium, increase thrombin generation. Most often this reaction appears on the 5-14th day of heparin therapy, the platelet count decreases more than 50% and new thrombosis are diagnosed. Diagnosis of this pathology is difficult, the main symptoms are nonspecific, especially for patients in critical injuries with initial thrombocytopenia. Use of SRA for diagnostics is limited, but using flow cytometry has opened up a new possibilities.

Aims: Study the functional activity of platelets of patient with confirmed HIT by flow cytometry with and without preliminary heparin-incubation

Methods: The diagnosis of HIT was confirmed on the test-system "HIT-Ab" (IL), the concentration of antibodies - 6.4 U/ml (threshold value < 1.0). The study by flow cytometry was carried out on FACS Canto II (BD Biosciences). The platelet population has been

identified by expression of CD61 and CD42b, which were selected as a main platelets markers. In this population we investigated CD62p (a marker of alpha-granules) and Annexin V (a marker of procoagulant activity).The results were compared with donor data.

Results: Comparing the response of platelets to heparin influence was found, that the number of CD62p-positive platelets increased from 26.1% to 47.2% after incubation. The MFI of this population increased from 131 to 761. Most of the patient's platelet initially expressed CD62p, which could be due to their pre-activation or transportation. When the donor's platelet was studied under the same conditions, the number of CD62p-positive platelets decreased after incubation from 6.3 to 3.8%. Reliable changes in procoagulant activity was not obtained.

Conclusions: Platelet flow cytometry may be an alternative and more convenient functional test for the advanced diagnosis of heparin-induced thrombocytopenia. The possibility of its use requires in further study.

PB1428 | First Performance Data of a New Latex Enhanced Immunoassay to Detect HIT Antibodies in Plasma Samples *

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Background: Heparin-induced thrombocytopenia (HIT) is a prothrombotic drug reaction caused by platelet activating antibodies with specificity for platelet factor 4 (PF4)/ heparin complexes. Immunoassays to detect those antibodies are essential in the workup of patients with suspected HIT.

Aims: Here, we show first performance data of a fully automated latex enhanced immunoassay for detection of PF4/ heparin complex specific antibodies in human plasma samples on a hemostasis analyzer. *

Methods: The assay is based on latex particles coated with monoclonal antibodies that mimic HIT antibodies with specificity for PF4/ heparin complex. PF4/ heparin antibodies contained in the sample compete with the monoclonal antibodies on the latex particles and decrease the turbidimetric signal which is conversely proportional to the antibody concentration in the sample.

The assay consists of three liquid ready to use reagents. Reagent one is a supplementary reagent containing a blocker to prevent HAMA and RF interference.

Reagent two is the latex reagent and the third one contains PF4 complexed with a polyanion to mimic the PF4/ heparin complex for signal generation. A calibrator automatically diluted by the system is measured to generate a calibration curve.

Results: First performance data of the prototype assay were collected regarding precision, HIL interference, HAMA/RF interference and method comparison data vs. heparin-induced platelet activation (HIPA) assay. Repeatability was between 2.31 - 8.62% CV. No interference up to 500 mg/dL hemoglobin, 30 mg/dL unconjugated and conjugated bilirubin, 1000 mg/dL triglycerides, 500 mg/dL cholesterol and 2000 IU/mL RF was observed. The immunoassay confirmed all 20 HIPA positive.

Conclusions: We conclude that our latex enhanced immunoassay reliably measures PF4/heparin antibodies in human citrated plasma by using only 10 µl sample volume. The assay might be a helpful tool for the workup of patients with suspected HIT.

PB1429 | Addition of Exogenous Platelet Factor 4 in the Heparin Induced Platelet Activation (HIPA) Assay for the Detection of Pathogenic Heparin-induced Thrombocytopenia Antibodies

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Background: Laboratory confirmation of heparin-induced thrombocytopenia (HIT) relies on functional assays highlighting the presence of heparin-dependent platelet-activating antibodies in the plasma or serum of patients. Functional assays using washed platelets, i.e., ^{14}C-serotonin release assay and heparin-induced platelet activation assay (HIPA) are considered gold standards because of their excellent specificity (> 99%) and good sensitivity (> 95%). However, several recent data suggest that the addition of exogenous platelet factor 4 (PF4) in functional assays using washed platelets could improve their ability to detect low levels of pathogenic HIT antibodies.

Aims: To investigate whether the addition of PF4 enables to detect the presence of low levels of platelet-activating antibodies in the plasma of patients investigated for HIT that failed to cause platelet activation in the conventional HIPA test.

Methods: Plasma samples from 28 patients with suspected HIT were studied. The presence of anti-PF4/heparin antibodies was evaluated with an immuno-assay specific to IgG (Zymutest HIA IgG, Hyphen Biomed). HIPA was performed as previously described and after adding 10 µg/ml of purified human PF4 (Hyphen Biomed) in the reaction mixture (HIPA-PF4).

Results: HIPA-PF4 detected platelet-activating antibodies in 11 (100%) of the 11 HIPA-positive plasma (HIPA^{pos}). Platelet-activating antibodies were detected in 5 (29%) of 17 samples that were positive for the immuno-assay but tested negative or undetermined in the HIPA (HIPA-PF4^{pos}). None of the HIPA-PF4 negative plasma was positive in the conventional HIPA (HIPA^{neg}, Table 1). Importantly, the addition of PF4 in HIPA allowed to detect significantly lower levels of platelet activating PF4-specific IgG than conventional HIPA (mean

TABLE 1

	Positive HIPA-PF4	Undetermined HIPA-PF4	Negative HIPA-PF4
Positive HIPA	11	0	0
Undetermined HIPA	2	0	0
Negative HIPA	2	2	10

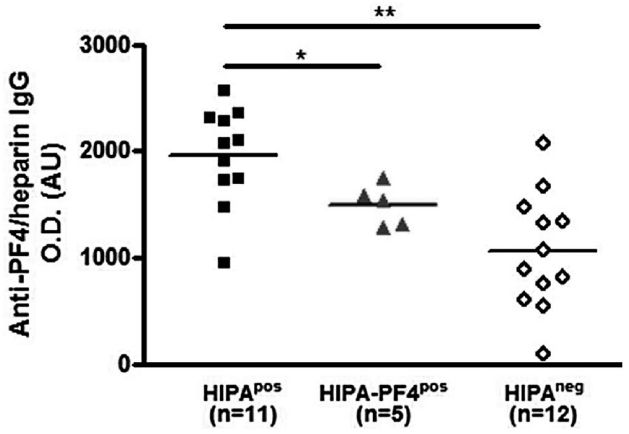

FIGURE 1 Levels of anti-PF4/heparin IgG and reactivity in the HIPA-PF4

optical density = 1493 ± 192 vs 1961 ± 463, Mann Whitney p=0.04, Figure 1).

Conclusions: Addition of exogenous PF4 might improve the detection of weakly activating HIT antibodies by HIPA and make the test more sensitive.

PB1430 | Effect of Synthetic Inhibitors on HIT Antigenic Complex - Therapeutic Approach Alternative to Anticoagulation

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Background: Heparin-induced thrombocytopenia (HIT) is a highly prothrombotic, antibody-mediated disorder that develops during anticoagulation therapy in ~1% of adults exposed to therapeutic doses of unfractionated heparin. Ultra-large immune complexes (ULICs) composed of HIT antibody, PF4 and heparin, glycosaminoglycans or polyanions such as polyphosphates, von Willebrand factor or DNA are central to the pathogenesis of HIT. Recently, we developed and reported highly biocompatible cationic polyanion inhibitors composed of a dendritic polyglycerol core assembled with trivalent cationic ligands with high charge densities (R) (Figure 1) that are protected with short polyethylene glycol (PEG) to generate a protective shell (a brush layer) against nonspecific interactions named as UHRAs.

Aims: The aim of this study was to evaluate the capacity of representative compounds to disrupt ULICs as potential therapeutic in HIT.

Methods: We characterized UHRA binding properties using isothermal titration calorimetry, their capacity to disrupt formation of ULICs by dynamic light scattering and their effect on antigen recognition by HIT antibodies by ELISA. Flow cytometry was used to assess the effect on platelet activation and the membrane-bound antigenic complex. A murine model of HIT employing transgenic mice expressing hPF4 and hFcγRIIA was used to assess the in vivo effect.

Results: UHRAs caused extensive, dynamic changes within multimolecular PF4 antigenic complexes that prevented or disrupted formation of the epitope necessary for binding of multiple pathogenic

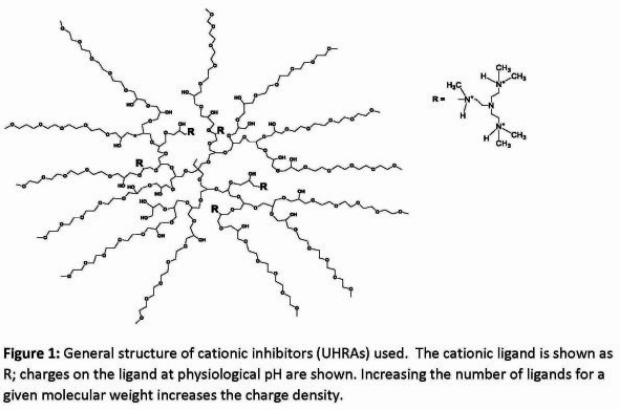

Figure 1: General structure of cationic inhibitors (UHRAs) used. The cationic ligand is shown as R; charges on the ligand at physiological pH are shown. Increasing the number of ligands for a given molecular weight increases the charge density.

FIGURE 1 General structure of cationic inhibitors (UHRAs) used

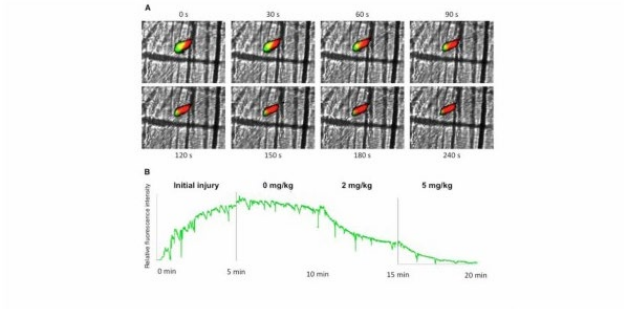

Figure 2: Disruption of HIT antigenic complex *in vivo*. A. Effect of UHRA (2 mg/kg) during the injection on perithrombus development of HIT antigenic complexes (green) and platelet adherence (red). **B.** Relative fluorescence intensity of bound KKO throughout the experiment (HIT antigen development during the initial injury - 0-5 min, stable phase - 5-10 min, incomplete removal after the infusion of UHRA (2 mg/kg) – 10-15 min, and complete removal of complexes after second infusion of UHRA (5 mg/kg).

FIGURE 2 Disruption of HIT antigenic complex in vivo

antibodies in vitro. Selected UHRAs were able to prevent thrombocytopenia in a murine passive model of HIT. Intravital imaging of cremaster muscle vasculature showed disruption of HIT antigenic complex assembled on arteriolar endothelium (Figure 2) and prevention of thrombus growth induced by HIT like monoclonal antibody KKO in murine model of HIT.

Conclusions: Disrupting ULIC formation prevents antibody-mediated cell activation and the subsequent development of a prothrombotic state in HIT and could potentially be used as a new therapeutic approach for HIT alternative to anticoagulation.

PB1431 | Anaphylactic Shock to Apixaban in a Patient Treated for Heparin-induced Thrombocytopenia (HIT)

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Background: HIT is rare but an important complication induced by treatment with LMWH.

Aims: Share a reported case.

Methods: Clinical file Information.

Results: A 79 years old female patient, hemodynamically stable, with hypertension and AF hypocoagulated with rivaroxaban that she takes correctly, presented to the emergency department with dyspnoea for small efforts.

At admission, it was detected increased BNP (15777 pg/ml), thrombocytopenia (platelet count of 119000/uL) and central pulmonary embolism with right cardiac overload and bilateral DVT of the lower limbs.

The patient started treatment with enoxaparin 1mg/kg/bid day. Three days later, the platelet count decreased to 39000/uL. There was a suspicion it might be a HIT because she had an orthopedic surgery a month before and, in this last hospital stay, was hypocoagulated with LMWH. The patient had a 4T score of 4, which corresponds to intermediate risk of having HIT.

LMWH was immediately discontinued and switched to apixaban because the patient was hemodynamically stable with no high bleeding risk. After the second take of apixaban, she developed dyspnoea and generalized exanthema. Transthoracic echocardiogram was normal, suspecting an apixaban anaphylactic shock.

Accounting for installed hemodynamic instability and AKI, we introduced argatroban. The patient showed big lability in the INR and aPTT, so it was decided to put an IVC filter and start acenocoumarol when platelets recovered, maintaining argatroban perfusion during the first three days.

To confirm diagnosis, a blood test analysis for PF4 antibodies was sent abroad and it came back positive. She maintained acenocoumarol and showed better response during the rest of the hospital stay.

Conclusions: This case raises interest in the rarity of HIT associated with LMWH. Few case reports describe the beneficial effect of DOACS in the treatment of HIT syndrome but they are reasonable options in clinically stable patients at average risk of bleeding.

TABLE 1 4Ts Score for Heparin-Induced Thrombocytopenia

Variable	Score
Thrombocytopenia	2 points:Platelet count fall >50% AND platelet nadir ≥20
Timing of platelet count fall	0 points: Platelet count fall <4 days without recent exposure
Thrombosis or other sequelae	0 points: None
Other causes for thrombocytopenia	2 points: None apparent
Total	4

TABLE 2 Index Acronyms

INR	International Normalized Ratio
AKI	Acute kidney injury
aPTT	Activated partial thromboplastin time
AF	Atrial fibrillation
BNP	Brain natriuretic peptide
DOACS	Direct oral anticoagulants
DVT	Deep vein thrombosis
IVC	Inferior vena cava
LMWH	Low-molecular-weight heparin
PF4	Platelet factor 4

PB1432 | A Challenging Case of Delayed-onset Refractory Heparin-induced Thrombocytopenia Complicated with Arterial Thrombosis

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Background: Heparin-induced thrombocytopenia (HIT) is an iatrogenic, clinically relevant complication, which is often misdiagnosed and might lead to life-threatening events.

Aims: The objective of this clinical case report is to highlight challenging aspects in the management of HIT.

Methods: A 64-year old woman was discharged following right hip replacement. In the post-operative setting she developed an atrial flutter, treated with nadroparine. Prior to discharge rivaroxaban prophylaxis was started.

On post-operative day17 she presented at the emergency department with acute skin necrosis on her right lower limb. Laboratory tests showed a significant drop in thrombocyte count. Platelet-factor-4 antibodies were positive, compatible with the diagnosis of HIT.

Results: *Delayed onset:* Thrombocytopenia classically occurs in HIT 5-10days after initiation of heparin. Despite our patient presented 17days after nadroparine-exposure, biochemical onset of the thrombocytopenia was unknown. Warkentin reported in his study that patients with delayed onset HIT had high-titers of platelet-activating IgG antibodies that exhibited heparin-dependent as well as heparin-independent platelet activation, potentially explaining why complications occurred in the absence of heparin.

Cross-reactivity fondaparinux: Although discontinuation of nadroparine (>2weeks) and adequate treatment with fondaparinux the patient's thrombocytopenia persisted and new thrombo-embolic events occurred. As previously reported in literature fondaparinux might cross-react with HIT-antibodies and therefore leading to therapy-refractority.

Administration of immunoglobulins (IVIG): On day6 and 7, IVIG was administered concerning the life-threatening thrombo-embolic complications, where-after rapid and sustained normalization of platelet count and clinical improvement was achieved.

DOAC's: Evidence supporting efficacy and safety of direct oral anticoagulants (DOAC) for acute HIT is increasing. The fact our patient developed HIT and thrombosis receiving rivaroxaban prophylaxis might be due to a non-therapeutic dose. After platelet count normalization our patient was successfully switched to dabigatran.

Conclusions: With the severity of this case, we want to increase awareness for atypical presentation of HIT and second-line treatment for patients not responding to standard therapies.

PB1433 | HIT Antibody-induced Aggregation of Breast Cancer Cells

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Background: Heparin-induced thrombocytopenia (HIT) antibodies develop in ~3% of patients receiving heparin (H). Both typical and autoimmune HIT antibodies can bridge between platelet factor 4 (PF4)/H complexes and platelets followed by inducing platelet activation/aggregation that can cause life-threatening. The autoimmune HIT antibodies select their own PF4 antigens and strongly activate platelets. However, little is known about the role of these antibodies in cancer patients.

Aims: We investigate if HIT antibodies mediate platelet binding to the breast cancer cell and enhance thrombosis complications.

Methods: As a model, we tested with murine antibodies including RTO as a control and KKO as a typical HIT antibody. The human breast cancer cell line (MDA MB 231) was used. Single-molecule force spectroscopy (SMFS) was utilized to determine binding strengths between antibodies and antigens coated cells and to identify changes in the mechanical properties of cells. Confocal laser scanning microscopy (CLSM) and microfluidic flow were used to visualize the binding of platelets to cancer cells in 2- and 3-dimension (D).

Results: KKO antibody showed higher binding force than and RTO when interacting with cancer cells coated with PF4/H complexes but not wiht PF4 alone. The bound HIT complexes soften breast cancer cells. KKO interacted strongly when cancer cells were coated with preformed PF4/H complexes or with PF4 of ≥ 20μg/ml while RTO interacts weakly. In the presence of KKO and PF4/H complexes, the density of bound platelets on cancer cells was significantly higher than that in the absence of the complexes and the antibodies brought cancer cells close to each other in 3-D.

Conclusions: HIT antibodies bind to the membrane of breast cancer cells followed by mediating between cancer cells and platelets.

INHERITED THROMBOCYTOPENIAS

PB1434 | A Novel Missense Mutations of the WASp Gene Cause a Familial X-linked Thrombocytopenia

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Background: X-linked thrombocytopenia (XLT) is a milder form of Wiskott-Aldrich syndrome (WAS) that is caused by mutations of the gene encoding WAS protein (WASp), characterized by persistent or intermittent thrombocytopenia with absent or minor infections and eczema. In clinical practice, the disease is often initially diagnosed as idiopathic thrombocytopenic purpura (ITP) before genetic diagnosis.

Aims: To add new data to the genetic diagnosis of XLT.

Methods: We describe a patient with thrombocytopenia for more than 5 months, who was found to have thrombocytopenia at 6 months after birth. The mean platelet volume (MPV) was normal. Genetic analysis of the coding sequences showed a novel WASP gene missense mutation (c.1315C>T) at exon 10. Family history showed that his mother, younger sister and grandmother all had a heterozygous genotype of the WASP gene. In addition, all the males with this gene mutation in the maternal family had different degrees of thrombocytopenia without obvious eczema and severe infection.

Results: A mutation analysis revealed a c.1315C>T mutation in exon 10 of the WAS gene, which led to a change in the 439th amino acid from arginine to tryptophan, and the mutation was inherited from the patient's carrier mother.The mutation was suspected to be likely pathogenic by multiple protein analysis software.

Conclusions: This case report adds a new WAS gene mutation and provided a new inherited evidence for the clinical diagnosis of chronic/persistent thrombocytopenia.

PB1435 | Diagnostic Approach to Inherited Thrombocytopenias in a Low-Income Setting

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Background: Inherited thrombocytopenias (IT) remain a diagnostic challenge due to clinical and genetic heterogeneity. Although more than 30 genes have been identified, the underlying abnormality is unknown in half of the patients. Advent of next-generation technologies have represented significant advances although access is limited in low-income economies.

Aims: To rationalize resources for IT diagnosis in Argentina.

Methods: First, we applied a diagnostic algorithm (Balduini, 2003) based on phenotypic characterization followed by candidate gene sequencing and, second, whole exome sequencing (WES) was performed in an international center in undiagnosed patients after this algorithm.

Results: We included 114 patients from 50 pedigrees, 25 (0-73) years old, 68 (4-172) x10^9/L platelets; 68%, 30% and 2% had large, normal-sized and small platelets; 21% had syndromic forms: 11% hearing loss, 6% nephropathy, 7% hematologic malignancy, 2% myelofibrosis. By applying the algorithm, a conclusive diagnosis was reached in 27/50 (54%) pedigrees, 38% MYH9-RD; 4% Bernard-Soulier syndrome (1 monoallelic, 1 classic); 4% Gray Platelet Syndrome; 4% ANKRD26-RT; 2% FPD/AML; 2% Wiskott-Aldrich Syndrome. WES was undertaken in 8/23 (35%) pedigrees without diagnosis following the algorithm and known disorders were identified in 4 (1 FPD/AML, 1 ANKRD26-RT, 2 BSS:1 monoallelic, 1 biallelic), whereas no pathogenic variants in either known or new genes were detected in 4. Undiagnosed patients after the algorithm in whom WES was not performed suffered from mild isolated macrothrombocytopenia without distinctive features. Altogether, by this combined approach (algorithm+WES), a definitive diagnosis was identified in 31/50 (62%) pedigrees, which does not differ from the yield of NGS panels.

TABLE 1 Diagnostic yield in inherited thrombocytopenias

	1° step Algorithm (n=50)	2° step WES (n=8)	Combined approach Algorithm+WES (n=50)
MYH9-RD	19 (38%)	0 (0%)	19 (38%)
Classic BSS	1 (2%)	1 (12.5%)	2 (4%)
Monoallelic BSS	1 (2%)	1 (12.5%)	2 (4%)
Gray platelet syndrome	2 (4%)	0 (0%)	2 (4%)
FPD/AML	1 (2%)	1 (12.5%)	2 (4%)
ANKRD26-RT	2 (4%)	1 (12.5%)	3 (6%)
Wiskott-Aldrich	1 (2%)	0 (0%)	1 (2%)
With diagnosis	27 (54%)	4 (50%)	31 (62%)
Without diagnosis	23 (46%)	4 (50%)	19 (38%)

Conclusions: Careful clinical phenotyping allowed diagnosis in a substantial proportion of patients and MYH9-RD was the disorder most easily recognized by the algorithm. Restricting the application of NGS to patients with negative results after the algorithm allowed to optimize resources and improved the diagnostic yield, representing a feasible approach in low-income settings.

PB1436 | Highly Disturbed Platelet Ultrastructure in Two Families with Novel IKZF5 Variants and Inherited Thrombocytopenia

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Background: Heterozygous variants in the *IKZF5* gene, encoding transcription factor Pegasus, were recently discovered to be causal of inherited thrombocytopenia (IT). Platelets from patients with *IKZF5* variants were characterized by a paucity of alpha granules, empty vacuoles and empty membrane structures. Megakaryocytic proplatelet formation was impaired and light transmission aggregometry results varied from normal to reduced response to all agonists. It is currently unknown whether *IKZF5* variants could also be associated with immunodeficiency or developmental impairment.

Aims: We report two families with novel variants in *IKZF5* and present the results of platelet studies together with the results of immunodeficiency examination from one proband.

Methods: The study was approved by the regional ethics committee. Written informed consent was obtained from participants. Whole-genome sequencing of 101 genes, associated with platelet- and

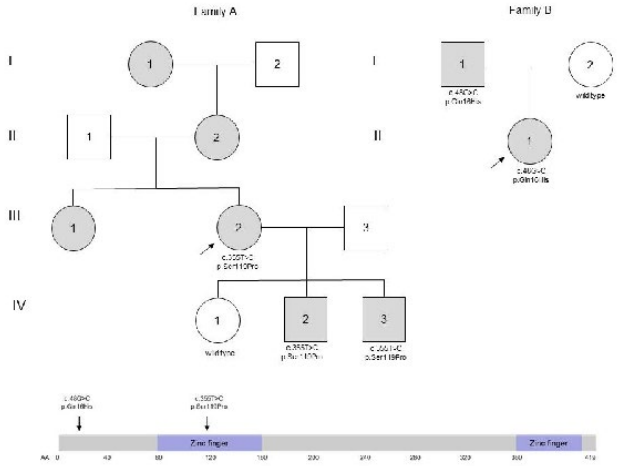

FIGURE 1 Pedigrees with co-segregation of IKZF5 variants. Grey color indicates thrombocytopenia, probands marked with an arrow

bleeding disorders, was performed. For downstream testing, flow cytometry, transmission electron microscopy (TEM) and immunofluorescence (IF) analysis of platelets were carried out.

Results: We analyzed 101 genes associated with inherited bleeding disorders in 90 index patients suspected of IT and identified two probands with specific variants in *IKZF5*. Platelet shapes, evaluated by TEM and IF, were highly abnormal appearing like elongated proplatelets with severe disturbances of the platelet cytoskeleton. By IF, NMMIIA and Filamin were diffusely distributed throughout the cell instead of presenting as a ring. Alpha granules were impaired evaluated by TEM. Expression of CD62P was reduced. In both families, a child carrying a rare *IKZF5* variants was affected by developmental delay. Due to recurrent infections, proband A was examined for immunodeficiency. The concentration of naive B-cells and T-cells were decreased, with evidence of reduced thymic output and impaired cellular immunity.

Conclusions: The unique morphology described in this study may help to improve the identification and classification of more cases with *IKZF5* associated IT. Variants in IKZF5 may be associated with other conditions.

PB1437 | Diagnosis of Inherited Platelet Disorders on a of Blood Smear: The One-Year Experience a Reference Laboratory

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Background: Inherited platelet disorders (IPDs) are rare diseases featured by thrombocytopenia and/or impaired platelet function. IPD diagnosis is challenging. The immune-morphological analysis of blood smears (by light- and immunofluorescence microscopy) is an effective and time-efficient tool to guide IPD diagnostic work-up.

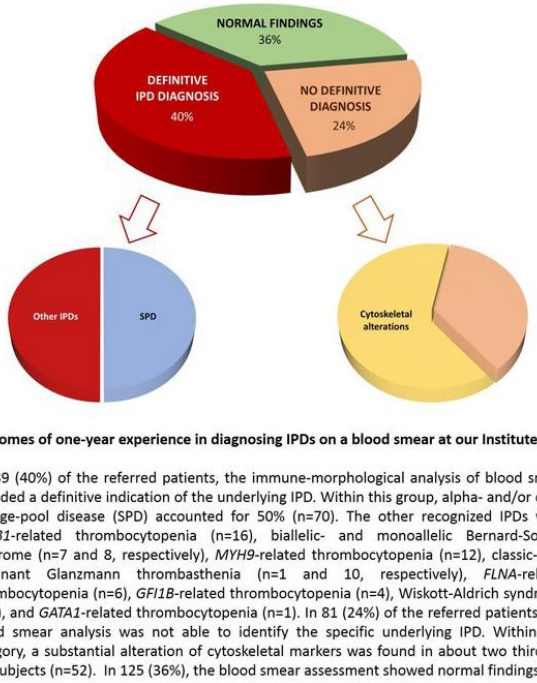

FIGURE 1 Outcomes of one-year experience in diagnosing IPDs on a blood smear at our Institute

It requires low blood volume (< 100 µL) and is applicable to shipped samples.

Aims: To report the one-year experience in diagnosing IPDs on blood smear at the reference laboratory of University Medicine Greifswald, Germany.

Methods: We systematically assessed outcomes of blood smear analyses referred for suspected IPD under clinical routine conditions from January to December 2019 (all study patients were excluded). For immunofluorescence assessment, a panel of 13 antibodies against specific platelet structures has been applied. The study complies with the Declaration of Helsinki.

Results: Out of 345 referred patients (female 62%, mean age of 27; 32% children), in 125 (36%) the blood smear assessment showed normal findings. 124 (36%) had low platelet counts and 76 (22%) overt alteration of platelet morphology (mainly platelet macrocytosis). Immune-morphological analysis of blood smears provided a definitive diagnosis in 139 (40%): alpha- and/or delta storage-pool deficiencies (n=70, 50%); *TUBB1*-related thrombocytopenia (n=16); biallelic-and monoallelic Bernard-Soulier syndrome (n=7 and 8); *MYH9*-related thrombocytopenia (n=12); classic- and dominant Glanzmann thrombasthenia (n=1 and 10); *FLNA*-related thrombocytopenia (n=6); *GFI1B*-related thrombocytopenia (n=4); Wiskott-Aldrich syndrome (n=4); *GATA1*-related thrombocytopenia (n=1). In 81 (24%) patients no specific diagnosis was possible, despite alteration of one or more platelet structures, among them alterations of cytoskeletal markers were found in 52 patients (64%) (e.g. alpha-, beta-1 tubulin or filamin A). The possible pathogenic role of such cytoskeletal defects currently represents a major research question.

Conclusions: Immune-morphological analysis of blood smears is a valid diagnostic tool for IPDs. The method is able to identify a subset

of patients probably worth of further investigations both for clinical and research purposes.

PB1438 | Clinical, Morphological and Functional Platelet Tests in Inherited Thrombocytopenia

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Background: Identification of new forms of inherited thrombocytopenia (IT) lead to their growing recognition. Distinguishing IT subgroups by clinical evaluation and platelet (PLT) studies is the first step to diagnosis and may suggest candidate disorders.

Aims: To characterize the most prevalent IT from a Portuguese Coagulopathies Center by the assessment of bleeding score (BS by ISTH-BAT), occlusion time (OT by PFA-200), PLT count, mean platelet volume (MPV) and immature platelet fraction (IPF), and to correlate these findings with IT variants.

Methods: The above-mentioned parameters were evaluated in 113 patients [31 ITGA2B/ITGB3-RT, 19 ANKRD26-RT, 16 TUBB1-RT, 15 ACTN1-RT, 13 MYH9-RD, 11 monoallelic Bernard-Soulier Syndrome (mBSS), 8 biallelic BSS (bBSS)] and 20 healthy controls.For the analysis, group comparisons were performed using Kruskal-Wallis test, followed by pairwise comparisons with Dunn's post-hoc test (adjusted with Bonferroni method). $p < 0.05$ was considered statistically significant.

Results: bBSS had significantly higher BS (8) as compared to mBSS (0), TUBB1-RT (0.5) and controls (0). All IT groups had significantly lower PLT counts compared with controls ($232x10^9$/L), except TUBB1-RT ($120x10^9$/L); the lowest PLT counts were observed in MYH9-RD ($25x10^9$/L) and bBSS ($45x10^9$/L). ANKRD26-RT had significantly lower MPV (10.4 fL) compared to other groups, except controls (11.1 fL); MYH9-RD showed significantly higher MPV (23.0 fL) compared to all groups, except bBSS (20.95 fL). All IT groups had higher IPF compared to controls (3.95%), except ANKRD26-RT (4.9%); MYH9-RD had the highest IPF values (61.1%). For PFA-Col/ADP and Col/Epi, bBSS (with the longest OT, > 300s), ITGA2B/ITGB3-RT, TUBB1-RT and MYH9-RD were significantly different from controls.

Conclusions: The BS and PLT parameters are useful for differential diagnosis of IT. Particularly, MYH9-RD and bBSS can be suspected based on the lower PLT counts and higher MPVs and OT can helpfully distinguish bBSS from other ITs. BS revealed limitations for subgroup discrimination.

PB1439 | Genotypic Analysis at Transition from Idiopatic Thrombocytopenic Purpura to Inherited Trombocytopenia

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Background: Inherited thrombocytopenia is a heterogeneous group of diseases with clinical and laboratory features. They may show autosomal recessive, dominant or X-linked recessive inheritance. The number of genes and mutations is gradually increasing. It may be caused by pathologies in the early and late stages of platelet production or the functional impairment of platelets may be seen.

Aims: The aim of this study is to present the genetic analysis results of patients who were sent to our clinic with the pre-diagnosis of ITP and considered inherited thrombocytopenias.

Methods: Patients were referred to our center from around seven cities and included those suspected of ITP. Genomic DNA was isolated from peripheral blood core cells. Libraries covering 12 genes were prepared according to the QIAseq Targeted DNA Panel (Thrombocytopenia Panel MPL, GP9, CYCS, ADAMTS13, ANKRD26, MPL, GP1BA, RUNX1, GP1BB, MYH9, WAS, GATA1) protocol and sequenced in Illumina MiSeQ system.

Results: Mutations were detected in the MYH9 gene in eight patients, the WAS gene in nine patients, the ANKRD26 gene in two patients, the GP9 gene in one patient, the GP1BB gene in one patient, the RUNX1 gene in one patient, the MPL gene in one patient, and the CYCS gene in one patient. Inherited thrombocytopenia mutation was found in 24 patients. Nineteen of the patients were male and five were female. Families were informed about the genetic transition and screened. Treatments such as apheresis platelet administration or allogeneic stem cell transplantation were planned according to their genetic diagnosis.

Conclusions: The first diagnosis of most patients presenting with thrombocy genetic analyzestopenia is ITP. However, the detailed history of the patient and his family, physical examination findings, and responses to treatments suggest inherited thrombocytopenia. Genetic analyzes is very important to make definitive diagnosis. It provides patients with the most appropriate treatment and provides genetic counseling to the family.

PB1440 | Clinical Use of Carica Papaya Leaf Extract in Thrombocytopenia in Bernard-Soulier Syndrome

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Background: Bernard-Soulier syndrome (BSS) is an inherited platelet bleeding abnormality, characterized by a prolonged bleeding time,

large platelets and thrombocytopenia. Many scientific works have been carried out to evaluate the benefit of different parts of papaya plant including fruits, seeds, leaves, rind, shoots, roots or latex.

Aims: To evaluate the efficacy of papaya leaves extract in management of thrombocytopenia and bleeding frequency in children with BSS.

Methods: Fifteen patients diagnosed as BSS were included in this study with age up to 15 years, during period from 2008 to 2018 at pediatric hematology unit of Zagazig University Hospital. Consent from patients and their life care were taken IRB of faculty of medicine of Zagazig university. Acceptance was confirmed. Full history and complete examination was taken especially for annual bleeding rate and frequency of platelet transfusion per year and Platelet counts before and after of start of papaya leaves extract (sweeten with honey given in dose of 5 ml to 10 ml according to age every day.

Results: There were significant decrease of bleeding episode per year after treatment with significant decrease platelet transfusion frequency. The platelet counts significantly increase after treatment with Papaya leaves extract.

Conclusions: Carica papaya leafs extract is effective,safe and cheap for treatment of Bernard-Soulier syndrome. Further studies should put in consideration.

PB1441 | MYH9-Related Inherited Thrombocytopenia: 14 Newly Diagnosed Chinese Pedigree

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Background: MYH9-related disease is a rare autosomal dominant inherited disease, characterized by thrombocytopenia, neutrophil inclusion bodies, and giant platelets. At present, only over 80 mutations in more than 200 families have been found in the world. Because of the low incidence, the basic characteristics of the disease remain unclear.

Aims: Through the 14 Chinese pedigree, we wanted to learn more about the clinical manifestation,laboratory tests, diagnostic method and mutation spectrum of the rare thrombocytopenia disease.

Methods: These patients visited Tianjin Blood Diseases Hospital for thrombocytopenia and the MYH9 gene mutations were detected by the next generation sequencing (NGS). We collected the clinical information about the patients and also did blood routine, urine routine, liver and renal function tests, wright stain of the blood smear, immunofluorescence staining of neutrophils and electron microscopic examination of platelets.

Results: Through NGS, we newly found 14 Chinese pedigree, including 16 patients of MYH9 mutations. All the mutations detected were missense mutations. Two mutations, c.4258C>G(p.Q1420E) in exon 31 and c.418G>A(p.G140S) in exon 3 were first reported around the world. The median age of these patients ranged from 6 months and 37 years old). The median platelet count ranged from 1-61 × 10^9/L. The bleeding symptoms were mild in these patients, including purpura, epistaxis and menorrhagia.One patients had unexplained abnormal liver function, mainly manifested as the elevation of liver enzyme. one patient was diagnosed with cataracts and another was found albumen and blood in urine. Giant platelets were found in the blood smear and neutrophils include bodies were found by the immunofluorescence staining. Chaotic internal structure of platelets was observed by electron microscope.

Conclusions: The abstract reported 14 newly diagnosed Chinese pedigree, including two new pathogenic mutations. The bleeding syptoms of these patients were mild. Immunofluorescence staining of neutrophil include bodies may be necessary for diagnosis of MYH9-related thrombocytopenia.

PB1442 | Refractory Thrombocytopenia with TUBB1 Variants: Clinical Features and Responses to Eltrombopag Treatment

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Background: Tubulin β-1 (TUBB1) is a major β-tubulin isotype expressed in megakaryocytes (MK) and platelets. TUBB1 mediates pro-platelet formation and transport of cytoplasmic contents to proplatelet. Studies have shown that TUBB1 genetic variations or polymorphisms can lead to thrombocytopenia, or are associated with treatment failures in immune thrombocytopenia.

Aims: Recently, thrombopoietin-receptor agonists (TPO-Ras) have been used in the treatment of thrombocytopenia. Whether TUBB1 variants impact the efficacy of eltrombopag therapy still need to be investigated.

Methods: Based on NGS method, we detected 81 cases with TUBB1 genetic variants out of 310 patients who failed to respond to a 4-week conventional corticosteroids therapy. Patients with severe thrombocytopenia among them received eltrombopag treatment. The clinical manifestations and treatment effects of these patients were analyzed retrospectively.

Results: 81 cases of TUBB1 genetic variants included common variants (MAF > 1%) R307H and Q43P, rare variants (MAF < 1%) R318W, T107P, R241T and newly detected variant G38R and T149I. Among these cases, 39 patients had moderate thrombocytopenia, the other 42 patients had severe thrombocytopenia (< 30 × 10^9/L). 31 of these patients were treated with eltrombopag for 4 - 6 weeks, of which only 5 achieved major responses, 7 transiently responded, and the remaining 19 were ineffective. Eltrombopag is effective mainly in R307H carriers, while patients with other variants responded significantly lower to eltrombopag than the reported average effectiveness (Table 1). Four patients with rare variants were treated with cyclosporine after ineffective eltrombopag therapy, and two of them partly responded (platelets > 50 × 10^9/L).

TABLE 1 Genetic features and response to eltrombopag treatment of TUBB1 variant carriers

Table1. Genetic features and response to eltrombopag treatment of TUBB1 variant carriers in patients with refractory thrombocytopenia

TUBB1 variant	Cases (% of rTP)	MAF in EAS (%)	Genetic status	Cases of eltrombopag	Major response	Transiently response	No response
R307H	47(15.2)	17.4	het	15	5	3	7
Q43P	22(7.10)	7.71	het	9	0	3	6
R318W	2(0.65)	0.41	het	2	0	0	2
T107P	1(0.32)	0.16	het	1	0	0	1
T149I	1(0.32)	NA	het	1	0	1	0
G38R	3(0.97)	NA	het	1	0	0	1
R307H+Q43P	5(1.61)	1.33	c.het	2	0	0	2
Total	81	-	-	31	5 (16.1%)	7 (22.6%)	19(61.3%)

rTP, refractory thrombocytopenia; MAF, minor allele frequencies; EAS, east Asian; het, heterozygous; c.het, compound heterozygous.

Conclusions: R307H is the most common TUBB1 variant in patients with refractory thrombocytopenia. Eltrombopag was partly effective in increasing platelet counts in patients with R307H. But patients with other TUBB1 variants, especially those with rare variants, do not respond well to eltrombopag. For these patients, immune modulatory therapies or other therapeutic options should be considered.

PB1443 | Phenotype and Characteristics of a Cohort of Macrothrombocytopenic Patients Related to the AlphaIIbbêta3 Variants

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Background: Variants of *ITGA2B or ITGB3*, genes coding for the alphaIIbbêta3 glycoprotein, have been identified as responsible for a mild form of inherited macrothrombocytopenia (IMT), named Glanzmann Thrombasthenia (TG)-like. These variants lead to constitutionally active alphaIIbbêta3 integrin, and difficulty of megakaryocytes to reorganize their cytoskeleton for proplatelets production.

Aims: Description of the clinical and biological phenotype of a cohort of patients bearing variants implicated in TG-like macrothrombocytopenia.

Methods: Three hundred thirty patients with inherited thrombocytopenia were analysed for *ITGA2B* and *ITGB3* variants by NGS sequencing. Variants identified were additionally looked for in available family members by Sanger sequencing. Clinical data were prospectively extracted from clinical files. Definitions for platelets size are: giant platelets: > or = 8 µm; macroplatelets: > or = 4 µm).

Results: We identified the bêta3 p.Asp749Asn variant in one woman and the alphaIIb p.Arg1026Trp variant in 15 patients (8 females) from 8 unrelated families. Five families were originated from French West Indies.

Patients phenotype associated (i) moderate thrombocytopenia (platelet count: mean: 74 ± 35.10^9/l; range: 30-92), (ii) few giant platelets (mean: 3%) and numerous macroplatelets (mean ± SD: 25 ± 22%) (iii) numerous degranulated or hypogranulated platelet (mean: 30%), with for some platelets only one single central granule and (iv) moderate bleeding tendency.

AlphaIIbbeta3 receptor expression on platelets was low compared to normal control: mean ± SD: 56%±22%. Platelet functions by aggregometry were not assessable due to low platelets counts.

Conclusions: We reported 16 patients with IMT harbouring alphaIIb p.Arg1026Trp or bêta3 p.Asp749Asn variants. The p.Arg1026Trp variant is overrepresented in patients originated from French West Indies. The diagnosis of TG-like IMT could be evocated by careful analysis of blood films, the most prominent findings being macroplatelets with degranulation, a few of them harbouring only one central granule and by evidence of alphaIIbbêta3 low expression on platelets. It should be confirmed by the genetic analysis.

PB1444 | Flow Cytometry of Platelets: A Way for Distinguishing Patients with MYH9-related Disease from Patients with Other Inherited Thrombocytopenias and Immune Thrombocytopenia

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Background: Platelet size is one of the important distinguishing features for different inherited thrombocytopenias (ITs) and immune thrombocytopenia (ITP). MYH9-related disease (MYH9-RD) is an IT that can be suspected if peripheral blood smear shows Dohle-like cytoplasmic inclusion bodies in neutrophils or if abnormal localization of non-muscle myosin heavy chain IIA is detected by means of immunofluorescence microscopy. Giant size of platelets is another diagnostic feature in MYH9-RD. But mean platelet volume can be underevaluated by the electronic counter in patients with extreme platelet macrocytosis. Flow cytometry of platelets is another way to assess platelet size among other parameters.

Aims: To assess the possibility of distinguishing patients with MYH9-RD from patients with other ITs and ITP based on parameters of flow cytometry of platelets.

Methods: We enrolled 24 patients (17 children and 7 adults) with MYH9-RD, 35 patients with other ITs and 111 patients with

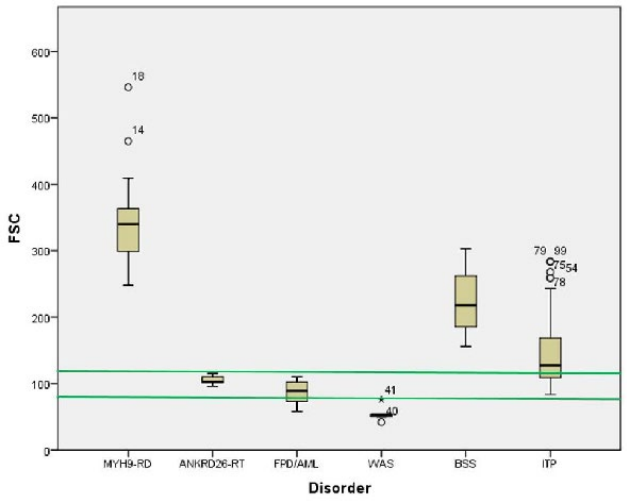

FIGURE 1 FSC prior to activation in different ITs and ITP. Dots with patients' index numbers mark graph outliers. Green lines indicate normal range

persistent and chronic ITP. Diagnosis was confirmed by identification of causative mutations in all cases of ITs. Forward scattering (FSC) parameter prior to activation was used for characterization of platelet size. ROC curve analysis was used to identify optimal cut-off values.

Results: Identified cut-off values for FSC had 96.8% specificity and 100% sensitivity in distinguishing MYH9-RD from other ITs and 93.1% specificity and 100% sensitivity in distinguishing MYH9-RD from ITP. AUC was 0.992 (CI 95% 0.973; 1.00) and 0.991 (CI 95% 0.978; 1.00) respectively. Based on FSC parameter patients with patients with Bernard-Soulier syndrome (BSS) had the nearest to MYH9-RD platelet size among all ITs, but BSS and MYH9-RD were easily separated based on expression of CD42b.

Conclusions: Diagnosis of MYH9-RD can be suspected not only after light or immunofluorescence microscopy of peripheral blood smear but also by means of flow cytometry of platelets. Patients with MYH9-RD can be distinguishing from patients with other ITs and ITP based on FSC parameter.

PB1445 | Determination of the Genotype of von Willebrand Disease Type 3

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Background: Von Willebrand disease (vWD) is an autosomal recessive inherited bleeding disorder caused by a defect of von Willebrand factor (vWF). vWF plays a crucial role in platelet adhesion to vessel walls after injury and is a carrier protein for factor VIII (FVIII). vWD has several subtypes that can be caused by functional or quantitative defects of vWF. It might be sometimes difficult to differentiate type 3 vWD (vWD3) from type 1 vWD (vWD1). We diagnosed vWD3 patients by undetectable vWF and propeptide (vWFpp). Patients with a higher level of vWFpp were excluded as vWD1 or acquired vWD.

Aims: To determine the genotype of vWD3 in our patients.

Methods: We have tested 9 samples from patients monitored in our center diagnosed as vWD3. We examined FVIII:C, vWF (Ag, RCo, propeptide, functional inhibitor and multimeric structure). Genomic DNA was analyzed by Sanger sequencing and MLPA for the *vWF* gene mutations.

Results: We detected vWF:Ag under 0.03 UI/dL (0.003-0.03), vWF:Rco under 0.06 UI/dL (0.005-0.06), undetectable vWFpp and multimers. The average FVIII:C was 3.4% (±1.4). Overall, we found 14 different mutations. Mutations were mostly located in the propeptide region. All patients were combined heterozygotes; we detected 2 different heterozygous mutations at every patient except one. Three of index patients had the same known deletion mutation in exon 18 p.Pro812Argfs*31. Every patient had at least one null mutation - nonsense or frameshift mutation. Two patients had large deletions of exons - delEx1_Ex3, delEx19_Ex52; one of them was not reported in the EAHAD database. We detected 3 missense mutations; one of them not described so far. This novel mutation was screened with prediction software programs and designated as disease causing.

Conclusions: All vWD3 patients were confirmed by causative mutations, most of them were null mutations. We have found nine novel mutations.

PB1446 | Variants of *GFI1B*: Phenotype/Genotype of a Series of 9 New Cases

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Background: Variants of *GFI1B*, the gene coding for the *Growth Factor Independent 1B transcription repressor*, have been recently identified as responsible of a mild form of inherited thrombocytopenia, sometimes characterized by a reduction of alpha granules.

Aims: The aim of this study is a better characterization of this *GFI1B*-Related Disease (RD).

Methods: High-throughput gene panel (NGS) sequencing was performed on patients with suspected inherited thrombocytopenia. Family relatives were diagnosed by Sanger sequencing. Clinically and laboratory findings were analysed.

Results: NGS panel sequencing identified eight missense variants of *GFI1B* in nine out of three hundred thirty probands. Two members family were additionally diagnosed. Whereas 2 variants were already known, the others were novel. The six new variants affected highly conserved amino-acid residues and were considered deleterious *in silico*. All the variants were in the first Zn Finger domain. Analysis of the laboratory findings revealed for all the patients but one a mild thrombocytopenia (mean±SD: 127 ± 90.10^9/l-[88-246]). Platelet morphology analysis showed the presence of some giant platelets (defined as platelet size over than red cell size) (mean 3%, [1-5]) and especially the presence of numerous macroplatelet (defined as platelet size under red cell size and over ½ red cell size) (mean±SD: 25 ± 16%, [10-36]). Only few data were found about a bleeding tendency: only one woman with menorrhagia.

Overall, the platelet CD34 aberrant expression analysed by flow cytometry was positive for all the five novel variants tested.

Conclusions: We identified six novel variations of *GFI1B* and confirmed previous results indicating that *GFI1B*-RD with variant located in the first Zn Finger domain was a mild macrothrombocytopenia with low risk of bleeding. The platelet CD34 expression was positive in every case, and seemed to be a good tool to apprehend the pathogenicity of *GFI1B* variants.

PB1447 | New Clues to *GNE* Gene Role in the Pathogenesis of Inherited Thrombocytopenias

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Background: The *GNE* gene encodes for a kinase with a key role in sialic acid biosynthesis and its mutations are classically associated with myopathy and sialuria, displaying an autosomal recessive and autosomal dominant inheritance pattern, respectively. Nevertheless, in a few recent cases, *GNE* variants have be associated with thrombocytopenia both with and without muscle weakness.

Aims: We present a two-year-old child of a consanguineous couple with a significant platelet count reduction, intending to disclose the molecular causes underlying the disease.

Methods: The proband and his family members, including both the parents and the two healthy sisters, underwent whole exome sequencing and Sanger sequencing analysis, respectively. We assessed variant pathogenicity by western blot, protein 3D-structure analysis and sialic acid assays to evaluate the mutation effect both on protein structure and function.

Results: The patient was found to carry a novel homozygous variant, c.1724C>G (p.Thr575Arg) of the *GNE* gene. No candidate variants were identified in the other known thrombocytopenia-causing genes. The pedigree analysis confirmed segregation within the family in accordance with an autosomal recessive pattern. The western blots of proband's cell culture unveiled a considerable reduction in the protein expression level in comparison to the wild-type control, suggesting that the mutant protein is unstable. Moreover, the pathogenic effect of p.Thr575Arg was further supported by the altered three-dimensional structure of GNE ATP pocket and a defective sialylation, which could affect platelet production and/or survival in the patient.

Conclusions: Considering the growing cases similar to our proband reported in the literature, we are confident that including *GNE* among the genes associated to inherited thrombocytopenias could significantly contribute to understanding the role of *GNE* in inherited thrombocytopenia, as well as patients' appropriate molecular diagnosis and clinical management.

PB1448 | Platelets Function and Cytoskeletal Organization in a Patient with a Novel Variant in ACTB Causing Macrothrombocytopenia

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Background: Inherited thrombocytopenia (IT) is a highly heterogeneous group of genetic disorders associated with a low platelets count. The clinical features of affected individuals vary considerably and range from asymptomatic to severe bleeding tendency. Several forms of IT are associated with variants in genes encoding for cytoskeleton components. The process of megakaryocyte maturation and the release of newly platelets are essential steps and both depend heavily on the actin and microtubules cytoskeleton components. The *ACTB* gene encodes for the cytoplasmic beta (β)-actin. Germline variants in the *ACTB* gene have been associated to several different genetic disorders and some patients have inherited thrombocytopenia.

Aims: To evaluate platelets function and morphology in a patient with thrombocytopenia and with a *de novo* heterozygous missense variant in the ACTB. To study actin and microtubules cytoskeleton interactions in this type of actinopathy in patient megakaryocytes and thrombocytes.

Methods: Platelets aggregation was analysed by Multiplate® aggregometry. Clot formation was evaluated by rotational thromboelastometry (ROTEM®). Platelets morphology was analysed using flow cytometry and immunofluorescence microscopy. Subsequently, the expression and interaction of cytoplasmic β-actin, F-actin and tubulin were analysed in patient-derived megakaryocytes and thrombocytes by confocal microscopy.

Results: No apparent *in vitro* defects in platelets function analysed with Multiplate® and ROTEM® were observed. 60% of platelets were large in size (6-9 μm diameter). The patient megakaryocytes were roundish in shape and the formation of proplatelets was reduced. Immunofluorescence analysis showed a disorganization of β and F actin cytoskeleton filaments, probably due to an uncorrected polymerization and depolymerisation of actin.

Conclusions: Examination of β-actin in patient thrombocytes and megakaryocytes suggests that this β-actin variant inhibits the final stages of platelet maturation due to dis-organization between β and F actin filaments. The abnormal expression and polymerization of ACTB and the resulting changes to the cytoskeleton were shown to be associated with macrothrombocytopenia.

PB1449 | Bernard - Soulier Syndrome (BSS): From Identification of the Variants to their Functional Studies

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Background: Inherited thrombocytopenias (ITs) are rare diseases displaying low platelet count and high heterogeneity. Nowadays, mutations in more than 30 different genes are known to cause IT, but they account for approximately 50% of the patients.

Bernard-Soulier syndrome (BSS) is an IT characterized by defects of GPIb-IX-V complex, the platelets' von Willebrand factor receptor. BSS occurs in two forms: biallelic severe and monoallelic moderate. Most of the mutations identified in the BSS genes (GP1BA, GP1BB, GP9) prevent the complex expression; consequently, platelets are defective to adhere to the vascular subendothelium.

Aims: Our work is aimed at understanding the role of the BSS variants identified by NGS, as disease-causing mutations.

Methods: We applied NGS analysis to identify mutations in our cohort of IT families (N = 141). After cloning wild type and mutant GP1BA cDNA into an expression vector, protein level was evaluated both by western blot and flow cytometry.

Results: We found more than 20 rare variants that could be categorized as causative of biallelic (N = 4) or monoallelic forms of BSS (N = 20), but also heterozygous variants (N = 10) of uncertain significance that could explain a series of ITs cases. The frequency of the variants identified in our cohort is higher than in the general population, moreover, most of them are novel missense substitutions. Focusing on the GP1BA gene, we found that the overexpressed mutant proteins are differently stable and/or expressed on cellular membrane, suggesting the pathogenetic role of some of them.

Conclusions: Our work extends the spectrum of mutations identified in BSS genes, underlying the importance of genetic testing in recognizing both monoallelic and biallelic forms of BSS. Moreover, we have identified private candidate variants that could be associated with the BSS monoallelic. Once confirmed their effect on GPIb-IX-V receptor, we could be able to explain thrombocytopenia in almost 5% of the cases without a molecular diagnosis.

PB1450 | Inherited Thrombocytopenia Associated with Haematological Malignancies

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Background: *ANKRD26*-Related Thrombocytopenia (*ANKRD26*-RT), Familial Platelet Disorder with propensity to Acute Myelogenous Leukemia (FPD/AML), and *ETV6*-Related Thrombocytopenia (*ETV6*-RT) are three rare autosomal dominant inherited thrombocytopenias caused by mutations of the ANKRD26, RUNX1, and ETV6 genes, respectively. Since they are associated with an increased risk of developing hematological neoplasms, such as myelodysplastic syndrome, acute myeloid leukemia, and acute lymphoblastic leukemia, they have recently been recognized by the WHO as a unique nosological entity called "myeloid neoplasms with germ line predisposition and preexisting platelet disorder"

Considering that these diseases lack specific pathognomonic signs that could help in differential diagnosis, the screening for mutations of ANKRD26, RUNX1, and ETV6 genes is the only approach available for identifying these patients.

Aims: The main goal of this study was to better characterize these three forms of IT providing insights into their pathogenic mechanisms.

Methods: Mutational screening was perform using a targeted Next Generation Sequencing approach developed in our laboratory for the simultaneous analysis of 28 IT genes.

Functional studies are based on gene reporter assays and immunofluorescence:

Results: Since identification of genetic variants does not necessarily confirm the diagnosis because the effect of missense or silent and intronic variants remains of uncertain significance, we have set up

- at least for ANKRD26 and ETV6 - luciferase based assay that allow us to identify 5 mutation hiting the 5'UTR of ANKRD26 gene in 44 families and 13 families carrying 6 different mutation on ETV6 gene. Moreover, we performed immunofluorescence assays showing that the mutant ETV6 proteins were unable to enter the nucleus and are retained in the cytoplasm.

Conclusions: Thanks to a consolidated collaboration with IRCCS San Matteo (Pavia, Italy), we have collected one of the largest cohorts of families with *ANKRD26*-RT, FPD/AML, and *ETV6*-RT provinding insights on molecular characterization of *ANKRD26*-RT and *ETV6-RT* and on pathogenetic mechanism of ETV6 mutations.

PB1451 | First Autosomal Recessive Variant in *TUBB1* Impairs Proplatelet Formation and Results in Inherited Macrothrombocytopenia

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Background: Tubulin β1, expressed in megakaryocytes (Mks) and platelets, forms microtubules which mediate pro-platelet formation. Only 4 families with heterozygous, autosomal dominant variants in *TUBB1* have been reported.

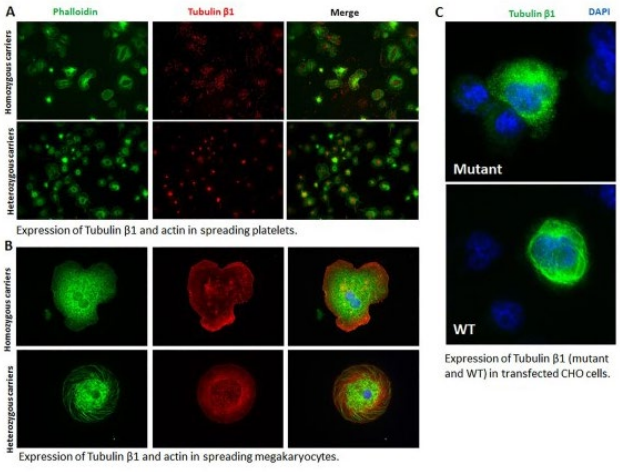

FIGURE 1

Aims: Characterization of a family with macrothrombocytopenia enrolled in the Spanish project "Functional/molecular characterization of patients with Inherited Platelet Disorder".

Methods: Platelet phenotyping included: blood count/smear, platelet aggregation (LTA), glycoproteins (GP) and activation by flow cytometry; cytoskeleton proteins in resting/spreading platelets by immunofluorescence. Patient's DNA was analyzed with a HTS-gene panel. Proplatelet formation was evaluated in Mks differentiated from CD34 + cells purified from blood. CHO cell transfection model was established to assess the pathogenicity of the candidate variant.

Results: The proband (52-year-old woman) and her brother displayed congenital macrothrombocytopenia ($50x10^9$/L) with 15% enlarged platelets (diameter:4.71 μm). GPs expression, fibrinogen binding, granule secretion and LTA were normal. HTS analysis revealed the novel homozygous *TUBB1* variant c.326G>A [p.Gly109Glu] in the proband and her brother. Unaffected family members were heterozygous carriers. Tubulin marginal band in resting platelets was undetectable in homozygous patients and normal in controls/heterozygotes. Platelet spreading on poli-L-lysine was unaltered in all cases. Tubulinβ1 formed aggregates in spread platelets/Mks in homozygous patients, while it was normally distributed in heterozygous carriers (FigureA&B). The localization of other cytoskeleton proteins (actin, actinin, DIAPH1, filamin and MYH9) was normal in all cases. Only homozygous patients displayed a defect in proplatelet formation (homozygous:< 5%; heterozygous/control:15% Mks forming proplatelets). p.Gly109Glu tubulinβ1-transfected CHO cells showed no incorporation of mutant tubulinβ1 into microtubules and the formation of punctuated aggregates similar to those observed in Mks/platelets from the homozygous patients (FigureC).

Conclusions: We report the first autosomal recessive variant in TUBB1 (p.Gly109Glu) associated with macrothrombocytopenia due to a defect in proplatelet formation. Tubulinβ1 is severely disorganized in platelets/Mks from homozygous carriers. However, platelet and Mk spreading is not impared, revealing that tubulinβ1 is not crucial for spreading. [PI17/01311-FMMAP172142019]

PB1452 | Identification and Functional Study of a New Germline Variant in *Mecom* Gene

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Background: Inherited thrombocytopenia (IT) is a group of rare genetic disorders characterized by reduced platelet count associated to variable bleeding tendency and/or other clinical defects. Next Generation Sequencing (NGS) led to the identification of novel

forms of IT and so far variants in over 30 different genes are known to cause IT that account for approximately 50% of patients suggesting that novel forms have yet to be identified.

Monoallelic variants in *MECOM* were recently associated with an IT called *MECOM*-associated syndrome and currently only three mutations have been evaluated through functional analysis.

Aims: Due to the uncertain significance of missense variants in functional domains of transcription factors we aim to investigate the functional effect of a novel variant in *MECOM*.

Methods: Mutational screening was performed using NGS panel. As described by literature we performed a luciferase reporter assay to test the transcriptional activity of identified variant on AP1 promoter, an indirect target of *MECOM*, in two different cell lines.

Results: We identified a monoallelic missense variant in *MECOM* (c.1901 C>T; p.P634L) in a patient with Congenital AMegakaryocytic Thrombocytopenia (CAMT). The variant identified is located in the Repression Domain and didn't affect the Zinc Finger 8 (ZF8) where all the previously known variants are reported. We showed that the transcriptional activity of p.P634L was significantly different from that of Wild Type form in both DAMI and HEK cell lines.

Conclusions: p.P634L is overall the fourth missense *MECOM* variant evaluated through functional studies. We showed that not only mutations hitting the ZF8 domain might cause CAMT. The observed alteration in the transcriptional activity of p.P634L was opposite between the cell lines highlighting the strong influence of the cell context in indirect reporter assays. For this reason a new, direct assay should be developed to analyze the functional effect of *MECOM* missense variants.

PB1453 | *Ex vivo* Observation of Platelet-granulocytes Interactions in Pediatric Patients with Congenital Platelet Defects

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Background: The role of granulocytes in thrombus formation and their interaction with platelets in normal and thrombocytopenic states are not fully understood and have been the focus of recent research.

Aims: Observation of thrombus formation and granulocyte activity in whole blood of normal and thrombocytopenic subjects in parallel-plate flow chambers (*ex vivo*).

Methods: Whole hirudinated blood of healthy pediatric donors (n = 12) or pediatric patients with Wiskott-Aldrich syndrome (WAS; n = 7), immune thrombocytopenia (ITP; n = 4), Glanzmann thrombastenia (GT; n = 4), MYH-9 linked macrothrombocytopenia (MYH-9; n = 4), ANKRD26-associated thrombocytopenia (ANKRD26; n = 2) was used in the experiments within 3 hours of blood collection. The samples were perfused through parallel-plate flow chambers coated with fibrillar collagen type I. Platelet aggregate formation and CD66b-positive cell activity were assessed by low-angle fluorescent and DIC microscopy. Numerical analysis of the images was performed in ImageJ software and a developed for this study Python 3.7 application.

Results: Formation of platelet aggregates and granulocytes' movement were observed simultaneously on the flow chamber surface. The movement of granulocytes appeared to occur along the collagen fibrils towards platelet aggregates. Collection of procoagulant platelets by granulocytes was frequently observed. The following criteria were derived: number of leukocytes per field of view (N_L), leukocyte crawling velocity (V_L), thrombus area (A), ratio of N_L to A (R_{AN}), percentage of spread leukocytes (S). All of the patients had decreased A (as expected) and increased S. N_L in patients with ITP, MYH9 and GT was significantly lower than in control group. V_L was significantly lower in WAS, ITP and MYH9. R_{AN} was higher than control in WAS, GT and MYH9.

Conclusions: During thrombus formation platelet-granulocyte interaction is characterized by neutrophil chemostaxis to activated platelets and their scavenging of procoagulant platelets. The relative activity of granulocytes in thrombi was generally higher in patients with thrombocytopenic disorders in comparison with healthy controls.

PLATELET ANTAGONISTS AND NOVEL THERAPEUTICS

PB1455 | High-Dose Dexamethasone Effectively Replaces Traditional Full-Dose Prednisone as the First-Line Treatment of Children Immune Thrombocytopenia: A Prospective Randomized Single-Center Study

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Background: To address the question whether it will be possible to apply the same HDD application as the first-line treatment of children ITP.

Aims: To evaluate results of HDD replacing full-dose prednisone as first-line treatment for newly diagnosed children primary immune thrombocytopenia (ITP).

Methods: A prospective randomized study. The cohort were randomized allocated to "HDD group" and "PDN group". The response rate of platelet count in two short-term points and two

long-term points, the bleeding score with the improving rate and Glucocorticoid related adverse effects in 1 month point after treatment were collected.

Results: 211 children were enrolled, allocated to HDD group (n = 110) and PDN group (n = 101).

(1) Platelet count response rate:: ① The one-week point: Total response (TR) (92.7% vs 93%, P = 1.000). Complete response (CR) (67.3% vs 78.2%, P = 0.001) and Partial response (PR) (25.5% vs 14.9%, P = 0.028).

②The one-month point: TR (93.6% vs 96.0%, P = 0.542), CR (76.3% vs 82.2%, P = 0.398) and PR (16.4% vs 13.8%, P = 0.702)

③The response rate in 6 m point and the 12 m point without differences.

(2) Although fewer bleeding events in the HDD group during 1 month treatment (10% vs 15%), without different (P = 0.105). Patients who couldn't reach to CR at 1 month that does not recover at 6 months was similar in two groups (39.3% vs 50%, P = 0.532), also at 12 months (36% vs 38.9%, P = 0.462)

(3) Glucocorticoid related adverse effects: high blood pressure, high intraocular pressure, abdominal discomfort, mood changes occurred in two group without differences (16.4% vs 14.9%, P = 0.850;4.5% vs 1% P = 0.449; 13.6% vs 19.8%,P = 0.267, 5.5% vs 12.9%,P = 0.266). Cushing face and infection in the PDN group were higher than HDD group (80% vs 10%, P = 0.001, 26% vs 11.8%, P = 0.012), with statistically differences.

Conclusions: With the same efficacy and less Glucocorticoid related side-effects, HDD could be the better first-line treatment for Children ITP comparing to traditional PND.

PB1458 | Tyrosine Kinase Inhibitors (TKIs) Targeting Syk and BTK Signaling Differentially Affect PI3K Organization and Inhibit Platelet Function

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Background: Tyrosine kinase inhibitors (TKIs) are highly successful, first-line therapeutics used in the treatment of hematologic malignancies and even offer promise against a range of immunological, inflammatory, and cardiovascular conditions. Unfortunately, TKIs targeting Bruton's tyrosine kinase (BTK), are associated with a number of side effects, particularly on platelets, such as high bleeding risks and cardiovascular complications, whereas Syk inhibitors do not, but maintain antiplatelet effects. It remains unclear whether and to what extent Syk and BTK inhibitors affect platelet function.

Aims: Determine the effects of 12 Syk and BTK inhibitors (Bay 61-3606, R406/fostamatinib, entospletinib, TAK-659, ibrutinib, acalabrutinib, ONO-4059/tirabrutinib, AVL-292/spebrutinib, CG' 806, BMS-935, BMS-986, fenebrutinib) on platelet functional responses.

Methods: Platelets are isolated and purified from human donors' whole blood and treated with the selected panel of TKIs (see Aims). Under each inhibitor condition, platelets are stimulated and assessed for adhesion on collagen/fibrinogen surfaces, P-selectin release, PAC-1 binding, ATP secretion, proteins' phosphorylation levels, and fluorescence imaging to evaluate each inhibitor' effects on platelet function.

Results: The selected TKIs (see Aims) significantly reduced GPVI-mediated platelet adhesion on collagen (27-70% reduction) and integrin-mediated platelet spreading on fibrinogen (from 33.43 μm^2 to 18.08-30.97 μm^2, average surface area). Downstream of GPVI-mediated activation, the selected TKIs significantly inhibited ATP secretion (53-67% reduction) and reduced the percentage of platelets exposing P-selectin (to 1.37-6.64% vs.19.2% of untreated platelets). Similarly, the selected TKIs significantly reduced the percentage of platelets binding integrin-mediated PAC-1 (to 0.91-9.09% vs. 43.33% of untreated platelets). All TKIs inhibited GPVI-mediated phosphorylation of PLCγ2, PKCδ and Akt T308, but differentially affected the phosphorylation of PI3K regulatory subunit, Akt S473, and Akt substrates. Fluorescence imaging of PI3K shows co-localization with tubulin when untreated, but is disrupted, disorganized, or absent in platelets treated with the selected TKIs.

Conclusions: Clinically utilized TKIs targeting Syk and BTK inhibit platelet functional responses and differentially alter PI3K signaling and organization.

PB1459 | Identification of the Sequence Specificity in C-Src SH3 Required for Binding to Integrin B3 as a Novel Anti-Thrombotic Target without Compromising Primary Hemostasis

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Background: Targeting platelet outside-in signaling inhibits thrombosis without compromising hemostasis. We previously showed that blockade of outside-in signaling was achieved by targeting the interaction of integrin β3 with c-Src. The c-Src SH3 is known to bind the canonical proline-rich motif and thereby regulates many biological processes. Therefore, it is essential to identify the sequences within SH3 responsible for the β3/SH3 interaction so that one may tell it apart from the canonical binding in the context of targeting strategy.

Aims: To identify the targets of integrin β3 in c-Src SH3 and evaluate their roles in anti-thrombosis and hemostasis in transgenic mouse model.

Methods: A synthetic small molecule known to interrupt the β3/SH3 interaction was used to mimic the β3 cytoplasmic domain. NMR, chemical shift perturbation and SPR were applied to identify the targets. Co-IP was assayed for β3/Src interaction and platelet

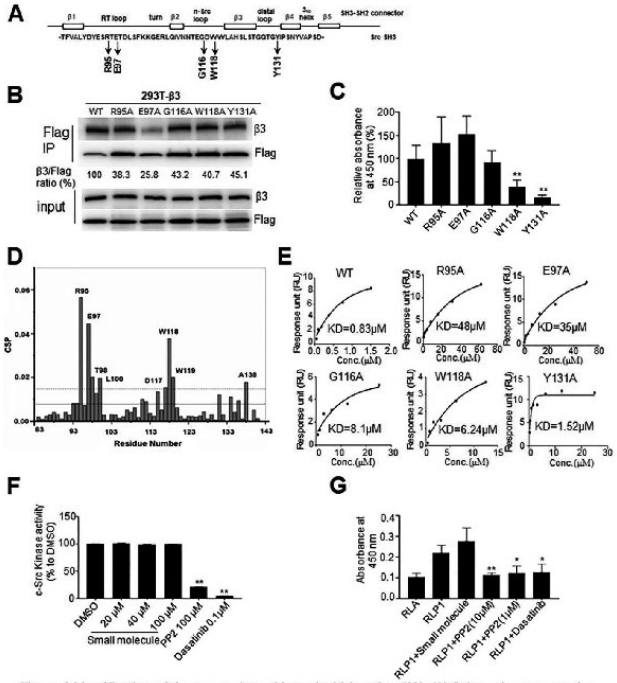

Figure 1 Identification of the target sites of integrin β3 in c-Src SH3. (A) Schematic representation of the structure of c-Src. (B) Co-IP results show the interaction of integrin β3 with WT or mutant (R95A, E97A, G116A, W118A and Y131A) c-Src in 293T cells. (C) ELISA results show the amino acids in c-Src SH3 critical for binding the RLP1 peptide containing the canonical PXXP motif. The wells of 96-well plate were coated with the Flag antibody and incubated sequentially with the purified WT or mutant (R95A, E97A, G116A, W118A and Y131A) c-Src with a Flag tag, biotin-RLP1 peptide and HRP-conjugated streptavidin. The binding intensity of the biotin-RLP1 was then measured (**: P<0.1). (D) Chemical shift perturbation shows the amino acids that displayed the significant chemical shift differences when the small molecule was titrated against ^{15}N-labeled c-Src SH3. (E) Surface plasmon resonance (SPR) shows the binding affinity of the small molecule with WT and mutant c-Src. (F) Enzyme-linked assay shows the c-Src kinase activity with or without the treatment of the small molecule, PP2 and Dasatinib (**: P<0.1). (G) ELISA assay to detect the effects of the small molecule on the interaction of RLP1 peptide with c-Src SH3 (*: P<0.5; **:P<0.1).

FIGURE 1 Identification of the target sites of integrin β3 in c-Src SH3

aggregation, adhesion and spreading on immobilized fibrinogen for platelet function. The transgenic mouse model was generated and the $FeCl_3$-induced carotid arterial thrombus formation and tail bleeding time assay were performed.

Results: The RT-loop of SH3, especially E97, was critically required for binding the small molecule and the β3 cytoplasmic domain but not necessarily for binding the canonical PXXP-containing RLP1 peptide and for the c-Src kinase activity that were primarily dependent upon the amino acids in the n-Src loop (Fig. 1). The c-Src^{E97A} mouse model was established. The β3/Src interaction in c-Src^{E97A} mice was interrupted and the platelet function was significantly diminished. Notably, c-Src^{E97A} mice exhibited a reduced thrombotic potential in the $FeCl_3$-induced thrombosis model with a normal hemostasis in the tail-bleeding assay (Fig. 2).

Conclusions: We identified that E97 in the RT-loop of c-Src SH3 was critical for β3 binding but was unlikely to contribute significantly to the canonical binding and kinase activity. The sequences around E97 may thus serve as potential anti-thrombotic targets.

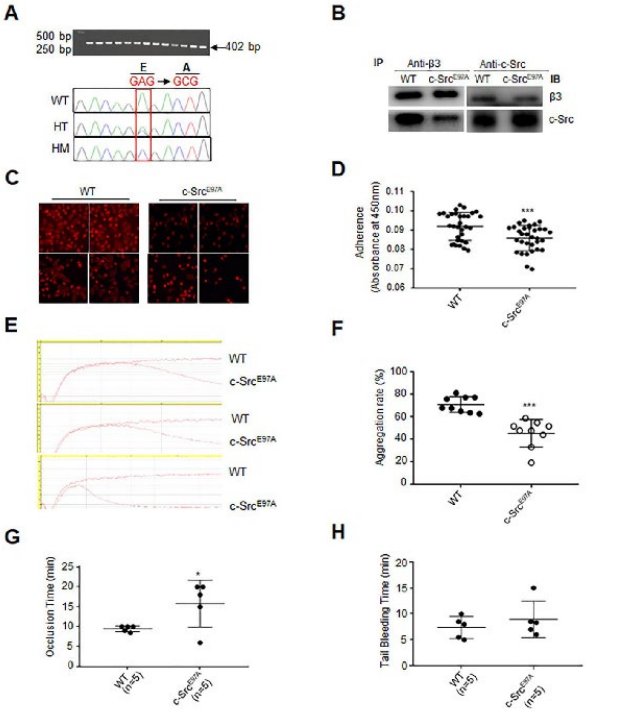

Figure 2 The effects of the c-Src^{E97A} mutation on the β3/Src interaction, platelet function, $FeCl_3$-induced thrombus formation and tail bleeding time in transgenic mice. (A) Genotypic identification of the c-Src^{E97A} transgenic mice. (B) Co-IP assays show the interaction between β3 and c-Src in platelets from WT and c-Src^{E97A} transgenic mice. (C) Spreading of platelets from WT and c-Src^{E97A} transgenic mice on immobilized fibrinogen. (D) Stable adhesion of platelets from WT and c-Src^{E97A} transgenic mice on immobilized fibrinogen (***: P<0.01). (E) Aggregation of platelets from WT and c-Src^{E97A} transgenic mice induced by thrombin. (F) Statistic results of the platelet aggregation (***: P<0.01). (G) $FeCl_3$-induced carotid arterial thrombus formation of the WT and c-Src^{E97A} transgenic mice (*: P<0.5). (H) Tail bleeding time of the WT and c-Src^{E97A} transgenic mice.

FIGURE 2 Effects of the c-SrcE97A mutation on the β3/Src interaction, platelet function, thrombus formation and tail bleeding in transgenic mice

PB1460 | Inhibition of Platelet Adhesion, Thrombus Formation and Fibrin Formation by a Potent αIIbβ3 Integrin Inhibitor

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Background: Ticks puncture human and animals' skin and secrete saliva into the bloodstream of the host, containing anticoagulant proteins. We studied disagregin, a potent platelet-inhibiting protein derived from the salivary glands of *Ornithodoros moubata*, an African soft tick. Conventional αIIbβ3 antagonists contain an RGD-sequence for integrin binding. In contrast, disagregin contains an RED-sequence, hypothesizing a differential mode of inhibitory action.

Aims: We aimed to compare the inhibitory effects of native disagregin and its RGD-mutant on platelet activation and to unravel the molecular basis of disagregin-αIIbβ3 integrin interactions.

Methods: Disagregin (MW: 6952 Da) was synthesized by solid-phase peptide synthesis using tert-butyloxycarbonyl(Boc)-chemistry and native chemical ligation. Effects of both disagregins on platelet aggregation were assessed by light transmission aggregometry in human platelet-rich plasma. Whole-blood thrombus formation was

investigated by perfusing blood over collagen type I with and without tissue factor (500 pM) at high wall-shear rate (1000s^{-1}) in the presence of disagregin, disagregin-RGD or eptifibatide, a clinically approved αIIbβ3 integrin inhibitor. Thrombi formed on microspots were assessed by 9 parameters, reflecting platelet adhesion, thrombus formation and fibrin formation. Parameters were analysed by Friedman tests.

Results: Disagregin showed dose-dependent inhibition of collagen- and adenosinediphosphate (ADP)-induced classical platelet aggregation with IC_{50} values of 65 and 100 nM, respectively. Multiparameter assessment of thrombus formation showed suppressed platelet adhesion and aggregate formation in presence of disagregin or disagregin-RGD, while eptifibatide treatment allowed platelets to form small aggregates.

Time to fibrin formation was delayed when adding disagregin or disagregin-RGD ($p < 0.01$) or eptifibatide ($p < 0.05$). However, surface covered by fibrin was lower in presence of disagregin and disagregin-RGD ($p < 0.01$), while addition of eptifibatide did not affect fibrin surface area coverage.

Conclusions: Both αIIbβ3-blocking disagregins have potent inhibitory effects on platelet adhesion, thrombus formation and fibrin formation in a dose-dependent manner. Eptifibatide was less effective, at concentrations fully blocking classical platelet aggregation.

PB1465 | Selatogrel, a Potent P2Y12 Receptor Antagonist, Dissolves Pre-Formed Platelet-thrombi *in vivo*

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Background: The combination of aspirin with a P2Y12 receptor (P2Y12R) antagonist has been shown to prevent major adverse cardiac events and is standard of care for patients with acute coronary syndrome. P2Y12R antagonism, and thus suppression of ADP-induced intra-platelet signaling, was shown to destabilize the interaction between GPIIb-IIIa and fibrinogen *in vitro*.

Aims: To investigate the effect of the P2Y12R antagonist, selatogrel, on stability of platelet-thrombi in experimental thrombosis models *in vivo*.

Methods: Experimental thrombosis was evaluated using Folt's model in guinea pigs and with real-time intravital microscopy in mice.

Results: Selatogrel administered subcutaneously stopped cyclic flow variations within 10 minutes after injection, confirming a fast onset of action. During ongoing thrombosis, selatogrel reversed blood flow to baseline levels, suggesting that existing platelet-thrombi were reduced in size. Quantification of laser-induced thrombus formation in mice using intravital microscopy demonstrated that selatogrel induced the dissolution of newly formed platelet thrombi. In a separate set of experiments, platelet thrombi were given time to consolidate for 30 minutes *in vivo*. Fibrin formation was not suppressed during thrombus consolidation. Infusion of selatogrel dissolved the consolidated platelet thrombi, confirming that P2Y12R-mediated intra-platelet signaling is essential for maintenance of the interaction between fibrin and the GPIIb-IIIa receptor. In addition, while selatogrel dissolved platelet thrombi, dissolution was never complete; mural platelet aggregates, functioning as hemostatic seals, were resistant in the presence of selatogrel.

Conclusions: Data presented here uncovered a novel role of P2Y12R in stabilization of consolidated platelet-thrombi. In addition, selatogrel dissolved platelet thrombi with no effect on hemostatic seals, which are critical to limit blood loss following vessel damage. Our data suggest that administration of selatogrel to patients with acute coronary syndrome might not only prevent further thrombosis but also reduce the size of pre-existing platelet-thrombi.

PB1466 | Method of Anticoagulation Influences Potency of Reversible P2Y12 Receptor Antagonists

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Background: Selatogrel represents a new chemical class of reversibly-binding P2Y12 receptor (P2Y12R) antagonists, which is being developed for subcutaneous administration in patients with acute coronary syndrome.

Aims: To characterize the potency of selatogrel *in vitro* with recombinant P2Y12R and with assay systems commonly used in the clinic.

Methods: Potency of selatogrel was profiled using radioligand (^{3}H-Selatogrel) binding with recombinant P2Y12R. We performed light transmission aggregation (LTA) with human platelet rich plasma (PRP) and VerifyNow® aggregation with human blood *in vitro*.

Results: Radioligand binding in the presence of increasing ADP concentrations verified that selatogrel is a competitive antagonist of ADP-binding to P2Y12R. Selatogrel potency was comparable between LTA and VerifyNow®. The method of anticoagulation affected the potency of selatogrel. Using citrated blood (i.e. low calcium concentration), the potency of selatogrel was reduced 3 fold relative to blood anticoagulated with a direct thrombin inhibitor, which preserves physiologic calcium concentration. Similar potency shifts were observed for the reversible P2Y12 antagonists ticagrelor, cangrelor and elinogrel. Radioligand binding experiments confirmed that low calcium concentration reduced binding affinity of ^{3}H-Selatogrel to P2Y12R. In addition, ADP stimulation of platelets at low calcium concentration resulted in a higher maximal extent of platelet aggregation, increased alpha granule secretion and elevated GPIIb-IIIa receptor surface expression relative to conditions with physiological calcium concentrations.

Conclusions: Selatogrel is a potent and reversible antagonist of P2Y12R. Radioligand binding experiments confirmed that selatogrel

prevents ADP binding to P2Y12R. In functional platelet assays at low calcium concentration, the potency of selatogrel was reduced due to lower P2Y12R binding affinity and increased platelet response to ADP. To avoid underestimation of reversible P2Y12R antagonist potency and thus recommendation of a supratherapeutic dosing regimen, clinical profiling of reversible P2Y12R antagonists should be performed in blood containing physiological calcium concentrations.

PB1467 | Idelalisib Selectively Inhibits GPVI-mediated Platelet Activation and Shows Anti-atherothrombotic Activity

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Background: Ischaemic heart disease and stroke have been the two major causes of death worldwide. As platelets play an essential role in the development and progression of these cardiovascular diseases, antiplatelet agents represent the treatment of choice in these patients. Glycoprotein VI (GPVI) is the major collagen receptor in platelets and has been proposed as a promising therapeutic because of their essential role in thrombosis while it is not critically required for haemostasis.
Aims: The main goal of the present study was to identify novel antiplatelet agent targeting GPVI signalling.
Methods: We performed a high-throughput screening (HTS) assay measuring intracellular calcium mobilization in real time upon platelet activation with the GPVI-specific agonist collagen-related peptide (CRP). Published Kinase Inhibitors Set (PKIS) library developed through drug discovery efforts by GSK was screened. Inhibitors showing activity were then tested by a series of thrombosis and haemostasis relevant in-vitro and in-vivo assays.

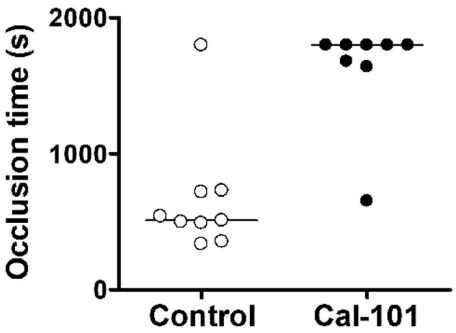

FIGURE 1 Ferric chloride-induced arterial thrombosis model

Results: Phosphoinositide 3-kinase (PI3K) selective inhibitor of P110δ isoform drug Idelalisib was identified as a hit that potently blocks intracellular calcium release, with an EC50 of 1.3 μM. We also demonstrated that Idelalisib efficiently blocks CRP-induced aggregation whereas collagen-induced aggregation is also moderately inhibited without significant effects in platelet viability. Moreover, platelet adhesion to collagen was highly reduced when platelets were preincubated with the inhibitor. To further investigate the potential of the inhibitor as antithrombotic, in-vivo assays using a ferric chloride-induced arterial thrombosis animal model were carried out. The results revealed that the occlusion time was significantly reduced when mice were treated with Idelalisib at 20 mg/kg (Figure 1).
Conclusions: The Idelalisib block effectively and selectively GPVI-induced platelet activation and shows antithrombotic potential in vivo. Further confirmation of the preclinical efficacy at platelet aggregation as well as better understanding of GPVI-related the mechanism of action might support the repurposing of these drug as antiplatelet agent.

PB1468 | Role of Multidrug Resistance Protein 4 on Both Platelet Nitric Oxide Resistance and ADP Induced Platelet Aggregation

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[1]*Sapienza University of Rome, Dept. of Experimental Medicine, Rome, Italy;* [2]*Regina Elena National Cancer Institute, Department of Research, Advanced Diagnostics and Technological Innovation, Rome, Italy;* [3]*University of Florida College of Medicine-Jacksonville, Division of Cardiology, Jacksonville, United States*

Background: MultiDrug Resistance-Protein 4 (MRP4) as part of the physiological mechanism of elimination of cyclic nucleotides in human platelets and its inhibition, when coupled with phosphodiesterase (PDE) inhibition, strongly impairs platelet aggregation.
Aims: To determine whether platelet nitric oxide (NO) resistance correlates with MRP4 expression and if cilostazol (an inhibitor of both MRP4 mediated transport and PDE) in vitro treatment reduces both NO resistance and ADP-induced platelet reactivity.
Methods: Platelet NO resistance was evaluated with ADP-induced aggregation in sodium nitroprusside (SNP)-treated platelets, obtained from aspirin treated patients.
The role of cilostazol on ADP-induced platelet aggregation was performed in high on aspirin residual platelet reactivity (HARPR) patients and compared to healthy volunteers (HV), and a control cohort (CTR).
Results: Aspirin treated patients were divided into quartiles for MRP4 expression. Aspirinated patients belonging to the 4th quartiles (> 0.015 ΔΔCt), with high levels of MRP4, had reduced SNP inhibition when platelets were stimulated with ADP compared to those belonging to the 1st-3rd quartiles (from 0.005 ΔΔCt to 0.014 ΔΔCt), with low levels of MRP4, (32 ± 18% vs 22 ± 15%; respectively;

p > 0.05). Cilostazol treatment enhanced NO inhibition by approximately 60%.

MRP4 inhibition by cilostazol significantly reduced ADP-induced platelet aggregation in the HARPR (36 ± 25% vs 74 ± 15%; p > 0.0001), in HV (15 ± 11% vs 55 ± 15%) and CTR (17 ± 16% vs 62 ± 17%) populations.

Conclusions: MRP4 inhibition by cilostazol can mitigate the hyperreactive platelet phenotype of HARPR patients by reducing residual ADP-induced platelet aggregation and increasing NO-dependent endothelial antiplatelet effects. Aspirin plus cilostazol may represent a novel antithrombotic strategy to reduce cardiovascular events, particularly among patients with MRP4 platelet overexpression.

PB1469 | Antithrombotic Therapy by Charged Nanoparticles

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Background: Current antiplatelet therapies remain ineffective for a large percentage of the population, while presenting significant risks for bleeding. We explore a novel way to inhibit arterial thrombus formation. Theoretically, a negative charge may influence the tertiary structure of von Willebrand Factor (vWF) to favor the globular configuration by biophysical means without the use of platelet inactivating drugs.

Aims: We tested this hypothesis experimentally for charged nanoparticles (CNP) to inhibit arterial thrombus formation in a microfluidic thrombosis assay (MTA).

Methods: We employed the MTA for efficacy screening of CNP size, surface charge, and blood concentration with polystyrene particles. Occlusion time (OT) in the MTA is measured as the time from first blood contact with the stenosis to the time of the initial maximum mass balance reading. An assessment of platelet surface coverage in the microfluidic was also performed. An effective particle was also assessed for its effects on hemostasis in a murine tail-bleeding model.

Results: The results of the MTA showed that CNP significantly extend occlusion time, with a maximum effect found at approximately 1.8 x 10^{11} CNP/mL of blood (Figure 1). The observed dosing profile

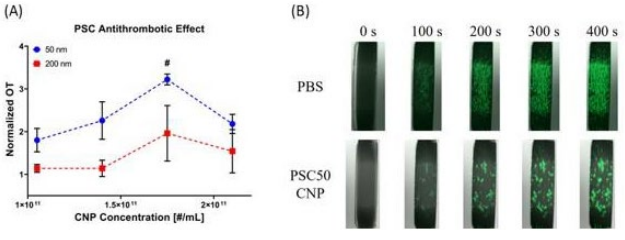

FIGURE 1 The CNP significantly retarded thrombus formation as quantified by (A) occlusion time and (B) platelet-calcein staining (1.8 x 1011 nanoparticles/mL)

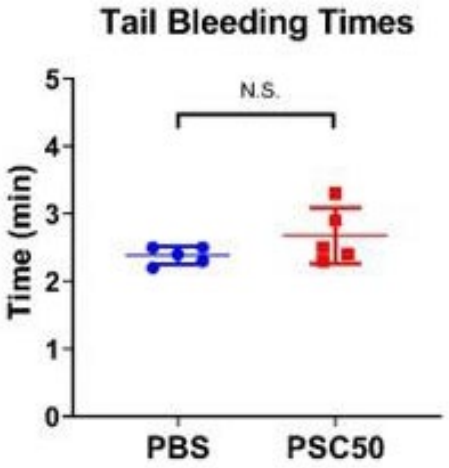

FIGURE 2 CNP vs. PBS in a murine tail bleeding model found no difference in bleeding, indicating that CNP do not significantly impact hemostatic function

was unusual in that higher doses resulted in less effect. The CNP decreased the rate of platelet adhesion and the total amount of platelet surface coverage, indicating that clots form slower and are potentially weaker than clots under normal conditions. Preliminary murine studies show no significant increase in bleeding due to CNP (Figure 2).

Conclusions: This study effectively demonstrates a new therapeutic for reducing acute arterial thrombosis based on preventing the shear elongation of circulating vWF. The effect is seen in multiple particles of differing size and charge, with a non-sigmoid dose-response curve, illustrating the non-pharmacologic mechanism of anti-thrombosis. CNP may be an inexpensive alternative to current antithrombotic agents without the inherent bleeding side-effects.

PLATELET FUNCTION DISORDERS, ACQUIRED

PB1470 | Complex Changes in Plasma Associated with Platelet Dysfunction in Trauma Patients

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Background: Hemorrhage is a leading cause of death after trauma, and coagulopathy contributes to death from hemorrhage. Platelet dysfunction plays a role in coagulopathy, and impaired platelet aggregation is associated with higher mortality. However, little is known about the cause underlying this platelet dysfunction. Proteomic analysis offers an unbiased approach to characterizing the changes in the plasma environment accompanying platelet dysfunction in trauma patients.

Aims: Characterize plasma protein changes in trauma patients with platelet dysfunction to generate a deeper understanding of the possible causes.

Methods: Blood was collected from severely injured trauma patients meeting full trauma team activation criteria at the time of Emergency Department (ED) arrival. Twelve patients with impaired platelet aggregation and twelve with normal aggregation were identified. Plasma samples underwent proteomics analysis by mass spectrometry. To account for different amounts of hemodilution prior to blood draw, individual protein concentrations were normalized to total protein concentration. Statistical comparisons were made by Wilcoxon rank-sum test with significance at $p < 0.05$.

Results: Compared to patients with normal platelet function, those with platelet dysfunction had lower gelsolin (median 1.21E6 vs. 1.83E6, $p < 0.001$), histidine-rich glycoprotein, alpha-2-HS-glycoprotein (31.1 vs. 2.3% positive, $p < 0.001$), and apolipoprotein A-IV (1.67E6 vs. 2.51E6, $p = 0.021$). They also exhibited higher plasma VWF (19.7 vs. 12.2 µg/mL, $p < 0.001$) and TSP-1 levels (2,609 vs. 128 ng/mL, $p < 0.001$). One-third of patients showed a higher density of less-adhesive, low-molecular weight multimers, and half showed enhanced satellite bands, a characteristic of multimer cleavage.

Conclusions: Trauma patients show evidence of platelet activation, VWF release and cleavage, and TSP-1 release. These changes describe a complex alteration in the VWF-platelet adhesion axis with mixed effects on function. Patients with platelet dysfunction also show lower levels of four proteins known to modulate coagulation and platelet function. Future study should describe the role of these changes in platelet dysfunction after trauma.

PB1471 | Platelet Dysfunction and Coagulation Activation in Intracerebral Hemorrhage

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Background: Intracerebral hemorrhage (ICH) has significant morbidity and mortality. Improving patient outcomes with ICH depends on our ability to recognize hematoma expansion (HE). The altered hemostatic functions have not yet been clearly defined. We hypothesize that changes in coagulation parameters, platelet function and fibrinolytic system are present and can provide biomarkers for the progress of HE, and furthermore may lead to targeted interventions to improve outcomes.

Aims: Determine hemostatic mechanisms occurring in ICH patients with HE using platelet function, coagulation activation and fibrinolysis assays.

Methods: Patients with acute ICH are studied after consent within the first 12 hours onset. Platelet function, coagulation factors and fibrinolysis activity are measured by thromboelastography (TEG) and ROTEM viscoelastic (VE) assays. Platelet function is also tested by PFA-100, and VerifyNow Aspirin and P_2Y_{12} point-of-care (POC) assays. Coagulation activation is measured by Thrombin Generation Assay and Tissue Factor activity. Fibrinolysis is assayed by Plasmin/antiplasmin (PAP) complex, Fibrinogen, D-Dimer and PAI-1 activity.

Results: Ten patients who presented with acute ICH were consented. Nine had hypertension and one history of hypertension. Seven of 10 had basal ganglia or thalamus lesions and 3/10 had cortical bleeding. Platelet dysfunction with inhibited ADP-induced aggregation was shown by TEG platelet mapping in 7/10 patients, with mean inhibition of 33.8% (range 6.8 - 96.5). Platelet dysfunction by POC assay was observed in 8/10 patients; the dysfunction was not accounted for by antiplatelet medication. Fibrinolysis was not detected by VE assays; however, 4/10 patients had elevated D-dimer, mean 1155 ng/mL D-DU (range 443 to 3040). Fibrinogen levels were normal.

Conclusions: This is the first evidence of platelet dysfunction found in 9/10 patients with acute ICH using VE and POC testing, while D-dimer was elevated in 4/10. The platelet dysfunction and coagulation activation are investigated as HE biomarkers in ICH.

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PB1472 | Platelet Purinergic Signaling Regulates Pulmonary Thrombosis in Sickle Cell Disease

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Background: Acute systemic painful vaso-occlusive crisis (VOC) is the predominant clinical event requiring sickle cell disease (SCD) patients to seek emergency medical care. VOC often serves as an antecedent to acute chest syndrome (ACS), which is a type of acute lung injury and one of the leading causes of mortality in SCD. Based on clinical epidemiology, ACS is preceded by thrombocytopenia and involves pulmonary thrombosis across pulmonary artery branches in 17% of ACS patients. However, the etiological mechanism that contributes to this sub-type of ACS pathophysiology remains largely unknown. Although adenosine diphosphate (ADP) release following hemolysis is known to activate platelets by stimulating their purinergic receptors P2Y1 and P2Y12, antagonists of P2Y12 have not shown any benefit and the role of purinergic signaling in SCD remains elusive.

Aims: To elucidate the role of platelet purinergic signaling in promoting pulmonary thrombosis in SCD humanized mice *in vivo*.

Methods: We used a novel intravital microscopy enabled experimental model of platelet-dependent pulmonary thrombosis in transgenic, humanized SCD mice, triggered by an intravenous (IV) administration of 2.5 mg/kg ADP. This method allows real time *in vivo* visualization of platelet-enabled pulmonary thrombosis in the lung of live SCD mice using multi-photon-excitation based quantitative fluorescence intravital lung microscopy (qFILM).

Results: We discovered that ADP-triggered pulmonary thrombosis involves entrapment of embolic platelet aggregates in the bottlenecks located between the pulmonary arterioles and capillaries. Surprisingly, ADP triggered pulmonary thrombosis in control mice but failed to induce pulmonary thrombosis in SCD mice. Remarkably, *in vivo* platelet aggregation studies confirmed that SCD mouse platelets were indeed refractory to ADP induced aggregation.

Conclusions: Our preliminary findings suggest an impaired platelet response to ADP in SCD and warrant the need for further studies to understand the role of purinergic signaling in the pathogenesis of ACS of SCD.

PB1473 | «Aspirin-Like» Platelet Defect Induced by Gabapentin: A Case-Report

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Background: Gabapentin is a structural analogue of gamma-aminobutyric acid used to treat peripheral neuropathic pain. This molecule has a favorable safety profile, although neurological and infectious side effects are described.

Aims: Here we report a case of "Aspirin-Like" platelet disorder with bleeding symptoms imputable to gabapentin.

Methods: A 44-year-old woman was referred to our hospital for unexplained mucocutaneous bleedings.

Results: She had a history of breast cancer, currently in remission but complicated by a chronic neuropathy treated since 9 months with gabapentin and trimestral injections of ketamine. Investigations revealed mucocutaneous bleeding symptoms present for the past 8 months, such as menorrhagia, oral bleedings, and easy bruising (ISTH-BAT score = 6), with no other clinical abnormalities.

Blood count revealed microcytic anemia secondary to iron deficiency. First line coagulation tests were normal, as well as Von Willebrand factor level. Platelet aggregation tests evidenced a complete absence of aggregation in response to arachidonic acid and epinephrine, major defect in response to ADP and subnormal response to collagen. This pattern was consistent with 'Aspirin-Like' platelet function defect.

Despite the lack of similar cases in the literature, the occurrence of bleedings one month after gabapentin introduction, without previous history, prompted us to discontinue this drug in this patient. The normalization of platelet aggregation tests performed 6 days after drug withdrawal combined with complete resolution of bleedings strongly supported the gabapentin imputability.

Conclusions: To our knowledge, we report the first case of gabapentin-induced thrombopathy. *In vitro*, only one team previously described a dose-dependent inhibitory effect of gabapentin on platelet aggregation induced by collagen, ADP and arachidonic acid (Pan CF, J Pharm Pharmacol 2007). Mechanisms underlying this acquired platelet function disorder are not clearly identified, but gabapentin appears to block thromboxane A2 signaling pathway. This case illustrates the importance of careful clinical monitoring in patients treated by gabapentin.

PB1474 | Uremia Induced Pre-Activation Results in Platelet Function Impairment in Chronic Kidney Disease

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Background: Patients with chronic kidney disease (CKD) are at high risk for thrombotic and cardiovascular complications. Counterintuitively, CKD patients also present with an increased risk of bleeding. Multiple platelet defects have been described, but data are conflicting.

Aims: To better understand the etiology of the altered platelet phenotype in CKD.

Methods: After written informed consent, blood was drawn from 29 patients with CKD stages 3-5 as approved by the local medical ethics committee. In case of hemodialysis (HD), blood was drawn prior to HD. Patients on anticoagulant or antiplatelet therapy were excluded from participation except for CKD5HD patients treated with 100 mg aspirin. Blood from healthy volunteers served as control. Platelet activation was measured using flow cytometry. Thrombus formation on a collagen type I surface under flow was used to assess the hemostatic potential.

Results: In total, we included 13 CKD3, 6 CKD4, and 10 CKD5HD patients (of whom 5 were on aspirin). Only CKD5HD patients without aspirin exhibited a reduced platelet count ($p = 0.013$ vs. healthy controls). Platelet counts of CKD5HD patients on aspirin were comparable to those of healthy controls and were increased compared to HD patients not on aspirin ($p = 0.044$). Platelet activation (integrin $\alpha_{IIb}\beta_3$ activation, P-selectin expression) upon collagen-related peptide was mildly decreased in all CKD groups. ADP and TRAP induced activation was comparable to that of healthy controls. Thrombus formation on collagen under flow was impaired in CKD4, CKD5HD and CKD5HD patients on aspirin, although thrombus formation

in CKD5HD with aspirin was increased compared to CKD5HD (p = 0.0033). Furthermore, platelet adhesion and aggregation under flow showed a significant correlation to glomerular filtration rate as a read-out of kidney function in CKD patients not receiving HD (r = 0.6276 and 0.5896, respectively).

Conclusions: Platelet function impairment in CKD correlates with disease severity and is likely a consequence of pre-activation of circulating platelets resulting in secondary exhaustion.

PB1475 | Bleeding Symptoms Associated with Platelet Function Abnormalities on Ibrutinib Therapy

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Background: Ibrutinib treatment in chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) is associated with bleeding at first stages, caused by action of ibrutinib on platelet signal transduction, but bleeding decreases with continued therapy.

Aims: To propose the predictive markers of bleeding on ibrutinib, as well as mechanisms responsible for decreased bleeding at later therapy stages.

Methods: Platelet functional activity was investigated in 50 CLL and 16 MCL patients on ibrutinib using flow cytometry and light transmission aggregometry. Blood was collected before ibrutinib treatment (point 0), then at 2, 4, 8 weeks (points 1-3) and 3-6 months (point 4) on treatment. The study was approved by the hospital's Ethical Committee.

Results: Prior to treatment, both CLL and MCL patients had decreased platelet counts, impaired aggregation with ADP, and decreased binding of CD62P, PAC1, and annexin V upon stimulation. Bleeding on ibrutinib was observed in 28 (56%) CLL patients, who had decreased aggregation with ADP and platelet count before therapy. Their platelet count on therapy did not change, aggregation with ADP steadily improved, and aggregation with collagen first decreased and then increased in anticorrellation with bleeding. The only platelet function marker associated with bleeding in CLL was aggregation with ADP. Bleeding in MCL was observed in 10 (62%) patients, who had decreased dense granule release before therapy. Aggregation with ADP and ristocetin in MCL increased on therapy, while collagen-induced aggregation evolved similarly to CLL. Decreased platelet count prior to ibrutinib treatment (114 ± 53 × 10^9/l versus 170 ± 70 × 10^9/l) was associated with a significant risk of developing bleeding (AUC = 0.71 ± 0.07) on ibrutinib in CLL but not in MCL.

Conclusions: We show that decreased platelet count and aggregation with ADP prior to ibrutinib therapy in CLL are predictive for on-therapy bleeding. Disappearance of bleeding in the course of ibrutinib therapy is associated with improved platelet reactivity towards ADP.

PB1477 | Severe Platelet Dysfunction in Patient with Philadelphia Chromosome-positive Acute Lymphoblastic Leukemia (Ph+ALL) after Tyrosine Kinase Inhibitor (TKI) Therapy

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Background: TKIs, targeting the BCR-ABL1 and SRC family kinases, have been incorporated into treatment regimens of Ph+ALL patients. Platelet activation in vivo is initiated by collagen and GPVI is one of the most potent receptor of signal generation. TKIs like imatinib are associated with abnormalities in platelet functional tests but not translated into significant clinical outcomes.

Aims: The aim of this study was to evaluate the correlation between platelet function, receptors expression and bleeding phenotype in an Ph+ALL patient in TKI monotherapy.

Methods: A 69 years old patient was admitted with Ph+ALL diagnosis and imatinib was used in frontline therapy. Blood samples were collected at different time points: complete blood count, coagulation tests, platelet function by light transmission aggregometry (LTA) and platelet expression profile of CD41, CD42a, CD49b, GPVI by cytofluorimetric assay were performed.

Results: During treatment with imatinib 600 mg/die platelet count increased from 13x10^3/µl to 174x10^3/µl but cutaneous bleeding still persisted. LTA studies revealed marked response impairment to

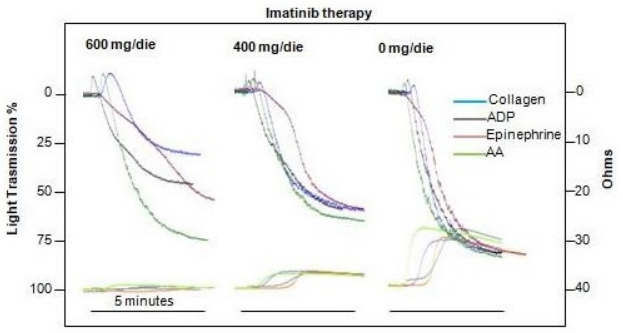

FIGURE 1 Imatinib therapy cause dysfuctional platelet aggregation: Serial LTA results in PRP from patient in Imatinib therapy at 600, 400 and 0 mg OD

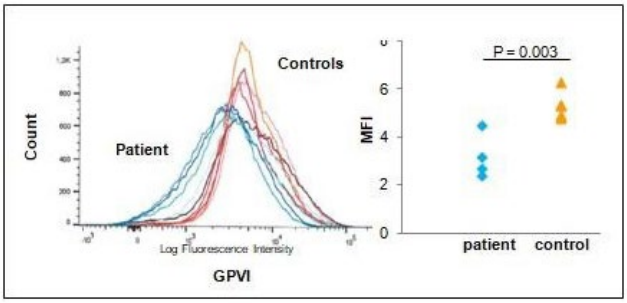

FIGURE 2 Results of GPVI patient surface expression compared with a group of healthy donors

collagen and ablated release reaction with all agonist used (Fig. 1). After imatinib dose adjustment (400 mg/die) platelet function remained reduced. Resumed the full dose, patient presented severe gastrointestinal bleeding so imatinib was stopped: no platelet functional abnormalities was identified beyond. At all time points, the expression of main platelet membrane receptors was not significantly different between patient and control group excepted for GPVI, whose MFIs values were 3.2 ± 0.91 and 5.3 ± 0.57 (p = 0.0033), respectively (Fig. 2).

Conclusions: We report for the first time a case of major bleeding in a patient treated with imatinib. Imatinib induce reversible platelet dysfunction and together with the low platelet GPVI expression results in a more profound inhibition of primary haemostasis contributing to the high bleeding phenotype. Therefore anti-platelet effects of imatinib should be take into account and should be carefully evaluated in presence of bleeding symptoms.

PB1478 | Dual Antiplatelet Therapy in One Patient with a Immune GPVI Deficiency

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Background: Patients with GPVI deficiency usually present low or no bleeding tendency suggesting that GPVI might be a promising target for the development of safe antiplatelet agents. However, combining a GPVI deficiency with additional risk factors of bleeding such as thrombocytopenia might worsens the bleeding phenotype.

Aims: We report the use of dual antiplatelet therapy in a patient presenting an acquired GPVI deficiency with no hemorrhagic complication.

Methods: Platelet functions and glycoproteins were analyzed according to standard procedures. Circulating antiGPVI antibodies were detected and purified before testing.

Results: A 64-year-old man was scheduled for inguinal hernia surgery. He reported epistaxis since childhood but no familial history of bleeding. He underwent a first inguinal hernia repair without bleeding 18 years ago. One year later he had hemorrhoids surgery and presented massive postoperative hemorrhage requiring blood transfusion. The origin of bleeding was related to rupture of surgical sutures. Five years later, he presented prominent face and neck hematoma caused by a horse hoof. The patient underwent endovascular vertebrobasilar stenting due to sequalae of this trauma, and received dual antiplatelet therapy (clopidogrel 75 mg/day, aspirin 100 mg/day, 40 days). No bleeding event was recorded during this period. Routine laboratory investigations were normal, apart from an isolated moderate thrombocytopenia (125G/L) with large platelets. Platelet aggregation in response to collagen (2 to 10 µg/mL) and convulxin (800 pM) was very low. A 80% deficiency of the GPVI/FcRgamma chain complex was found on immunoblots and an anti-GPVI antibody was identified. No GPVI gene abnormality was found, but the patient was heterozygous for a mutation in the alpha-actinin gene ACTN1c.682G>A that could account for macrothrombocytopenia.

Conclusions: This is the first report of a patient with an acquired GPVI deficiency who received dual antiplatelet therapy without bleeding. This observation brings new arguments in favor of the safety of therapeutic GPVI antagonists.

PB1479 | Quality of Life Today in Patients with Chronic Immune Thrombocytopenic Purpura

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Background: Immune thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by isolated thrombocytopenia in the absence of other causes or disorders. Bleeding and fatigue are the most common manifestation. The medications used to treat ITP include: corticosteroids, intravenous immunoglobulin, immunosuppressants, splenectomy and thrombopoietin receptor agonists.

Aims: Presenting the main challenges related to the treatment of ITP as resulting from our own experience and to show the impact on the quality of life

Methods: We present two cases of ITP that required different treatments for the control of haemorrhagic phenomena and to show the impact on the quality of life of patients. The quality of life was related to the number of bleeding episodes, the length of hospitalization and the side effects of the treatments performed.

Results: Case 1: A 20 years old woman diagnosed with ITP at 13 years old. She received corticosteroids that did not lead to a sustained response and after six months, the splenectomy was performed. The disease relapsed with the severe menorrhagia, severe anemia (Hb-1.9 g/dl). Corticosteroids/immunosuppressants were administrated

with severe side effects (Cushing syndrome, depression, infections). When she was 19 years old, romiplostim was started. Initially it was difficult to maintain the platelets above 50.000/mmc but she had not bleeding episode, no hospitalization.

Case 2: A 74 years old woman and when she was 62 years old was diagnosed with ITP. She received corticosteroids and then the splenectomy was performed. The disease relapsed with the severe purpura and corticosteroids were administrated with severe side effects (diabetes mellitus, arterial hypertension). When she was 72 years old eltrombopag was started with a good response at doses of 25 mg/day and no bleeding episode.

Conclusions: The main goal of treatment today should be to improve the quality of life of patients with ITP, the thrombopoietin receptor agonists being a solution in this regard.

PB1480 | Dysregulation of Platelet Serotonin and Platelet 14-3-3-Related Biomarkers in Sudden Infant Death Syndrome

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Background: Sudden infant death syndrome (SIDS) is the leading cause of postneonatal infant mortality. Subsets of SIDS infants show elevated serum serotonin (5-hydroxytryptamine [5-HT]) and abnormalities in 5-HT and 14-3-3 pathways in regions of the brainstem that mediate protective responses to life-threatening challenges during sleep. Platelets, which carry > 98% of 5-HT in blood, share with neurons many of the same 5-HT and 14-3-3 pathways.

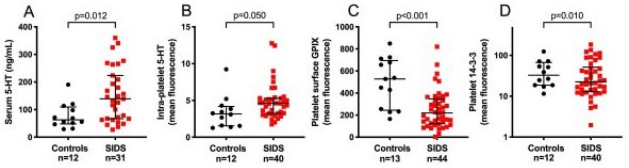

FIGURE 1 Platelet 5-HT and 14-3-3-related biomarkers in SIDS and controls. Medians ± IQR. P for ANCOVA adjusted for collection date and post-conceptional age

Aims: To determine if platelet 5-HT pathway and/or 14-3-3 pathway biomarkers are associated with SIDS.

Methods: Blood of infants dying suddenly and unexpectedly was collected under the auspices of California law and analyzed by whole blood flow cytometry to measure serotonin pathway markers (*e.g.* intra-platelet 5-HT, $5HT_{2a}$ serotonin receptor) and 14-3-3 biomarkers (*e.g.* 14-3-3 zeta, 14-3-3-linked platelet surface glycoprotein [GP] Ibα and GPIX). Serum was analyzed for 5-HT and related molecules. Case and control adjudications were based upon autopsy reports, clinical information, and death scene investigations and were performed blinded to laboratory results.

Results: Serum 5-HT was significantly elevated in SIDS *vs.* controls (p = 0.012, Figure, Table), replicating our previous findings (Haynes *et al.* PNAS 2017;114:7695). Intra-platelet (dense granule) 5-HT mean fluorescence intensity (MFI) was significantly higher in SIDS *vs.* controls (Figure). Platelet surface GPIX, which is linked to 14-3-3 via GPIbα, was lower in SIDS than in controls (p < 0.001) and 14-3-3 zeta itself was also lower in SIDS than in controls (p = 0.010) (Figure). GPIbα, which is susceptible to proteolysis, was not different between SIDS and controls. Other platelet end points were not significantly different between SIDS and controls.

Conclusions: The presence of both platelet and brain 5-HT and 14-3-3 abnormalities in SIDS suggests a global dysregulation of these pathways. Serum and platelet biomarkers may aid in the forensic determination of SIDS and have the potential to be predictive of risk for SIDS in living infants.

TABLE 1 Platelet 5-HT and 14-3-3-related biomarkers in SIDS and controls. PCA, post-conceptional age

	Mean ± SD				
Biomarker	**SIDS**	**Control**	**SIDS log-transformed**	**Control log-transformed**	**P-val, 2 sample t-test of log-transformed**
Serum 5-HT ng/mL	153.6 ± 97.0	77.8 ± 46.2	4.81 ± 0.71	4.21 ± 0.56	0.012
	Adjusted Mean ± SE				
Biomarker (adjusted for)	SIDS	Control	SIDS log-transformed	Control log-transformed	ANCOVA of log-transformed
Intra-platelet 5-HT MFI (PCA)	4.84 ± 0.39	3.66 ± 0.77	1.48 ± 0.08	1.13 ± 0.15	0.050
Platelet surface GPIX MFI (collection date)	242.4 ± 27.6	526.6 ± 52.8	5.21 ± 0.13	6.21 ± 0.24	<0.001
Platelet 14-3-3 MFI (PCA, collection date)	36.71 ± 5.75	64.08 ± 2.23	3.14 ± 0.14	4.05 ± 0.29	0.010

PB1481 | Assessment of Platelet Function after Shear Stress Simulation Using an ex vivo Model for Extracorporeal Circulation

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Background: A combination of an ex vivo model for shear stress and easy-to-use laboratory method to determine platelet function in whole blood (WB) may be helpful to better understand the pathophysiology of both bleeding and thrombosis duringextracorporeal circulation (ECC).

Aims: To investigate platelet activation status in WB and platelet rich plasma (PRP) samples after shear stress simulation using ex vivo chandler-loop model.

Methods: Two polyvinyl chloride tubes coated with heparin were filled with citrated or heparinized WB or PRP samples from healthy donors. To stimulate shear stress tubes were rotated in a temperature-controlled water bath (99 rounds per minute, one hour, 37°C). Samples were obtained before and after rotation for flow cytometer analyses. They were incubated with buffer, ADP or TRAP and double-stained for CD41, CD62P and CD63.

Results: A reduction in alpha granule release was observed in citrated WB samples upon ADP and TRAP stimulation after ECC-simulation compared to steady-state samples (median FI of CD62P: after ADP stimulation 0.96 vs. 1.35, p = 0.0006; and after TRAP stimulation 1.14 vs. 2.19, respectively, p = 0.0006). Similar results were obtained with PRP samples (median FI in CD62P: after activation with ADP 0.96 vs. 1.22, p = 0.0064; after activation with TRAP, 0.93 vs. 1.20, p = 0.0175). Furthermore, significant decrease in delta-granule response to TRAP was observed in WB (median FI in CD63: after TRAP activation: 1.01 vs. 1.23, p = 0.0262). Interestingly, significant reduction was observed in alpha granule release in heparinized blood samples obtained from chandler-loop compared to control samples (median FI in CD62P: after activation with ADP 1.05 vs. 1.45, p = 0.0973; with TRAP 1.06 vs. 3.06, p = 0.0064).

Conclusions: Our data confirm that increased shear stress conditions can cause dysfunction in delta as well as alpha granules. The usefulness of this new method to predict bleeding and thrombosis is under investigation in an ongoing clinical study of patients with heart assist devices.

PB1482 | First Evidence: Rivaroxaban, Apixaban and Edoxaban Reduce Thrombin-Dependent Platelet Aggregation

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Background: Rivaroxaban, apixaban and edoxaban are direct oral anticoagulants (DOACs), whose target specificity is to activate factor X (FXa), which is why they are referred to as Xabans. In contemporary cardiology, we see an effort to combine DOACs with antiplatelet therapy. Therefore, it is relevant whether Xabans have additional effects on platelet function. If so, it could affect the bleeding risk and influence the choice of treatment based on the patient risk profile.

Aims: The aim of the present study was to assess the effects of selected Xabans on in vitro platelet aggregation in patients with non-valvular atrial fibrillation (NVAF).

Methods: We assessed light transmittance platelet aggregation (LTA) with 100 nmol/L γ-thrombin. The LTA was performed at two time-points.

Results: Fifty four patients with NVAF were enrolled. All patients began treatment with rivaroxaban, apixaban or edoxaban as an initial anticoagulant treatment. Apixaban doses were 2.5 mg (n = 13, 70%) or 5 mg (n = 7, 30%) twice a day. Rivaroxaban doses were 15 mg (n = 6, 43%) or 20 mg (n = 8, 57%) once a day. Edoxaban doses were 30 mg (4, 20%) or 60 mg (16, 80%) once daily. The thrombin-induced platelet aggregation was significantly lower two hours after selected xaban was taken compared to baseline measurement. In addition, we have done 16 subgroup analyses in order to determine the impact on aggregometer measurement. We did not find any significant difference between the groups.

Conclusions: The thrombin-induced platelet aggregation is reduced in NVAF patients receiving Xabans. This should be taken into account in future studies of the combination of DOACs and antiplatelet therapy. The study was supported by grants VEGA 1/0187/17 and APVV-17-0054.

PB1483 | First Evidence: Edoxaban Affects TRAP-dependent Platelet Aggregation

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Background: Edoxaban is an oral anticoagulant drug and a direct factor Xa inhibitor, which is used to reduce the risk of stroke and systemic embolism in patients with non-valvular atrial fibrillation (NVAF). It is also labelled for the treatment of deep venous thrombosis and pulmonary embolism following initial parenteral anticoagulation. Edoxaban inhibits free factor Xa without the need of antithrombin. This inhibition of factor Xa in the coagulation cascade leads to decreased thrombin generation, and therefore, a reduction in thrombus formation and progression.

Aims: This prospective study aimed to assess the effects of edoxaban on in vitro platelet aggregation in patients with NVAF.

Methods: The local Ethical Committee of the Jessenius Faculty of Medicine in Martin approved this study (EK 1702/2015). It examined quantification of platelet aggregation assessed by light transmission aggregometry in 20 patients treated with edoxaban. The anti-Xa activity (ng/L) was assessed using edoxaban-calibrated anti-Xa chromogenic assays (BIOPHEN Heparin kit, BIOPHEN Edoxaban Plasma Calibrator, BIOPHEN Edoxaban Plasma Calibrator; Aniara Diagnostica, West Chester, OH).

Results: The thrombin receptor activating peptide (TRAP)-induced platelet aggregation was significantly lower two hours after taking edoxaban compared to baseline value (44.7% ± 32.03% vs. 73.3% ± 25.55%; $p < 0.0001$). In addition, we also performed 15 subanalysis in order to identify the differences in the outcome of different comorbidities, age, dosage, liver and kidney function tests, and concomitant treatment.

Conclusions: Results of the subgroup analyses were consistent with the findings of the main analysis; there was no apparent heterogeneity across the prespecified subgroups. The TRAP-induced platelet aggregation is reduced in non-valvular AF patients receiving edoxaban.

This work was supported by grants VEGA 1/0187/17 and APVV-17-0054.

PLATELET FUNCTION DISORDERS, HEREDITARY

PB1484 | Successful Perioperative Hemostasis for Glyoblastoma Surgery in a Patient with Glanzmann Thrombasthenia

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Background: Glanzmann Thrombasthenia (GT) is a rare inherited autosomal recessive bleeding disorder of platelet aggregation caused by a quantitative or qualitative defect of platelet membrane glycoprotein IIb/IIIa. Perioperative bleeding is a frequent complication in GT, and can be life threatening if not properly managed. Hemostatic strategies include surgical local measures, antifibrinolytics, platelet (PLT) transfusions and rFVIIa. Complex neurosurgical interventions in GT patients have never been reported in the literature.

Aims: To report on management of neurosurgical intervention in Glanzmann Thrombasthenia.

Methods: A 60 year GT patient, treated with multiple PLT transfusions for recurrent mucocutaneous bleeding since childhood, on May 2019 was admitted with severe headache, aphasia and confusion. An expansive left temporo-occipital lesion with perilesional edema and brain midline shift, suggestive of high grade glioma, was diagnosed by CT scan and MRI. Before surgery antiplatelet antibodies were absent and PLT count was normal ($180x10^9$/L).

Results: On June 7 2019 the patient underwent surgery. Before surgery IV tranexamic acid (TA) (subsequently repeated every 4 hours) and 2 PLT pools were administered followed by another PLT pool 90 minutes later. Despite of these, a severe bleeding with

TABLE 1 Pre and Intraoperative hemostatic management

	TA administration	PLT	RBC units/FFP
Preoperative hemostasis	TA 10 mg/kg iv,1 h before surgery	2 PLT pools immediately before surgery	
Intraoperative hemostasis	TA 10 mg/kg iv, every 4 h	1 PLT pools 90 min after the beginning of surgery; 2 PLT pools (for severe bleeding)	3 RBC units (for severe bleeding) in association with FFP 1.000 mL (as plasmatic RBC reconstitution)

TABLE 2 Postoperative hemostastic management

	TA administration	PLT	RBC units (to maintain hemoglobin levels> 10 g/dL)
First 24 h post-surgery	TA 10 mg/kg iv (1 dose)	1 PLT pool every 4 h (started 2 h after the end of surgery)	1 RBC unit
1° Postoperative day		1 PLT pool every 6 h	1 RBC unit
2° Postoperative day		1 PLT pool every 8 h	
3° Postoperative day		1 PLT pool every 12 h	

hypotension occurred and the patient was transfused with 2 more PLT pools, 1000 mL of fresh frozen plasma (FFP) and 3 red blood cell (RBC) units until an adequate hemostasis was achieved and the lesion was successfully removed *(see Table 1)*. The CT scan performed immediately after the surgery did not show any acute bleeding complications, and after this finding TA was stopped. Afterwards hemostasis was maintained only with PLTs *(see Table 2)*. Subsequent CT and RM scans revealed a regular postoperative course, without intracranial bleeding. Ten days after surgery the patient was discharged in good condition. Histologic examination confirmed diagnosis of glioma.

Conclusions: In our GT patient an aggressive hemostatic perioperative management was effective in limiting the bleeding risk.

PB1485 | A Whole Blood Flow Cytometric Method for Diagnosis of Platelet Function Disorders

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Background: Light transmission aggregometry (LTA) is the standard for diagnosis of platelet function disorders (PFDs), but LTA requires a customized aggregometer, large volume of blood and often cannot be used in the setting of thrombocytopenia. Flow cytometry based platelet activation test (PACT) requires very small volume of blood, can be interpreted irrespective of the extent of thrombocytopenia, can be performed on whole blood in a flow cytometer which is increasingly available in diagnostic laboratories for various applications.

Aims: Aims of this study were to develop simplified PACT using whole blood for diagnosis of PFDs and to compare its performance with the current standard LTA.

Methods: Platelet function was assessed after informed consent by LTA (Chrono-log Corporation, Havertown, USA) and PACT on Navios flow cytometer (Beckman Coulter, Miami, USA) in 27 normal controls and cohort of 33 patients with clinical bleeding suspected to be PFD after exclusion of coagulation factor deficiencies. LTA was performed using agonists Ristocetin, ADP, Epinephrine, Collagen, Arachidonic Acid, thrombin receptor-activation peptide-6 (TRAP) and Thromboxane A2 receptor analogue (TRA). ATP release response to collagen and thrombin were assessed. PACT was performed using agonists ADP, convulxin, TRAP and ristocetin. Activation markers like P-selectin (CD62P), antibody against GpIIb-IIIa activation epitope (PAC-1) and anti-Von Willebrand Factor

TABLE 1 Diagnosis and Platelet Activation Response to various agonist / activation markers in PACT

Diagnosis	Number of cases / controls	Glycoprotein expression	Activation Marker Anti-Von Willebrand Factor Antibody:		Activation Marker PAC-1:		Activation Marker CD62P:		
			Response to Normal dose Ristocetin	Response to Low dose Ristocetin	Response to ADP	Response to convulxin	Response to ADP	Response to convulxin	Response to TRAP
Bernard Soulier Syndrome	6	Absent expression of CD42b	Absent	Absent	Present	Present	Present	Present	Present
Glanzmann Thrombasthenia	5	Absent expression of CD41	Present	Absent	Absent	Absent	Present	Present	Present
Glanzmann Thrombasthenia Variant	9	Normal / dim expression of CD41	Present	Absent	Absent	Absent	Present	Present	Present
Acquired Platelet Dysfunction with Eosinophilia (APDE)	2	Normal expression of CD41 and CD42b	Present	Absent	Variable	Variable	Variable	Variable	Variable
Mixed Granule Deficiency	4	Normal expression of CD41 and CD42b	Present	Absent	Present	Present	Variable	Variable	Variable
Von Willebrand Disease Type 2B	2	Normal expression of CD41 and CD42b	Present	Present	Present	Present	Present	Present	Present
Normal platelet function with thrombocytopenia	5	Normal expression of CD41 and CD42b	Present	Absent	Present	Present	Present	Present	Present
Normal Controls	27	Normal expression of CD41 and CD42b	Present	Absent	Present	Present	Present	Present	Present

antibody were used. Both tests were compared for agreement using Cohen's Kappa analysis.

Results: LTA and PACT had almost perfect agreement (kappa = 0.89, standard error 0.13). Using LTA as reference method, sensitivity and positive predictive value of PACT was 96.4%, while specificity and negative predictive value was 96.9%. In 12% of the patients, LTA was inconclusive due to thrombocytopenia while PACT could assess the platelet function. Table 1 shows findings of PACT in study population.

Conclusions: PACT can be alternative to LTA for diagnosis of PFD, especially in the setting of thrombocytopenia, pediatric patients or in the setup where aggregometer is not readily available.

PB1486 | Platelet Activation Markers and Impaired Procoagulant Response in Pediatric Patients with Inherited Platelet Function Disorders

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Background: Inherited platelet function disorders (IPFD) are a group of diseases with unclear mechanisms of hemorrhagic complications. There is limited and conflicting information on platelet properties in IPFD except for a limited number of classical mutations.

Aims: To evaluate CD62p (P-selectin) and phosphatidylserine (PS), markers of intravascular platelet activation associated with an impaired platelet function reactivity in IPFD.

Methods: 50 pediatric patients with diagnosed IPFD and hemorrhagic symptoms were examined. The median age was 7.5 years (range 0.9-18 years). The gender composition was 23 boys and 27 girls. All patients have bleeding of different severity: scoring from 2 to 16 according to Bleeding Assessment Tool ISTH. They have different hemorrhagic phenotype: petechiae and ecchymosis, nosebleeds, bleeding after tooth extraction, menorrhagia, hemarthrosis and subarachnoid hemorrhages. We investigated platelets in diluted blood using flow cytometry at rest and after activation with CRP plus TRAP-6. Control group (CG) included 50 healthy children (median age was 10 years (range 2-17 years). The analyses of statistical differences were performed using a nonparametric Mann-Whitney test.

Results: Forward scattering in patients wasn't significantly different from the CG at rest and after activation ($p = 0.43$ and $p = 0.24$, respectively, Figure A, B). CD62p at rest was significantly higher in patients when compared with a group of healthy volunteers ($p < 0.001$), but no significant differences were observed after activation ($p = 0.21$) (Figure C, D). PS at rest was significantly higher in patients ($p < 0.001$), but after activation was significantly lower in patients when compared with the CG ($p = 0.035$) (Figure E, F).

Conclusions: A significant increase of CD62p and PS at rest may indicate intravascular activation of platelets in patients, which may cause a decrease in overall functional reactivity upon activation contributing to the disease pathophysiology. This is in line with decrease of PS expression in patients after activation.

PB1487 | Hemarthrosis and Chronic Synovitis in Glanzmann Thrombasthenia

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Background: Glanzmann thrombasthenia (GT) is a rare bleeding disorder characterized by a lack of platelet aggregation because of glycoprotein IIb/ IIIa deficiency. Although it's a rare bleeding disorder worldwide, the case is different in this part of the world with a prevalence of 1/28000 in AlMadinah, Saudi Arabia. Hemarthrosis is an uncommon presentation in GT, has been reported in a few cases, chronic arthropathy even rarer.

Aims: Report of five GT patients with hemarthrosis, three of them had chronic synovitis.

Methods: Patients information documented from the medical record system in Madinah hereditary blood disorders center, Maternity and children hospital, Al Madinah, Saudi Arabia.

Results: Out of 62 GT patients, five (8%) were presented with hemarthrosis, 2 females and 3 males. Three patients were complicated by chronic synovitis (chronic arthropathy).

A 19 years old man had presented with an ankle hemarthrosis in 2 episodes at the age of 5 and 7 years. A 9 years old girl had presented with a knee hemarthrosis at the age of 5 years. Both did not developed chronic synovitis.

A 10 years old boy had developed bilateral ankle joint chronic arthropathy after 3 hemarthrosis episodes over 3 years. He has been subjected to chemical synovectomy, where he had no hemarthrosis episodes afterword for 14 months. His brother is 9 years old had right ankle hemarthrosis in 2 episodes at the age of 7 and 8 years, when MRI has shown chronic synovitis, where chemical synovectomy performed, subsequently, no hemarthrosis developed.

An 11 years old girl has had chronic synovitis after a documented hemarthrosis in the right ankle joint. chemical synovectomy performed a month ago.

All five patients are type I GT, diagnosed through flowcytometry and three of them confirmed with cytogenetics.

Conclusions: Though hemarthrosis and chronic synovitis is rare presentation in GT, but need to be considered.

PB1488 | Defective Endocytosis of Albumin, Fibrinogen and IgG in Platelets with *RUNX1* Haplodeficiency

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Background: Patients with *RUNX1* mutations have thrombocytopenia, impaired platelet function, and decreased granule contents and secretion. Our studies in a patient with *RUNX1* mutation showed that platelet albumin and was decreased (Sun et al, *Blood*103: 948-54, 2004). Human platelets alpha granules contain numerous proteins, including PF4 and VWF, which are synthesized by megakaryocytes (MK), and others (fibrinogen, albumin and IgG) that are not synthesized but incorporated by endocytosis.

Aims: To understand the mechanisms leading to decreased platelet albumin and granule defect in *RUNX1* haplodeficiency (RHD).

Methods: We studied endocytosis of fluorescent-labeled albumin, fibrinogen and IgG using flow cytometry in washed platelet suspensions (0-90 min) and in PMA-treated megakaryocytic HEL cells (up to 24 hours).

Results: In normal platelets the protein uptake was time- and concentration-dependent. The uptake of albumin, fibrinogen and IgG was decreased in father and daughter (both with RHD), with the mean fluorescent intensity (MFI) decreased > 50% of that in normal platelets. In HEL cells, uptake of albumin and fibrinogen was time- and concentration-dependent, with marked increased between 120 min and 24 hrs. In contrast to the findings in platelets, on siRNA RUNX1 knockdown the uptake/retention of albumin and fibrinogen was higher than in control siRNA cells at 120 min and 24 hrs.

Conclusions: This is first evidence that platelets with RUNX1 haplodeficiency have defective endocytosis of albumin, fibrinogen and IgG. The handling of these proteins by platelets and megakaryocytic cells is different and it is altered with RUNX1 knockdown in MK cells. These studies provide insights in to diverse a-granule abnormalities in RHD and into the handling of proteins by platelets and MK about which little is known.

PB1489 | Usefulness of ISTH-BAT in Clinical Assessment of Inherited Platelet Disorders: Experience of the Spanish National Project

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Background: Inherited platelet disorders (IPDs) are a heterogeneous group of congenital rare diseases, characterized by variable bleeding. IPDs comprise both inherited thrombocytopenia (IT) and/or inherited platelet function disorders (IPFDs). Even under expert analysis, diagnosis of IPD remains a challenge due to clinical and molecular heterogeneity. Bleeding assessment tools (BATs) are recommended to evaluate the bleeding phenotype (BP).

Aims: Evaluate the BP in our IPDs patients by the ISTH-BAT and compare the BP between ITs and IPFDs, and investigate potential association between ISTH-BAT bleeding score (BS) and findings in platelet function tests.

Methods: We performed a retrospective study of 138 patients recruited in the multicenter Spanish Project in IPD of the Society of Thrombosis and Haemostasis (SETH). BP was related to bleeding score (BS) as evaluated by the ISTH-BAT. Platelet phenotyping included blood cells count, blood film, light transmission aggregometry (LTA), and we also assessed genetic diagnosis by means of HTS (high-throughput sequencing) targeted gene panel.

Results: Sixty six percent of the patients were female; median age was 31 (0-71) years; 85 cases (62%) presented with IT, while 53 cases (38%) have IPFDs. Abnormal LTA profile was demonstrated in 69 patients (50%). Median BS was 5 (0-25) and the most common bleeding complication was epistaxis. The BS was higher in adults vs. children's [6 (0-18) vs. 1 (range, 0-6),p< 0.05], female vs. male [5 (0-25) vs. 3 (0-21),p=0.017] and IPFDs vs. ITs [7 (2-25) vs. 2 (0-21),p< 0.05]. In the

cohort of IT patients, higher BS was associated to impaired LTA [8 (0-25) vs. 1 (0-8) in IT with normal LTA profile,p< 0.001].
Conclusions: The BS determined by ISTH-BAT was associated with impaired platelet function. This study suggests that ISTH-BAT could be useful to identified patients with significant abnormalities in platelet function, which are candidates to further platelet phenotyping.
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PB1490 | Periodic rFVIIa Prophylaxis due to Recurrent Haemarthroses into the Right Ankle Joint in a Patient with Glanzmann's Thrombasthenia

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Background: Glanzmann's thrombasthenia is a genetic disease that can be inherited in an autosomal recessive manner. The first-line treatment for severe bleeding are platelet concentrate transfusions or, in the case of transfusion resistance, recombinant factor VIIa (rVIIa) treatment.
Aims: The aim of the study is to evaluate the clinical effects of the prophylactic use of rFVIIa in the treatment of recurrent haemarthroses into the right ankle joint in a 16-year-old patient with Glanzmann's thrombasthenia.
Methods: A 16-year-old boy with Glanzmann's thrombasthenia (GT) was hospitalized due to recurrent bleeds into the right ankle and restricted mobility of the joint. The disease began with subcutaneous haemorrhages at the age of 7 months. Up to the age of 14, he had mainly nosebleeds and subcutaneous haemorrhages requiring frequent hospitalizations. During the last hospitalization at age14 due to bleeding into soft tissues, an X-ray of the right ankle joint was normal - 0 points according to the Pettersson scale. From then onwards, the boy did not report for checkups.
He was hospitalized again at the age of 16 due to oedema, haemarthroses into the right ankle and restricted mobility of the joint. In an X-ray of the ankle joint, 10 points were found on the Pettersson scale, which was why radiosynovectomy of the right ankle was performed together with rFVIIa. Due to extensive lesions in the right ankle joint, prophylactic administration of rFVIIa (90mcg / kg 3 times per week) was prescribed.
Results: In an X-ray performed after 6 months, no changes were observed. During this time the boy did not have any haemarthroses or accumulation of joint lesions.
Conclusions: Periodic rFVIIa prophylaxis in the treatment of haemarthroses in a patient with Glanzmann's Thrombasthenia effectively reduces bleeding and protects the patient from arthropathy or its accumulation.

PB1491 | The contribution of flow cytometry in the diagnosis of inherited *thrombocytopathies*

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Background: Thrombocytopathies constitute a heterogeneous group of pathologies affecting several stages of the platelet activation process, leading to hemorrhagic syndromes of varying severity. These platelet functional abnormalities can be acquired or constitutional. These diseases are caused by genetic abnormalities that affect protein expression or functionality in the platelet membrane.The biological diagnosis of these thrombocytopathies is based on platelet exploration, requires excellent pre-analytical quality and platelet aggregation being the gold standard for diagnosis. Currently, flow cytometry occupies a paramount place in the diagnosis of these pathologies. The technique presents a twofold benefit: it is simple and rapid, in addition to being advantageous for children compared to the platelet aggregation test: small sample volume, no interference related to plasma opalescence … .
Aims: To diagnose inherited thrombocytopathies by flow cytometry.
Methods: The work, which lasted for a one-year period, took place in the hemobiology and blood transfusion service of Annaba University Hospital, Algeria. A flow cytometry study on the Beckman Coulter FC500 automated system was performed on cases diagnosed by platelet aggregation on TA 8V thrombo-aggregometer (SD Medical). The objective was to highligh the deficiency in membrane glycoproteins responsible for thrombocytopathy. The following agonists and markers were used: Ristocetin, ADP, Collagen, Arachidonic acid, CD41, CD61, CD42a and CD42b.
Results: Our study has enabled us to diagnose five cases of inherited thrombocytopathy, including four patients with Trambasthenia of Glanzmann (Type I: GpIIbIIIa < 5%) and one patient with the Bernard Soulier syndrome (Anomaly of GPIbIX).
Conclusions: We can effectively conclude that the analysis of platelet flow cytometry has significantly improved the thrombocytopathy diagnosis.

PB1493 | Glanzmann Thrombasthenia in Pakistani Patients: Biochemical Analyses & Identification of Novel Pathogenic Variants in the Fibrinogen Receptor αIIbβ3

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Background: Glanzmann's thrombasthenia (GT) is a rare autosomal recessive disease which occurs more frequently in populations in which consanguineous marriages is practiced. GT is characterized by a quantitative or qualitative defect of the platelet αIIbβ3 (GPIIb/IIIa) receptor caused by pathogenic variants of the *ITGA2B* and *ITGB3* genes, respectively. Patients present with a moderate to severe bleeding tendency, having normal platelet count, morphology and normal first line coagulation tests. The disease is diagnosed and classified by platelet aggregometry and by flow cytometry. Molecular genetic analyses confirm the diagnosis and help to understand the pathomechanism.

Aims: Aim of the study was to identify the biochemical and molecular genetic defects in patients with Glanzmann thrombasthenia in Pakistan.

Methods: The data of the patients were collected from NIBD Karachi, Fatimid foundation Karachi and Chughtai's Lab, Lahore. Patients diagnosed with platelet functional defects were identified. CBC (complete blood count) was done to identify platelet morphology and counts. Prothrombin time (PT), Activated partial thromboplastin time (APTT), Fibrinogen levels and platelet aggregation studies were performed. Further investigations were initiated using flow cytometric analysis (platelet CD41 and CD42 glycoprotein expression). In addition, molecular genetic analyses were performed using Sanger sequencing method.

Results: In 20 out of the 26 patients suffering from platelet functional defects in our cohort GT was diagnosed or suspected: 12 (46.1%) males and 8 (30.7%) female patients. Pathogenic or likely pathogenic variants in the candidate genes *ITGA2B* and *ITGB3* were identified in 15 patients. History of consanguinity was found in 88.4% of the patients. Eight novel pathogenic/likely pathogenic variants were identified. Family genotyping was performed in 2 families.

Conclusions: GT is commonest platelet disorder identified among inherited platelet disorders. Variant analyses reveal missense mutations were the most frequent. Flowcytometric analyses reveals GT type 1 as the most common disorder found in this cohort.

PB1494 | Inherited Platelet Disorders: Identification of Novel Disease Causing Variants Using Next Generation Sequencing

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Background: Inherited platelet disorders (IPDs) comprise a highly heterogeneous group of disorders affecting platelet number and/or function. For molecular genetic analysis we now established a panel-based next generation sequencing (NGS) approach comprising 90 genes associated with IPDs. Patients of our study (n=21) had moderate to severe bleeding symptoms; especially mucosal bleedings (epistaxis, menorrhagia), hematoma and increased bleeding post-operatively. Von Willebrand factor and coagulation factor disorders were excluded.

Aims: To identify the disease-causing variant in a pilot study of 21 patients with a history of recurrent bleeding episodes and functionally suspected IPD.

Methods: Platelet studies: Morphology (blood smear), aggregometry, flow cytometry. Molecular genetic analyses: Targeting enrichment (Nextera Rapid Custom Enrichment) followed by sequencing on MiSeq (Illumina). Data analysis: SeqPilot (JSI), Alamut® Visual, pathogenicity prediction (SIFT, MutTaster, PolyPhen2, CADD), occurrence in Human Gene Mutation Database. For verification: Sanger sequencing and family genotyping if possible.

Results: NGS achieved a mean coverage from 98% (20x) for the investigated genes and a mean read depth from 578 per nucleotide. We identified 17 variants which were classified as "pathogenic" or "likely pathogenic" in 8 genes. Correlation with platelet functional analyses enabled us to identify class 4 and class 5 pathogenic variant/s for 13 patients.

Conclusions: Panel enrichment is a feasible tool to investigate IPDs in combination with platelet functional analyses. The disease causing variant could be identified for 13 of 21 patients using panel diagnostic. For those patients for whom the platelet phenotype indicated a specific platelet disorder, we could identify the disease causing variant in the suspected candidate genes in all cases. For the patient cohort for whom the platelet phenotype did not indicate to a specific platelet disease, we could only identify the disease causing variant in about 33%. The patients for whom the disease causing variant could not be identified, Whole Exome Sequencing will be performed.

PB1495 | Clinical and Molecular Characterization of Chinese Patients with Wiskott-Aldrich Syndrome

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Background: Wiskott-Aldrich syndrome (WAS) and X-linked thrombocytopenia (XLT) are caused by mutations of the *WAS* gene. The genotype-phenotype association of WAS and XLT have not been fully elucidated.

Aims: To analyze the mutations of *WAS* gene and identify the potential correlation between genotype and phenotype.

Methods: We collected clinical data of 17 WAS/XLT patients, analyzed mutations of WAS gene at the genomic DNA by the next generation sequencing (NGS), and further determined the potential correlation between genotype and phenotype.

Results: There were 13 unique mutations identified, including 4 novel mutations, from 17 unrelated affected members. Most mutations (8/13, 62%) were missense or nonsense mutations, and were preferentially located in the amino-terminal part of the protein, exons

1 and 2, whereas two frameshift mutations (p.Arg308Lysfs*28 and p.Glu488Glafs*171) were located in the carboxyl-terminal region, exons 9 and 11 respectively. Nearly all the patients with XLT had missense mutations, while only half of the patients with missense mutations exhibited the XLT phenotype. In contrast, patients with nonsense mutations, deletions, insertions, and complex mutations developed the classic WAS phenotype.

Conclusions: *WAS* gene analysis can make a definite diagnosis of WAS and identify mutation carriers, beneficial for timely treatment and genetic counseling for children with WAS.

PB1496 | Genetic Characterization of Inherited Platelet Disorders in a Single Portuguese Center for Coagulopathies

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Background: Inherited Platelet Disorders (IPD), a group of rare and heterogeneous diseases due to qualitative and/or quantitative platelet (PLT) defects, are increasingly recognized. Various strategies to IPD diagnosis were adopted but the best approach in still unknown.

Aims: Characterization of individuals with IPD followed in a Portuguese Center for Coagulopathies.

Methods: Probands from 94 unrelated families with PLT defects not explained by acquired causes, were evaluated by PLT morphometric and functional studies (lumiaggregometry and glycoprotein expression by flow cytometry); the study was oriented to Sanger sequencing (in presence of strong suspicion for a genetically homogeneous disease) and/or to Next-Generation-Sequencing (NGS). 93 affected and 40 unaffected family members (segregation studies) were also evaluated.

Results: Candidate variants in genes encoding for IPD were identified in 75 families (79.8%) (161 patients); 19 families (20.2%) had likely benign (6 families) or no candidate variants (13 families). Sanger sequencing confirmed the diagnosis in 91.7% of families with phenotypically identified IPD and NGS enabled the identification of candidate variants in 31 out of 48 families (64.6%). ITGA2B/ITGB3-RT (13 families, 17.3%), ANKRD26-RT (10.7%), ACTN1-RT (9.3%), MYH9-RD (9.3%), Glanzmann Thrombasthenia (8%), TUBB1-RT (6.7%), HPS (6.7%), bBSS (6.7%) and mBSS (5.3%) were the most prevalent IPD identified, representing 80% of the 75 families with molecular diagnosis being followed in our Centre.

Conclusions: The integration of clinical data with morphologic and functional tests, allowed a large number of phenotypically identified patients, including rare IPD such as ITGA2B/ITGB3-RT (investigated for a long time by systematic screen for αIIbβ3 deficiency in familial thrombocytopenia) with subsequent molecular identification of almost all the cases (>90%) by Sanger sequencing. The adopted strategy could justify a slightly different prevalence of various IPDs relative to other series and allowed the molecular identification in most (80%) of the families studied.

PB1497 | Utility of the ISTH Bleeding Assessment Tool and the Pediatric Bleeding Questionnaire in predicting platelet function defects in pediatric patients with excessive bleeding

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Background: The ISTH Bleeding Assessment Tool (ISTH-BAT) and the Pediatric Bleeding Questionnaire (PBQ) were initially developed for persons with suspected von Willebrand disease. The diagnostic utility of these bleeding scores in patients with inherited platelet function disorders (IPFD) is still elusive.

Aims: To investigate the utility of the ISTH BAT and the PBQ in prediction of platelet function defects in children with excessive bleeding and suspicion for IPFD.

Methods: We enrolled 170 patients with bleeding complaints and without evidence of thrombocytopenia and coagulopathies. The ISTH-BAT and the PBQ scores were applied by a trained investigator prior to light transmission aggregometry (LTA) and platelet flow cytometry (PFC) with activation. A ROC-curve analysis was performed to evaluate whether the ISTH BAT and the PBQ scores could discriminate between patients with and without demonstrable defects in platelet function tests.

TABLE 1 Bleeding scores in patients with GT, patients with other defects in platelet function tests and patients with no pathological findings

	GT patients	Patients with other defects	Patients with normal tests	p-value (Kruskal-Wallis test)
Number of patients (N)	21	42	107	-
M/F	7/14	20/22	50/57	-
Median age (IQR), year	5 (1-10)	7 (4-13)	9 (5-13)	0.006
Median PBQ score (IQR)	7 (5.5-9.5)	3 (2-4)	2 (2-4)	< 0.00001
Median ISTH BAT score (IQR)	8 (6.5 - 10.5)	3 (2-4)	2 (2-4)	<0.00001

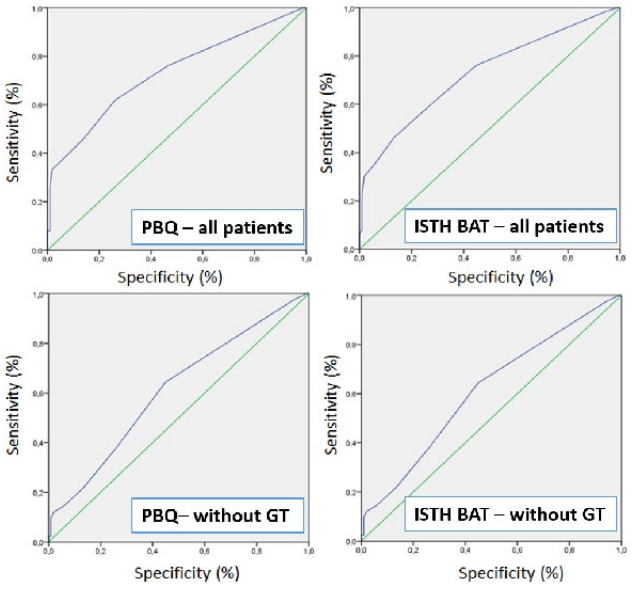

FIGURE 1 ROC curve for presence of a defect in LTA and/or PFC and the bleeding scores

Results: Diagnosis of Glanzmann thrombasthenia (GT) was made in 21 patients. Other defects in platelet function tests were revealed in another 42 kids and there were no pathological findings in 107 patients. Both ISTH BAT and PBQ scores differs between GT patients and patients with other defects and between patients with other defects and group with no pathological findings (p< 0.05 in all pairwise comparisons). But difference between two last mentioned groups was less than 1 point (0.85 (CI 95% 0.19;1.50) for PBQ and 0.85 (CI 95% 0.18; 1.52) for ISTH BAT). AUC was 0.728 (CI 95% 0.647; 0.809) for PBQ and 0.730 (CI 95% 0.648; 0.812) for ISTH BAT for all patients. But after exclusion of GT patients AUC was only 0.610 (CI 95% 0.510; 0.711) for PBQ and 0.610 (CI 95% 0.508; 0.711) for ISTH BAT.

Conclusions: The ISTH-BAT and the PBQ questionnaires are reasonable tools for documenting of bleeding history. However, both scores are not predictive for non-severe platelet functional defects in patients with excessive bleeding.

PB1498 | Correlation GLATIT Scores and the Number of Bleeding Episodes in the Assessment of the Prognosis of Inherited Platelet Disorder

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Background: Evaluating the inherited platelet disorder prognosis represent a very important element in the management and monitoring of the disease.

Aims: We applied the GLATIT score and calculated the number of bleeding episodes, as well as the study of their correlation.

Methods: We studies the contribution of GLATIT score the number of bleeding episodes in the prognosis of inherited platelet disorder, for the 60 patients with Inherited Platelet Disorder (34 TG, 18 JBS and 8 MH).

TABLE 1 Average number of bleeding episodes / year in the 3 prognostic classes GLATIT (34 T.G, 18 J.B.S, 8 M.H)

GLATIT Score	N= 60	Mean	
Minor	23	2	
Moderate	5	2	
Severe	32	4.5	P=0,00021

TABLE 2 Mean number of bleeding episodes per year during T.G and J.B.S disease according to prognostic classes GLATIT

	TG (34)	JBS(18)
Phenotype	Number/Mean	Number/Mean
Minor	9 (3)	7 (1)
Moderate	4 (2)	1 (2)
Severe	21 (4.5)	10 (4)
	p<0,05	p<0,05

Results: The study of the number of hemorrhagic episodes according to the prognostic classes of the GLATIT score gives the following results:

The number of bleeding episodes is 2 times higher in the severe prognostic class GLATIT (4.5 episodes per year) compared to the two moderate and minor prognostic classes (2 episodes per year). The difference is statistically significant (P = 0.00021).

During the TG, the average number of bleeding episodes per year is three in the GLATIT minor prognostic class, 2 in the moderate class and 4.5 in the severe class. The difference is statistically significant (P < 0.05).

For J.B.S disease, the average number of bleeding episodes per year is one in the GLATIT minor prognostic class, 2 in the moderate class, and 4 in the severe class. The difference is statistically significant (P < 0.05).

Conclusions: There is a good correlation between GLATIT scores and the number of bleeding episodes. In addition to hemorrhagic scores, the concept of bleeding rate, can be used to identify the risk of bleeding. This simple parameter gives an idea of the repetition of Haemorrhagic syndromes fand to evaluate its prognosis.

PB1499 | Utility of the ISTH Bleeding Assessment Tool (BATs) in Diagnosis of Glanzmann Thrombasthenia Patients - Experience from Pakistan

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Background: Glanzmann's thrombasthenia (GT) is a rare autosomal recessive disorder characterized by a deficiency or functional defect

of the platelet GPIIb/IIIa (αIIbβ3) receptor leading to moderate to severe bleeding episodes. ISTH-BATs (Bleeding assessment tools) have been proven beneficial in the diagnosis of patients with different bleeding disorders. However, use of ISTH-BATs in diagnosis and prediction of bleeding risk in GT patients is still under study.

Aims: The aim of the study was to assess the diagnostic utility of ISTH-BATs in the diagnosis of Glanzmann's thrombasthenia patients by comparing it with healthy controls.

Methods: We included all patients from neonates to 18 years of age referred with a suspected platelet function disorder to the The Children's Hospital, Lahore and NIBD, Karachi, Pakistan from January 2010 to June 2019. The clinical details of all these consecutive patients were collected prospectively on the proforma based on ISTH-BATs questionnaire. The diagnosis of GT was done using light transmission aggregometry followed by Flowcytometry. The same proforma was obtained from 75 healthy controls.

Results: Out of 427 patients who presented with suspected platelet function disorders 133 were diagnosed as Glanzmann's thrombasthenia. The mean age calculated was 7.29 years and male to female ratio was 1:1.1. 76.6% patients were born to parents with consanguineous marriages. Epistaxis was the commonest symptom with highest score (p value< 0.003). Cutaneous and oral cavity bleeds were significantly more severe and frequent in patient group as compared to controls (p value < 0.005). Gastrointestinal bleed was present in 13.5% patients. Other bleeding symptoms were observed less frequently. The median ISTH-BAT score in GT patients was 8 while in control group it was 1.

Conclusions: ISTH-BAT scores were quite significant in GT patients as compared to controls. We recommend the integration of standardized bleeding assessment tools questionnaire in diagnostic evaluation of patients with a suspected platelet function disorder.

PB1500 | Efficacy of Novoseven in the Treatment of Bleeding Episodes During Glanzmann's Thrombasthenia

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Background: In the various monitoring centers for our patients, the aim of treatment has been twofold for all patients: To stop hemorrhagic syndrome and prevent the occurrence of hemorrhagic syndrome. It is based on local means associated with hemostatic treatments, anti-fibrinolytics, transfusion of platelet concentrates and recombinant factor VII.

Aims: Efficacy of recombinant factor VII during Glanzmann thrombasthenia.

Methods: We compared our results with the study by M.C. Poon 2010.

Results: In our series, during Glanzmann's thrombasthenia. The use of VIIr has shown its effectiveness in the treatment of epistaxis in 78% (7/9) of the cases compared to 84% (26/31) in the Ponn MC study of 2010.

TABLE 1 Effectiveness of factor VIIr, comparison with data from the international T.G.

	GTR Poon MC Haemophilia 2010 (N = 55)	ETCOA 2014 Western Algerian Constitutional Thrombopathy Study.
Nature of bleeding		
Epistaxis	26/31 (84%)	7/9 (78%)
Mouth bleeding	6/7 (86%)	5/6 (83%)
Gastrointestinal	7/8 (88%)	4/4 (100%)
Surgery	2/3 (67%)	2/3 (67%)

For bleeding in the mouth, VIIr was effective in 83% (5/6) of our patients compared to 86% (6/7) in the Ponn MC 2010 study. This means that our results agree with the data from Glanzmann Thrombasthénia Registry.

In our series, the use of VIIr during TG in gastrointestinal bleeding is effective in 100% of cases, ie in 4 out of 4 patients. Our results join those of the Ponn MC study at 88% (7 / 8), despite the small number in the 2 series.

The management of major surgical procedures by VIIr during TG is comparable in the 2 series (Study of Western Algerian Constitutional Thrombopathy 2014 and Ponn MC 2010) with an efficiency rate of 67% (2/3).

Conclusions: During TG, the efficacy of recombinant factor VII was established in patients with anti-GP IIbIIIa or anti-HLA alloimmunization or an absence of response to platelet transfusions or in the context of emergency in the absence of platelet concentrates.

PB1501 | Use of a Whole Exome "Slice" for Evaluation of Inherited Platelet Disorders

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Background: Platelet disorders represent a prevalent but inadequately understood subset of bleeding diatheses, often with little understanding of causative genetics. The low rate of molecular diagnosis indicates a critical need for genetic diagnostic tools to improve risk stratification and therapeutic personalization. Thus, institutions have begun utilizing next generation sequencing tools to bridge this knowledge gap.

Aims: Review our institutional experience using a whole exome sequencing (WES) platelet "slice" in patients with suspected platelet disorders to determine the frequency of variant identification, rate of diagnosis assignment, and clinical phenotype associated with variants in the 66 tested genes.

TABLE 1

	Number of Patients	Percentage of Patients
Any Variant Identified	79	82%
Multiple Variants Identified[1]	42	44%
VARIANT TYPES		
Diagnostic[2]	10	10%
Likely Diagnostic[2]	10	10%
Pathogenic but Non-Diagnostic	2	2%
Variant of Unknown Significance[3]	72	75%
No Variant Identified	17	18%

[1] In patients with multiple variants, 15 patients (15%) had both a diagnostic variant of unknown significance.

[2] Diagnostic or likely diagnostic variants were identified in the following genes: *MYH9*, *TUBB1 TBXAS1*, *THPO*, *RUNX1*, *ETV6*, *GFI1B*, *ANO6*, *WAS*, *ITGA2B*, *STXBP2*, and *ANKRD26*.

[3] 3In-silico predictions of these patients' variants predicted 18 patients with pathologic variants, 27 with conflicting predictions of pathogenicity, and 49 with benign variants. Some patients with multiple variants had variants that fell into different categories.

TABLE 2

Variants Associated with MDS/Leukemia	Diagnostic	Likely Diagnostic	VUS
ETV6	0	2	1
RUNX1	0	1	1
ANKRD26	2	1	1
Abbreviations: MDS - myelodysplastic syndrome			

Methods: We retrospectively reviewed our institution's platelet WES slice comprising 66 genes. Testing was performed clinically on 96 patients with genetically undefined disorders of platelet function and/or thrombocytopenia. Each WES slice was reviewed by faculty in Human Genetics and Hematology.

Results: Of the 96 patients analyzed, 20 had variants that were deemed either definitively or likely diagnostic of their clinical phenotype (Table 1). Of these, 11 were related to thrombocytopenia, 4 to platelet function, and 5 could impact platelet number and function. Six definitively or likely diagnostic variants identified were associated with familial myelodysplastic syndrome (MDS)/leukemia (Table 2). Three additional variants of uncertain significance were identified in MDS/leukemia-associated genes which will be further investigated.

Conclusions: WES slices can provide otherwise elusive molecular diagnoses in patients with platelet related disorders. In addition to providing patients/families with a definitive answer, positive results also provide critical information for genetic counseling. Moreover, some diagnoses have far reaching implications as exemplified by the variants seen in this cohort in *ETV6*, *RUNX1*, and *ANKRD26*, which have been associated with myelodysplastic syndromes and leukemia. Analyses such as these also provide an avenue for research studies designed to determine the importance of a specific VUS, such as those seen here in MDS/leukemia-related genes, pushing our understanding forward.

PB1502 | Defective RAB31-mediated Endosomal Trafficking in *RUNX1* Haplodeficiency

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Background: Transcription factor RUNX1 regulates genes critical for megakaryocyte (MK)/platelet formation and function. *RUNX1*haplodeficiency (RHD) is associated with thrombocytopenia, and defective platelet granules and function. Platelet expression profiling of a RHD patient revealed decreased *RAB31*expression. RAB GTPases regulate vesicle trafficking, granule biogenesis and secretion. The role of RAB31 in MK/platelets is unknown.

Aims: To study the regulation of RAB31 by *RUNX1*and role of RAB31 in platelets/MK.

Methods: We performed studies in platelets from RHD patients and in PMA-treated megakaryocytic HEL cells.

Results: By real-time PCR, platelet *RAB31* mRNA was decreased by 60-80% compared to 5 healthy controls in our RHD patient (P1) and in two additional patients (P2 and P3). Platelet RAB31 protein was decreased in P2 and P3. *RAB31* promoter region revealed 4 RUNX1 consensus sites. CHiP and EMSA revealed RUNX1 binding to sites II and IV; their mutation decreased promoter activity. RUNX1 downregulation (siRNA) and overexpression altered *RAB31* promoter activity and protein expression in HEL cells. RAB31 regulates vesicular trafficking between the Golgi/TGN and endosomes, and from early endosomes (EE) to late endosomes (LE). We investigated RUNX1 and RAB31 role in endosomal dynamics using immunofluorescent staining for markers of EE (EEA1) and LE/multivesicular bodies (LE/MVB, CD63). Either RUNX1 or RAB31 siRNA yielded a striking enlargement of EE (marker EEA1), partially reversed by RAB31 reconstitution. Mannose-6-phosphate receptors (MPRs) regulate lysosomal protein trafficking; RAB31 regulates transport of MPRs from TGN to endosomes. In HEL cells, siRNA *RUNX1*knockdown increased MPR (immunoblotting). In preliminary studies RAB31 deletion in HEL cells (5 independent knockout clones) increased MPR suggesting that RUNX1 and RAB31 downregulation alters MPR handling.

Conclusions: RAB31 is a direct transcriptional target of RUNX1. *RAB31* or *RUNX1* downregulation results in impaired endosomal maturation/trafficking and handling of proteins.These effects may underlie the α-granule protein defects in RHD patient.

PB1503 | Clinico-pathological Spectrum of GlanzmannThrombasthenia

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Background: Glanzmann'sThrombasthenia (GT) is a rare autosomal recessive inherited platelet function disorder, which is characterized by decrease or absence of functional platelet αIIbβ3 or GPIIb/IIIa integrin receptors. The clinical presentation and bleeding tendencies in GT are variable.
Aims: Analysis of clinical spectrum of patients with GT and its correlation with the laboratory parameters.
Methods: Across sectional study of 19 patients diagnosed with GT in the Department of Haematology, from January 2014 to December 2019 was undertaken. A diagnosis of GT was confirmed by clinical history and laboratory investigations comprising of peripheral blood smear examination, coagulation screen, platelet aggregometry and flowcytometry.
Results: The median age our patients were 8 years (10 months-40 years). There was an unusual male preponderance with a male: female 13:6. Majority of patients presented with epistaxis, gum bleeding, ecchymosis and prolonged bleeding from trauma site. Platelet aggregation studies showed absent or decreased aggregation with ADP, Collagen, Arachdonic acid and Epinephrine in 100% of patients whereas 21% showed decreased aggregation with Ristocetin. Flowcytometric analysis showed absent expression of CD41 in all the cases analysed and normal expression of CD42b suggestive of GT. Eight of our patients required occasional transfusion of platelet rich plasma.
Conclusions: Basic haemostatic screening tests and platelet aggregometry are sufficient to establish the diagnosis in majority of the patients with GT, however more sophisticated tests like flowcytometry is required for confirmation of GT.

PB1504 | Global Tests of Primary Hemostasis for the Diagnosis of Mild/Moderate Bleeding Disorders (MMBD): Rehabilitating the Skin Bleeding Time

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Background: Bleeding time (BT) and platelet function analyzer (PFA-100®) are global tests of primary hemostasis altered in thrombocytopenia, platelet function disorders (PFD) and VonWillebrand disease (VWD), largely used in MMBD diagnostics. It was suggested that PFA-100®C-ADP is altered in VWD but not in PFDs while the BT would be prolonged in both.
Aims: To compare the sensitivity of PFA-100® and BT in discriminating VWD from PFD in a large cohort of subjects with suspected MMBD.
Methods: We retrospectively analyzed PFA-100®, BT, platelet count, VWF:Ag and VWF:RCo results from all consecutive subjects with suspected MMBD studied at our Center between 2004 and 2019. Inclusion criteria were platelets 150-400x10^9/L, clinical suspicion of MMBD, no recent drug intake.
Tests were carried out by the same group of skilled investigators throughout the period.
Results: 1,015 subjects (589 women) were included and in retrospect classified as PFD (n=89, 36±1.7 years), VWD (n=279, 34±1 years) and HC (n=647, 42±1 years). Platelet count was significantly lower in PFD compared to VWD and HC (174.7±25.9 vs 265.3±5.9 and 252.6±5.0×10^9/L, p< 0.0001). PFA-100®CT was significantly and similarly prolonged in PFD (C-ADP: 156.1±9.0 seconds; C-EPI: 229.1±8.7) and VWD (C-ADP: 182.0±5.7; C-EPI: 241.1±4.7) compared to HC (C-ADP: 95.4±1.3; C-EPI: 147.1±2.2). BT was significantly prolonged in PFD (15.0±1.3 min) but not in VWD (7.5±0.3 min) compared with HC (6.8±0.2 min)(p< 0.0001).
PFA-100® showed high-moderate accuracy in discriminating VWD from HC (AUC=0.8829) and moderate in discriminating PFD from HC (AUC=0.80), while BT low in discriminating VWD from HC (AUC=0.65) but high in discriminating PFD from HC (AUC=0.89).
Finally, BT showed moderate accuracy (AUC=0.8007) in discriminating VWD from PFD, while PFA-100® with both cartridges or only the cADP cartridge was inaccurate (AUC=0.5691 and AUC=0.5736, respectively).
Conclusions: Our data show that the C-ADP PFA-100® cartridge does not differentiate VWD from PFD patients while the BT does.

PB1505 | Strategies for Analysis of Novel Molecular Variants in the *RUNX1* Gene as a Cause of Familial Platelet Disorder with Predisposition to Acute Myeloid Leukemia (FPD/AML)

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Background: Germline mutations in *RUNX1* are associated with an autosomal dominant disorder characterized by thrombocytopenia,

platelet dysfunction and predisposition to acute myeloid leukemia (AML). Overestimating the pathogenicity of new variants can lead to unnecessary worry about a potential risk to develop malignancies.

Aims: To evaluate the pathogenicity of new variants in *RUNX1* identified in three unrelated women (P1, P2, P3) with a life-long history of mild thrombocytopenia and moderate bleeding.

Methods: We performed platelet phenotyping (flow cytometry, aggregation studies), and molecular analysis by high-through-sequencing (HTS). Ultrapure platelets were isolated by filtration+immunoselection (3 patients, and 5 controls). Platelet RNA was analyzed using Clariom-D Array (≈540 000 transcripts). In P1 we also evaluate proplatelet formation in megakaryocytes (Mks) differentiated from CD34+ peripheral blood cells.

Results: Only P1 and P2 showed functional platelet abnormalities (reduced aggregation and granule secretion). By HTS we identified new heterozygous variants in *RUNX1*: c.802C< T in P1; c.586A>G in P2; c.476A>G in P3. The analysis of the expression of 15 genes recognized as targets of RUNX1 (including *MYL9*, *MYH9*) was confirmed to be altered in P1 and P2 (30.8% and 38.5% similarity to controls), but not in P3 (92.7% resemblance). Similarly, the evaluation of 100 genes reported to be downregulated in the transcriptome of a RUNX1 patient (*Sun*, *JTH 2007*), showed 74.7%, 67.7% and 7.1% of them had lower expression in P1, P2, and P3, respectively. Culture of CD34-Mks from patient P1 showed reduced proplatelet formation. Transcriptome analysis (P1 and P2 *vs*. controls) revealed that 25% of top 75 downregulated genes encode for cytoskeleton proteins.

Conclusions: Our study supports the pathogenicity of the *RUNX1* variants identified in P1 and P2, but not in P3. Platelet transcriptome analysis can be a useful tool in the pathogenic characterization of new molecular variants affecting transcription factor genes in patients with inherited thrombocytopenias. [PI17/01311 -FMM AP172142019].

PB1506 | Identification of a Novel Molecular Variant in the *PTGS1* Gene that Causes a Loss of a Glycan in the Catalytic Domain, Defects in Platelet Thromboxane A2 Synthesis, and Bleeding

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Background: Thromboxane A2 [TxA2] is generated from arachidonic acid [AA] by cyclooxigenase-1 (COX1) and thromboxane synthase. To date, few patients with mutations in *PTGS1*, which encodes COX1, have been identified (< 5cases).

Aims: Characterization of a patient with aspirin-like platelet defect enrolled in the Spanish project "Functional/molecular characterization of patients with Inherited Platelet Disorder".

Methods: The index case is a 13-year-old adopted girl of Asian origin, referred because of moderate chronic bleeding (BAT-ISTH=6). We performed regular platelet phenotyping (aggregation [LTA], western-blot [WB], immunofluorescence [IF] assays). TxA2 and eicosanoids synthesis was measured by enzyme-immunoassayand and LC-MS. Patient DNA was analyzed by HTS-gene panel. Recombinant WT and mutant COX1 generated by site directed mutagenesis were expressed in 293T HEK cells. N-glycosylation analysis included PNGase-F treatment.

Results: The index case had normal platelet size and count. LTA was absent with AA (1.6 mM), but normal with U46619 (5 mM) suggesting a defect in TxA2 synthesis, which was confirmed in LTA supernatants (< 10% *vs*.controls) as well as in agonist-stimulated whole blood (10-50%). COX1 level (WB & IF) was normal, but enzyme displayed faster electrophoretic mobility. HTS analysis revealed a heterozygous variant c.428A>G, p.Asn143Ser located in the catalytic domain of COX1 which affects one of the three predicted N-glycosylation sequons

(Asn-X-Ser) of *PTGS1*. HEK 293T cells transfected with p.Asn143Ser mutant showed no AA-induced TxA2 synthesis (WT:500 ng/mL; Asn143Ser:≈50-75 ng/mL) and a COX1 band with faster electrophoretic mobility. In cells co-transfected with WT and p.Asn143Ser mutant, PNGaseF treatment results in a single migration band, confirming a defective N-glycosylation. Moreover cells transfected with p.Ser145Ala mutant generate another hypoglycosylated variant that also exhibited defective TxA2-synthesis.

Conclusions: We have identified a novel COX1 variant p.Asn143Ser which did not alter enzyme expression, but abrogated TxA2 synthesis and impaired platelet function. These data emphasize the relevance of N-glycans in the catalytic domain of this key constitutive enzyme. [PI17/01311-FMM AP172142019]

PB1507 | Variants in the LLR5 Domain Confer to GPIbα Increased Affinity for VWF: A Novel Case of Platelet-type von Willebrand Disease

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Background: Gain-of-function variants in *GP1BA* cause platelet-type von Willebrand disease (PT-VWD), a rare inherited autosomal dominant bleeding disorder characterized by enhanced platelet GPIbα-von Willebrand factor (VWF) interaction and thrombocytopenia. To date, only 6 variants causing PT-VWD have been described, 5 in the VWF-binding domain (β-switch) of GPIbα, and one in the macroglycopeptide. We have studied a boy with easy bruising, enhanced ristocetin-induced platelet agglutination (RIPA=0.6 mg/ml) and normal platelet count, characteristics that oriented towards PT-VWD or type 2B-VWD.

Aims: To perform differential diagnosis between PT-VWD and type 2B-VWD, to identify the gene variant and to investigate its impact on GPIbα.

Methods: GP1BA gene was analyzed by PCR and direct sequencing. CHO cells expressing the GPIbβ and GPIX subunits were transfected with plasmids expressing WT GPIbα, p.G233V mutant GPIbα (a typical β-switch gain-of-function variant) or the novel variant. Binding of VWF to platelets and GPIbα-transfected CHO cells induced by ristocetin was assessed by flow cytometry; rolling of GPIbα-transfected CHO cells on immobilized VWF was assessed by microcinematography in a flow chamber.

Results: Patient platelets mixed with control plasma showed enhanced VWF-binding, orienting towards PT-VWD. *GP1BA* sequencing revealed a novel heterozygous c.C455A substitution, corresponding to a p.R111Q in the leucine-rich-repeat (LRR) 5 domain of GPIbα. CHO cells expressing WT GPIbα barely bound VWF (5.8±3.2%), while those expressing p.G233V and p.R111Q GPIbα showed enhanced VWF-binding (G233V: 23.6±4.9%, $p< 0.05$ vs WT; R111Q: 11.8±1.9%, $p< 0.05$ vs WT)*. CHO cells expressing WT GPIbα rolled significantly faster on a VWF-coated surface (average speed:14.1±2.7 mm/sec), then those expressing p.G233V and p.R111Q GPIbα (G233V:4.9±0.4 mm/sec, $p< 0.05$ vs WT; R111Q:9.1±0.5 mm/sec, $p< 0.05$ vs WT and G233V).

Conclusions: We describe the first p.R111Q variant in the LLR5 domain of GPIbα inducing PT-VWD, demonstrating that gain-of-function variants outside the β-switch may confer increased VWF-binding activity to GPIbα and generate a mild PT-VWD phenotype.

PB1508 | Molecular Insights into Glanzmann's Thrombasthenia through Family Forums for Gene Sequencing and Discovery

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Background: Glanzmann′s Thrombasthenia (GT) is a challenging syndrome as platelet receptor expression does not always correlate with the bleeding phenotype. Molecular analysis and variant mapping is proving useful in our understanding of which mutations may have an impact on receptor function as well as total protein expression.

Aims: To further research on rare blood disorders, patients have created forums where they can access knowledgeable providers, educational and diagnostic services. Comprehensive Health and Education Services (CHES) has been a leader in helping families drive genetic sequencing for families with a shared diagnosis, such as the recent Glanzmann's Retreat in Austin Texas 2019. The aim was to provide molecular diagnosis and variant identification for providers, patients and family members.

Methods: Over 400 individuals with RBD have been evaluated through CHES forms, including 56 individuals with Glanzmann's or potential carriers. Diagnosis through flow cytometry was performed by the primary physician and whole gene sequencing was performed using a validated Next Generation Sequencing (NGS) clotting protein whole gene panel which contains ITGAIIb and ITGAbeta3 along with 20 additional clotting genes. Each individual had whole gene sequencing on all 22 genes on the panel.

Results: As in previous reports for RBD in the US, we found both compound heterzygotes as well as patients with homozygous variants. Bleeding scores correlated when there was a complete absence of receptors, but varied considerably in the case of the missense mutations. Number of individuals and type of mutations include: missense variants: 39,

frameshifts ITGB3:5-ITGAIIb:11, early stop: 4, splice site variants: 19 deletions or CNV:2, premature start in ITGAIIb: 3, and start loss in ITGB3: 3.

Conclusions: Patient AND family members are critical to determine which molecular variants are pathogenic in rare disorders, particularly FVII deficiency.Patient AND family members are critical to determine which molecular variants are pathogenic in rare disorders, particularly FVII deficiency.

PB1509 | Glanzmann Thrombasthenia-like Syndrome: Report of 2 Cases

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Background: Glanzmann thrombasthenia (GT) is a rare autosomal recessive bleeding disease characterized by normal platelet count and defect in platelet aggregation. GT is due to quantitative or qualitative deficiencies of the fibrinogen receptor, αIIbβ3, an integrin coded by the ITGA2B and ITGB3 genes. Rare cases mutations in ITGA2B and ITGB3 cause GT with macro-thrombopenia named Glanzmann thrombasthenia-like syndrome.
Aims: We report 2 cases of GT with macro-thrombopenia in the same family.
Methods: Our study was done in patients with GT and their family members. We carried out the platelet count, the primary hemostasis balance with platelet functions studies by aggregometry, flow cytometry, MAIPA and gene sequencing.
Results: The two propositus have mucocutaneous haemorrhages and moderate macro-thrombocytopenia. The platelet functions studies showed normal platelet aggregation with ristocetin and very reduced platelet aggregation with multiple agonists. The GPIIb-IIIa expression rate's by flow cytometry is 12,7% and 26,3%. These two patients did not develop any post-transfusion anti-αIIbβ3 antibodies. No mutation was found.
Parents are heterozygous while the third child is healthy
Conclusions: Two members of this family have a Glanzmann thrombasthenia-like syndrome.

PB1510 | LAD III, Platelet Pathology Related to GT: A Case Report

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Background: Leukocyte adhesion deficiency type III (LAD-III) is characterized by both severe bacterial infections and a severe bleeding disorder. LAD-III is rare autosomal recessive disorder and it is caused by mutations in the *FERMT3* gene. The immune defects are supplemented by a Glanzmann thrombasthenia-like bleeding tendency.
Aims: Recognize the LAD III which can wrongly evoke the diagnosis of Glanzmann's disease.
Methods: We carried out the platelet count, the primary hemostasis balance with platelet functions studies by aggregometry, flow cytometry and gene sequencing.
Results: The male propositus has recurrent mucocutaneous haemorrhages and bacterial infections. In the family history two sisters is died. Propositus has normal platelet morphology and normal platelet count. Platelet function studies showed no platelet aggregation with multiple agonists and normal platelet aggregation with ristocetin. The GPIIb-IIIa expression rate's by flow cytometry is approximately 50% at the propositus and the parents. No mutation was found.
Propositus has CD18 expression defect.
Conclusions: Differential diagnosis with GT is important because treatment and prognosis are differents.

PB1511 | Platelet Reactivity, Independed of Platelet Count, Is Associated with Bleeding Severity in Patients with Gaucher Disease

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Background: Gaucher disease (GD) is characterized by the accumulation of glucosylceramide-laden macrophages in a variety of organs, most notably of the reticuloendothelial system, leading to hepatosplenomegaly, anemia, thrombocytopenia, and bone involvement. Bleeding tendency is a prominent feature of patients with GD, which is occasionally not proportional to the platelet counts, raising the suspicion for platelet function disorder.
Aims: To assess flow cytometry (FACS) based platelet function in patients with GD and to relate the platelet function to bleeding phenotype and GD-related status.
Methods: Bleeding severity (assessed by standardized self-administered bleeding score) and platelet function (assessed by whole blood flow cytometry (FACS)) with and without agonist stimulation were evaluated in adult patients with GD. Whole blood FACS analysis of platelet surface P-selectin and activated glycoprotein (GP)IIb-IIIa was performed with and without agonist stimulation based on a previously described protocol. Forty controls were done to establish in-house normal ranges. Logistic regression analysis was used for predictors of abnormal tests and positive bleeding phenotype. The study was approved by SZMC IRB.
Results: We have enrolled 104 GD patients [55 females, median 46 (4-91) years] with a median platelet count of 166 (29-467) X10^9/l. The bleeding phenotype was positive in 35 of 58 patients who completed the bleeding questionnaire; 24 with mild and 11 with severe bleeding phenotypes. Abnormal ADP and thrombin receptor activating peptide (TRAP) stimulated patients were recorded in 72 patients. ADP- stimulated P-selectin percent platelets and TRAP-stimulated activated GPIIb-IIIa were associated with platelet count (Table). Adjusted to platelet counts, females and low percent of TRAP

TABLE 1 Association of abnormal low platelet reactivity with platelet counts

	Total	Platelet count <50 × 10^9	Platelet count 50-99 × 10^9	Platelet count 100-149 × 10^9	Platelet count > 150 × 10^9	P value
Patients tested	104	2	11	32	58	
Abnormal ADP-(20 μM) stimulated activated GPIIb-IIIa	14	1	1	7	5	0.14
Abnormal ADP (20 μM) stimulated P-selectin platelets	33	1	7	14	11	0.006
Abnormal TRAP-(20 μM) stimulated activated GPIIb-IIIa	54	0	10	21	23	0.002
Abnormal TRAP-(20 μM) stimulated P-selectin platelets	41	0	4	17	20	0.22

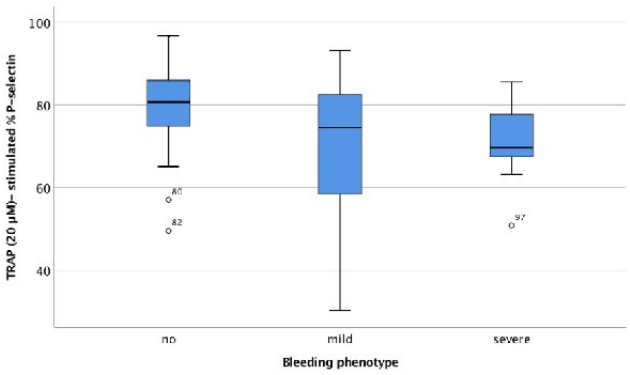

FIGURE 1 TRAP stimulated % P-selectin platelets asscoiated with bleeding phenotype

stimulated P-selectin platelets were associated with positive bleeding phenotype (figure).

Conclusions: Patients with GD have a high rate of abnormal FACS-platelet function. The association of platelet reactivity with bleeding phenotype independent of platelet counts may be helpful to assess the risk of bleeding in patients with GD.

PB1513 | Current Data About Arterial Thrombotic Events in Cerebrovascular Circulation Caused by Sticky Platelet Syndrome in the National Registry of Thrombophilic States in Slovak Republic

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Background: Sticky platelet syndrome (SPS) is a prothrombotic disorder of haemostasis with familial occurrence characterized by hyperaggregability of platelets in response to low concentrations of adenosine diphosphate (ADP) or epinephrine (EPI) or both and is associated with arterial and venous thrombosis. SPS can affect people in all age categories. Because of the seriousness of the symptoms and the risk of disability of young people, it is necessary to take it into account in the differential diagnosis of the underlying cause of unexplained thrombotic events. Transient ischemic attack (TIA) and ischaemic stroke (IS) are one of the most common clinical manifestations of the SPS.

Aims: The aim of this study is to provide insight into the background of the thrombotic events caused by SPS, especially with a focus on thrombotic events in cerebrovascular circulation.

Methods: The authors analyzed the presence of SPS in patients with history of thrombotic episodes and described individual types of SPS and further characteristics in patients after TIA or IS.

Results: In this comprehensive study, the authors revealed the influence of the SPS on the development of thrombosis. In the selected groups of individuals, they also assessed the different types of SPS.

Conclusions: SPS is a serious risk factor for development of thrombotic events and therefore we should take it into account in management of patients with unexplained cause of thrombosis.

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PB1514 | Targeted High-throughput Sequencing for Genetic Classification of Patients with Inherited Platelet Disorders

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Background: Inherited platelet disorders (IPD) comprise rare and heterogeneous disorders. Despite extensive platelet function analysis, many patients with bleeding disorders remain without substantiated

diagnosis. However, correct classification of the underlying genetic cause is essential for optimal treatment and genetic counseling.

Aims: We applied a panel-based next generation sequencing (NGS) approach based on an ISTH-based gene list to identify genetic variants in 38 IPD patients.

Methods: 59 genes known to be involved in platelet biogenesis or function were selected. Twenty-six children or adolescents and twelve adults were included into the study. Each patient had been evaluated in specialised centres due to a bleeding diathesis and considered to have a familial, syndromal or functionally diagnosed platelet disorder with early onset in life. Of these, 18 patients (47%) suffered from isolated thrombocytopenia, 16 (42%) from a platelet function disorder, and four from a combined defect (11%).

Results: For three patients, DNA quality was insuffucient for panel-analysis. In 40% of the remaining 35 analyzed patients, we detected 18 variants (phase#1 variant assessment in the 12 genes *ACTN1*, *AP3B1*, *FLNA*, *GFI1B*, *HPS1*, *HPS4*, *HPS6*, *MPL*, *MYH9*, *TBXA2R*, *TPM4*, and *TUBB1*), of which nine were novel. In 10 patients we found 13 variants of uncertain significance (VUS, class 3). Applying adequate functional tests and co-segregation analyses, 2 variants were attributed to be benign (class 1,2), 4 variants were reclassified as (likely) pathogenic (class 4,5). In total 9/35 patients received a definite genetic cause to their disease, providing a molecular diagnosis for 26% of patients.

Conclusions: This novel panel is capable of expanding genetic testing to patients that have failed standard approach to diagnosis following published guidelines, but requires accompanying functional tests and segregation analyses. We are currently updating the panel to the new ISTH gold standard.

PB1515 | Association of Genetic Variability in Selected Genes in Patients with Deep Vein Thrombosis and Platelet Hyperaggregability

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Background: Platelet hyperaggregability after low concentrations of platelet agonists adenosine diphoshate (ADP) and/or epinephrine (EPI), referred to as sticky platelet syndrome (SPS), was first described by Holiday at the Ninth Conference on Stroke and Cerebral Circulation in Arizona in 1983. Since both genders are affected, SPS has a clear autosomal pattern of inheritance, although the exact genetic cause has not been identified yet.

Aims: The aim of this study was to evaluate the genetic variability of the selected single nucleotide polymorphisms (SNPs) and examine the association between these SNPs and risk for deep vein thrombosis (DVT) in patients with SPS.

Methods: All patients were enrolled into this study between March 2011 and September 2017. Platelet aggregability was tested with platelet-rich plasma using platelet aggregometry (PACKS-4 aggregometer, Helena Laboratories, USA). High-resolution melting (HRM) analysis on LightCycler 480 II (Roche Diagnostics, Mannheim, Germany) was used for SNP (single nucleotide polymorphism) genotyping.

Results: We examined 84 patients with SPS and history of DVT and 101 healthy subjects. We were interested in two SNPs within PEAR1 gene (rs12041331, rs12566888), two SNPs within MRVI1 gene (rs7940646, rs1874445), one SNP within JAK2 gene (rs2230722), one SNP within FCER1G gene (rs3557), one SNP within PPBP (rs442155), four SNPs within ADRA2A (rs1800545, rs4311994, rs11195419, rs553668), and one SNP within SHH gene (rs2363910). We identified two protective SNPs within PEAR1 gene and one risk SNP within ADRA2A gene (PEAR1: rs12041331; rs12566888; ADRA2A: rs1800545). A haplotype analysis of 4 SNPs within ADRA2A gene identified a risk haplotype aagc (p=0.003). Moreover, we identified one protective haplotype within PEAR1 gene (AT, p=0.004).

Conclusions: Our results support the idea that genetic variability of PEAR1 and ADRA2A genes is associated with platelet hyperaggregability manifested as VTE. The study also suggests a possible polygenic type of SPS heredity. The study was supported by grants VEGA 1/0187/17 and APVV-17-0054.

PB1516 | Gynaecological Outcomes in Women with Inherited Platelet Function Disorders: Preliminary Data from the ISTH REDCap Registry

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Background: The presentations and management of women with inherited platelet function disorders (IPFD) in the gynaecology setting can be varied. Clinical experience in this area is limited due to the rarity and variety of these disorders.

Aims: To build knowledge and experience of different presentations, complications and management approaches in women with platelet function disorders.

Methods: Worldwide data were captured through the ISTH Registry completed by healthcare professionals using their patients' clinical records. Registry data was anonymized and statistics are observational.

Results: Data from 38 women, with a mean age 26 years, was analyzed. Mean age at diagnosis of IPFD was 16 yrs. Heavy menstrual bleeding (HMB) was the commonest main presenting symptom leading to diagnosis in 53%. Average age at menarche was 13 yrs with 64% of women experiencing HMB from the start of menarche and 28% presented acutely to emergency departments for management. Prior to treatment, mean pictorial blood loss assessment chart (PBAC) score was 459 and mean ISTH score was 6.

Medical management of HMB included tranexamic acid, oral combined pill, progestogens, levonorgestrel-releasing intrauterine system and/or desmopressin (DDAVP, second/third-line only). A single agent of treatment was used as first-line management in 62% with an effective treatment rate of only 14%. The proportion of women receiving two or more combination medical therapy increased at second-line, then third-line. At third-line therapy, endometrial ablation was also used in women who had completed their family. Rate of effective treatment increased with line of therapy, reaching 97% in the third-line. In acute HMB, 30% of women needed additional platelet transfusion and/or rFVIIa.

Conclusions: Data from this international registry show that women with IPFD usually present with HMB from menarche. Medical management can be challenging, necessitating a combination of antifibrinolytic, hormonal and haemostatic therapy. In retractable cases, endometrial ablation is an option for those who have completed their family.

VON WILLEBRAND FACTOR BIOLOGY

PB1518 | The Method of Purification of von Willebrand Factor

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Background: The von Willebrand's disease (VWD) together with Hemophilia A is the most common hereditary diseases of the hemostasis system. The most Factor VIII (FVIII) concentrates are designed to treatment of hemophilia A and, consequently, have low von Willebrand factor (vWF) content. The use of such concentrate in the treatment of VWD is mainly associated with an increased risk of thrombosis. On the other hand, the use of FVIII drugs can cause a serious complication of therapy - the emergence of anti-FVIII antibodies.

Aims: To development a method of purification of vWF.

Methods: The Cryoprecipitate was initial material. The activity of factors VIII and IX was determined using a coagulation test that measures the partial thromboplastin time (aPTT); vWF - the cofactor activity of ristomycin; fibrinogen concentration - by the method of Klaus; the total concentration of proteins - the Bradford method. The ion-exchange and dye-ligand affinity chromatography performed.

Results: We have developed a method of purification of vWF with a triazine dye. The first chromatographic step was performed using the DEAE-Sepharose FAST FLOW anion exchanger. At this point, we achieved a 79-fold purification (from 0.12±0.03 IU/mg protein to 9.52±0.46 IU/mg protein). In the second step, Diosorb-Procion Blue MXR absorbent was used (with a total purification rate of 284.42 times: from 0.12±0.03 IU/mg protein to 22.13±1.46 IU/mg protein). The final product yield was 33.75%. The ratio of FVIII/vWF:Rcof complex in the eluate Diasorp-Procion Blue MXR was 1.96±0.12.

Conclusions: We have developed a new method purification of vWF by the use dye-ligand affinity chromatography method that may be a fairly reliable method of this protein purification. We achieved a purification rate of 284.42 times.

PB1520 | Golgi SNARE STX5 Regulates Golgi Morphology and WPB Biogenesis

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Background: Von Willebrand factor (VWF), a multimeric hemostatic protein primarily synthesized in endothelial cells (ECs), facilitates the arrest of platelets from the circulation at the site of vascular damage and initiates the primary hemostatic plug. VWF is temporarily stored in endothelial storage organelles, the Weibel-Palade bodies (WPBs), whose biogenesis and morphology are strongly related to VWF anterograde trafficking and Golgi morphology. We have previously identified Sec22b, an ER-to-Golgi SNARE, as a novel regulator of WPB elongation though its involvement in Golgi integrity and VWF anterograde transport.

Aims: In this study we aimed to further elucidate the molecular determinants of WPB morphology by looking into the role of endothelial Sec22b interaction partners, focusing on cognate SNAREs.

Methods: Using a mass spectrometry-based approach we identified a vast array of proteins within the Sec22b interactome that potentially regulate WPB biogenesis and morphology, including the Golgi

Qa-SNARE STX5. To investigate the role of STX5 in WPB biogenesis we depleted STX5 in endothelial cells using shRNA silencing approach and followed WPB biogenesis and ER and Golgi morphology using confocal and electron microscopy. Additionally, we explored the effect of STX5 depletion on the exocytotic behavior of WPB by quantifying the VWF release under basal and stimulated conditions.
Results: STX5 depleted ECs exhibited decreased WPB length, ER dilation and extensive Golgi fragmentation. This subsequently led to reduced intracellular VWF levels and reduced stimulus-induced VWF secretion. Finally, we examined the whole proteome changes in shSTX5 and shSec22b KD ECs and found that STX5 downregulation resulted in a drastic alteration of the proteome, that negatively affected several intracellular compartments.
Conclusions: Taken together our study has identified STX5 as a novel SNARE protein that plays a major role in WPB formation and VWF release.

PB1521 | Inertial Forces Generated by a Vortex System Promote Cleavage of von Willebrand Factor

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Background: Upon exposure to non-physiological flow, through disease or implanted medical devices, the excessive cleavage of von Willebrand Factor (VWF) can lead to impaired hemostasis and bleeding.
In this current study, we used a motorized benchtop vortexer as an inexpensive, easy to use tool that requires small sample volume, to study the time-dependent behavior of VWF under various flows conditions.
Aims: Investigate flow patterns within a vortex system and evaluate the effects on VWF structure and function over time.
Methods: VWF was vortexed at three mixer settings in the presence or absence of ADAMTS13 for 0-60 minutes in a 96-well plate. Rotational speeds and flow patterns were determined from captured images using a high-speed camera and evaluated using computational fluid dynamics (CFD). Western blots and multimer analysis were used to investigate VWF structure.

TABLE 1 Characterization of the Flow Conditions within the Vortex system

Vortex setting	Calculated Vortex Rotational Speed (rad/s)	Min shear rate (s/1)	Max shear rate (s/1)	Flow behaviour
1	34	2	200	laminar
4.5	112	20	700	transitional
7	150	100	3000	turbulent

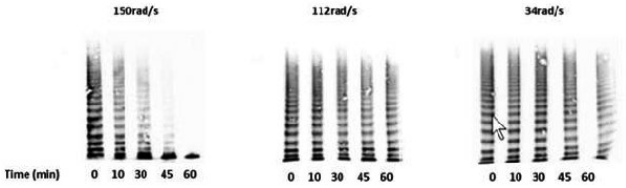

FIGURE 1 Representative multimer analysis demonstrating loss of HMWM after 10 min under turbulent 150 rad/s flow conditions

VWF function were assessed by VWF activity, collagen-binding and Ristocetin-induced-platelet-aggregations (RIPA).
Results: Three vortex mixer settings led to laminar (34-rad/s), transitional (112-rad/s), and turbulent flow (150-rad/s), as determined with a high-speed camera and CFD. Turbulent flow lead to cleavage of VWF at lower shear rates than previously reported in cone-and-plate and microfluidic devices under laminar flow (Table 1).
The cleavage and loss of HMWM was observed in samples exposed to turbulent flow with respect to time (Figure 1).
VWF function was impacted after exposure to turbulence for >10 minutes with a time-dependent decrease in antigen levels, VWF activity, collagen binding and RIPA. No significant functional changes were seen in VWF exposed to laminar or transitional flow.
Conclusions: Turbulent flow in the vortex system leads to cleavage of VWF and loss of HMWM over time, correlating with a decreased ability to bind platelets and collagen. Utilizing the vortex system, we can study turbulent flow's impact on blood, while using small samples, enabling the study of flow features seen in disease and medical devices.

PB1522 | Diagnosis of von Willebrand Disease in Algeria Laboratory and Clinical phenotype: Single Center Experience

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Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder, in Algeria the reduced number of patients is an evidence of a screening problem due to the lack of national recommendations and diagnosis guidelines. A large number of testing is necessary to diagnose and classified of VWD.
Aims: Of this study is to diagnose the VWD in Algeria.
Methods: For each patient was performed an activated partial thromboplastin time APTT, Prothrombin time, assay of Factor VIIIc (FVIIIC), assays of von Willebrand factor antigen VWF:Ag (STAGO), ristocetine cofactor VWF:RCo by visual method (SIEMENS), collagene binding test VWF:CB (STAGO) and FVIII binding test VWF:VIIIB (STAGO), blood typing ABO and blood count. The Bleeding severity was determined using ISTH-BAT.
Results: We diagnose 96 patients with VWD. 11% of the patients had VWD type 3, 33% had VWD type 1, 33% had VWD type 2 and

22% had low VWF. The frequency of bleeding family history was 59%, consanguinity was 44%. The ecchymose and epistaxis was the most common bleeding and 97% of patients had bleeding score (BS) ≥ 3. 47% of patients had blood group O. 67% of patients had low level of VWF:RCO (< 30%), 38% had low level of VWF:Ag < 30%) and 29% of patients had low level of VWF:CB (<30%). Distinction between blood groupe O and non O is important in low VWD (VWF]30-50%]).

Conclusions: The diagnosis of VWD is problematic, being subject to overdiagnosis and underdiagnosis or misdiagnosis. The existence of an isolated prolongation of the TCA or of thrombocytopenia and personal or family bleeding history (ISTH-BAT score ≥ 3) justified the performance of specific tests (FVIIIC, VWF: RCo, VWF: Ag, VWF: CB). The phenotypic diagnosis of Von Willebrand disease is very important for optimal patient management.

PB1523 | Structural Analysis of the VWF molecule with Compound in Cis Heterozygous p.R924Q and p.A2178S Mutations Causing a Type 2B-Like von Willebrand Disease

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Background: We recently identified in a 55-year-old man with hemorrhagic diathesis a compound heterozygous mutation in the VWF molecule, p.R924Q/ p.A2178S (DM-VWF), localized in the D'D3 and D4 domain of VWF. Although these mutations do not involve the A1 domain, commonly responsible for the type 2B VWD, the phenotype of this mutant clearly showed a type 2B-like phenotype.

Aims: Expression of the recombinant VWF with the observed mutations in cell cultures in order to study structural and functional features of this 2B-like VWD form.

Methods: WT and DM-VWF were expressed in HEK293T cells and purified by SE-FPLC. Transmission Electronic Microscopy (TEM) micrographs of all recVWF species, were collected with an acceleration voltage of 120 keV at a magnification of 30,000× (Libra 120; Carl Zeiss Meditec). Atomic Force Microscopy (AFM) was carried out with a silicon nitride cantilever of nominal force constant k =0.1 N/m (MikroMasch® NSC16). The region from S764 to V2191 was modeled on the I-TASSER threading modeling server (Iterative Threading ASSEmbly Refinement) to assess the putative structural effects of the compound heterozygous in cis mutation.

Results: TEM micrographs of the recombinant constructs in the absence of shear showed that WT-VWF has a globular shape, whereas the DM-VWF molecules are stretched and form reticular structures. AFM analysis obtained similar results. The molecular modeling agreed with these findings, as the S764-V2191 DM-VWF region showed a more "open" conformation than the WT form and less compact assembly of b5-b12 strands in the D3 domain.

Conclusions: The compound heterozygous p.R924Q/ p.A2178S mutation causes a less compact conformation of the VWF-monomer. The R924Q mutation eliminates several polar contacts with G913, H916 and K843, contributing to generate a less compact assembly of b5-b12 strands in the D3 domain of VWF. These conformational changes are compatible with the functional type 2B-like VWD features observed in the patient.

PB1524 | The Scavenger Receptor Scara5 Mediates Clearance of the von Willebrand Factor Propeptide

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Background: Despite the widespread use of the VWF propeptide (VWFpp) to antigen ratio as a marker for increased VWF clearance, little is known about the mechanisms regulating clearance of the VWFpp including the specific receptor(s) involved.

Aims: To investigate a role for the scavenger receptor Scara5 in mediating clearance of the VWFpp.

Methods: Mouse VWFpp (mVWFpp) and propeptide-deleted mVWF (Δpro-mVWF) were expressed in a HEK293T expression system. The interaction between mVWFpp and mouse Scara5 (mScara5) was investigated using solid-phase binding assays. Clearance studies in $VWF^{-/-}$ and $Scara5^{-/-}/VWF^{-/-}$ mice involved infusion of mVWFpp via tail vein injection (200U mVWFpp/kg).

Results: Solid-phase binding assays demonstrated dose-dependent binding of mVWFpp to mScara5 (K_{Dapp} 2.5±0.4 µg/ml, 27 nm). Binding was strictly calcium-dependent (binding buffer ± $CaCl_2$; 100±4.9% vs 12.9±2.8%, p=0.0001) and was abrogated by the presence of EDTA (9.2±1.4%, p=0.0001). Co-incubation of mVWFpp with Δpro-mVWF significantly inhibited mScara5 binding (100±3.3% vs 14.8±2.2%, p=0.0001) indicating that mVWFpp competes with mVWF for mScara5 binding. To further probe the mechanisms governing this interaction, a role for N-linked glycans was examined. Enzymatic removal of the N-linked glycans completely abolished mScara5 binding (100±1.6% vs 2.6±0.2%, p=0.0003). Moreover, a specific role for the glycan at Asn666 was identified. Targeted removal of this glycan significantly reduced binding (100±2.2% vs 28.9±9.3%, p=0.0001) while removal of the N-linked glycan at Asn156 had no significant effect (111.0±6.0, p=0.404). Subsequent *in vivo* clearance studies in $Scara5^{-/-}/VWF^{-/-}$ mice demonstrated a 2-fold increase in mVWFpp half-life ($VWF^{-/-}$ vs $Scara5^{-/-}/VWF^{-/-}$; 3.5 minutes vs 6.8 minutes, p=0.0043). Interestingly however, Scara5 deficiency was not associated with an increase in endogenous mVWFpp plasma levels in $Scara5^{-/-}$ mice ($VWF^{-/-}$ vs $Scara5^{-/-}$; 1.0±0.12U/ml vs 1.2±0.2U/

ml, p=0.37). Cumulatively, these data demonstrate that although mScara5 can bind and clear mVWFpp from plasma, its relative physiological contribution to mVWFpp clearance may be minor.

Conclusions: The endocytic scavenger receptor Scara5 mediates mVWFpp clearance.

PB1525 | Monitoring Recombinant and Plasma-Derived VWF Concentrates: An *in vitro* Study

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Background: Several different plasma-derived and recombinant von Willebrand Factor (VWF) concentrates are used to prevent and treat bleeding in von Willebrand disease patients. Measuring VWF antigen levels and VWF activity can be useful in monitoring replacement therapy however several different assays could exist for measuring these parameters.

Aims: The aim of this study was to assess the performances of different laboratory assays in monitoring VWF replacement therapy using 3 plasma-derived and 1 recombinant VWF concentrates.

Methods: Human VWF deficient plasma (Cryopep®) was alternatively spiked with four different concentrates of VWF (plasma-derived : Eqwilate® (Octapharma®), Wilfactin® (LFB®), Voncento® (CSL Behring®); and recombinant : Veyvondi® (Takeda®)) and human standard plasma (Siemens®). We measured VWF antigen (VWF:Ag) and different VWF activities using 4 different analyzers: VWF:Ag and VWF:GPIbR (HemosIL®) on AcuStar and ACL-Top (Werfen®), VWF:CB (HemosIL®) on AcuStar, VWF:Ag and VWF:Rco on STAR-Max (Stago®), VWF:Ag and VWF:GpIbM (Innovance®) on CS-2100i (Sysmex®). Assays were performed in triplicates using 4 different final concentrations of VWF (1, 0.5, 0.25 and 0.1 IU/mL).

Results: For all VWF concentrates tested, there was no significant difference between techniques when measuring VWF:Ag at all 4 concentrations.

Except for human standard plasma for which inter-assay variability showed high agreement (Fig. 1), results for VWF activity measurements showed variations depending on the assay and molecule tested.

Wilfactin® activity levels were significantly higher with ACL-Top than all 4 other assays especially at high concentrations (Fig. 1 Fig. 2). VWF:GpIM and VWF:Rco assays showed significantly lower activity levels than the target in plasma spiked with Wilfactin®, Voncento® and Eqwilate® ($p < 0.05$).

For Veyvondi® no agreement was found between all assays at every concentration tested ($p < 0.05$).

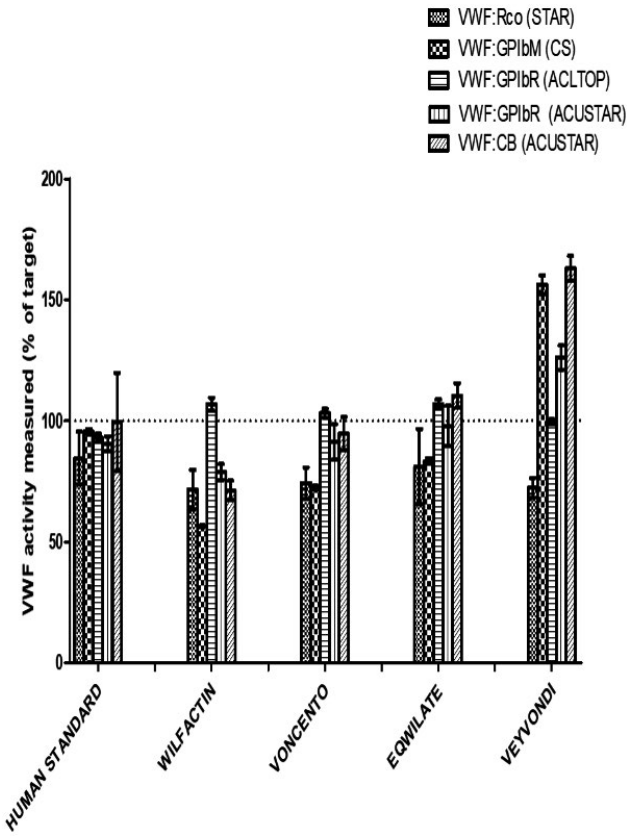

FIGURE 1 VWF activity measured for 1 IU/mL final concentration of VWF

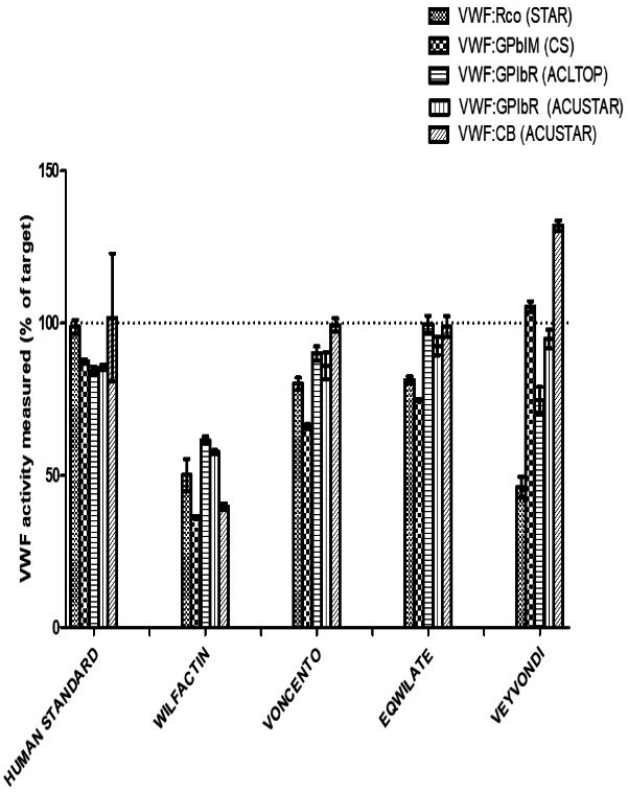

FIGURE 2 VWF activity measured for 0.5 IU/mL final concentration of VWF

Of note, VWF:CB was consistently higher whereas VWF:Rco provided the lowest results.

Conclusions: VWF activity assays are not all equivalent when monitoring different VWF concentrates and precautions should be taken accordingly.

PB1526 | Excessive Angiogenesis in von Willebrand Factor Knockout Mice is Accompanied by Increased Perivascular Coverage of Neovessels

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Background: Von Willebrand Factor (vWF) is an essential coagulation protein that is becoming recognized as a regulator of angiogenesis. Patients with acquired or congenital vWF deficiency exhibit dysregulated angiogenesis in the form of angiodysplastic lesions in the gut. The mechanisms that regulate angiodysplasia are unknown and current studies focus almost exclusively on the role of endothelial cells in vWF deficient angiogenesis.

Aims: Thus the goal of our study was to characterize the role of perivascular cells (pericytes) during angiogenesis in WT and vWF knockout (KO) mice using the aortic ring model.

Methods: Abdominal aortas were collected from mice aged 4 to 8 weeks, cut into 0.1 cm rings, and cultured for up to 21 days in collagen matrices. Neovessels sprouting from the rings were counted on selected days.

Results: On days 3, 7, and 10 vWF KO rings contained significantly more sprouts than WT: ($p< 0.05$, n= 30 rings). This difference resolved 14 days after embedding and the number of vessels remained similar until the experiment's end. At every time point WT and vWF KO vessels exhibited similar thickness, length, and number of branch points. In the aortic ring model the proliferative stage of angiogenesis peaks around day 7. Pericyte coverage and morphology were evaluated in 7-day-old cultures by immunofluorescent staining for the pericyte marker NG2. Images were captured by confocal microscopy and the area of NG2 staining was analyzed with Image J. vWF KO sprouts demonstrated significantly higher pericyte coverage than WT sprouts
($p< 0.05$, n=52 images KO, n=43 images WT), with no discernible difference in pericyte morphology or localization along the vessels.

Conclusions: These data suggest that pericyte migration, adhesion and/or proliferation may be enhanced in the absence of vWF expression. This further suggests that pericytes play a role in the excessive angiogenesis that occurs in vWF KO conditions.

PB1527 | Explanations for Rise of Levels of Factor VIII and von Willebrand Factor Antigen with Age

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Background: Factor VIII (FVIII:C) and von Willebrand Factor antigen (VWF:ag) levels increase with age in the general population.

Aims: To evaluate whether the increase of FVIII:C and VWF:Ag with age in the population is mediated by estrogen use, body mass index (BMI), comorbidities, impaired kidney function, or high levels of C reactive protein (CRP).

Methods: Analyses were performed in a sample from the general population in the Netherlands (n=2823; table 1). Individuals with active cancer and women who were pregnant or within three months postpartum were excluded. FVIII:C was measured on an automatic coagulometer (STA-R) and VWF:Ag with an immunoturbidimetric method (STA Liatest), according to the manufactures instructions. Estimated glomerular filtration rate (eGFR) was calculated by the MDRD equation. Information on estrogen use, BMI, and comorbidities (diabetes, liver and kidney disease, rheumatoid arthritis, multiple sclerosis, hyperthyroidism, hypothyroidism, bronchitis, emphysema, heart failure, angina, myocardial infarction, transient ischemic attack, ischemic and hemorrhagic stroke, peripheral vascular disease) was obtained from self-administered questionnaires. Linear regression analysis was used to evaluate the change in FVIII:C and VWF:Ag levels with age. Analyses were adjusted for sex, estrogen use, BMI, comorbidities, CRP (log transformed), and eGFR.

Results: FVIII:C and VWF:Ag increased with age, i.e., increase per decade of age (β) for FVIII:C: 8 IU/dL, 95%CI 7-9 and for VWF:Ag: 11 IU/dL, 95%CI 10-13. Adjustment for eGFR led to marginally different estimates: FVIII:C: β 7 IU/dL, 95%CI 6-8, VWF:Ag: 10 IU/dL,

TABLE 1 Clinical characteristics

	Population controls (n=2823)
Male, n (%)	1364 (48)
Age at enrollment (years), mean (SD)	49 (12)
Major illness, n (%)	444 (16)
Estrogen use (in women), n (%)	425 (31)
BMI (kg/m^2), mean (SD)	25.60 (4)
C reactive protein (mg/L), mean (SD)	2.83 (5.68)
eGRF (mL/min), mean (SD)	87 (17)
FVIII activity (IU/dL), mean (SD)	112 (38)
VWF antigen (IU/dL), mean (SD)	112 (47)

TABLE 2 FVIII activity and VWF antigen levels in age groups

Age (years	Number of controls, n (%)	Mean FVIII (IU/dL)	FVIII Mean difference (IU/dL)	Adjusted[a] FVIII Mean difference (IU/dL)	Mean VWF:Ag (IU/dL)	VWF:Ag Mean difference (IU/dL)	Adjusted[a] VWF:Ag Mean difference (IU/dL)
18-30	223 (8)	101 (35)	Reference	Reference	97 (35)	Reference	Reference
30-40	480 (17)	101 (32)	0(-6 to5)	-2(-8 to 3)	97 (34)	2(-5 to 8)	-1(-9 to 6)
40-50	677 (24)	105 (35)	4(-2 to 9)	0(-6 to 5)	101 (36)	5(-1 to 11)	0(-7 to 7)
50-60	847 (30)	114 (36)	13(8 to 18)	5(0 to 11)	115 (43)	19(13 to 26)	10(3 to 16)
60-70	596 (21)	129 (43)	27(22 to 33)	18(11to 24)	136 (62)	39(33 to 46)	27(20 to 35)

[a] Adjusted for sex, estrogen use, BMI, comorbidities, CRP and eGFR.

95%CI 8-11. Further adjustment for sex, estrogen use, BMI, comorbidities, and CRP (individually or in the full model) did not affect the association between biomarkers and age (full model: FVIII:C: β 5 IU/dL, 95%CI 4-7, VWF:Ag: 8 IU/dL, 95%CI 6-9). Table 2 shows FVIII:C and VWF:Ag in age groups.

Conclusions: The increase of FVIII:C and VWF:Ag with age is only marginally mediated by estrogen use, BMI, comorbidities, eGFR, or CRP.

PB1528 | Comparison of an Automated von Willebrand Factor Activity Assay by HemosIL Werfen® with Ristocetin Cofactor Assay by Aggregometry in Thrombocytic Patients with Myeloproliferative Neoplasms or Secondary Thrombocytosis

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Background: VWF is composed of multimers and the high-molecular-weight multimers (HMWM) carry the most important hemostastic effect. Patients with myeloproliferative neoplasms (MPN) and high platelet count (> 1500 G/l) are at risk of bleeding because of a decrease in HMWM, i.e. acquired von Willebrand disease. This is suspected when VWF ristocetin cofactor activity (VWF:RCo) is decreased. For functional assessment of VWF, aggregometry (*RCo(Agg)*) is considered to be the gold standard. However, this assay suffers from poor reproducibility and sensitivity at low levels of VWF and is labour intensive. Various assays for RCO have been developed in recent years.

Aims: To compare the performance of HemosIL RCo, Werfen® versus aggregometry in patients with thrombocytosis (platelets > 450G/L) in the context of MPN (T-MPN) and in patients with secondary thrombocytosis (ST).

Methods: Samples from 36 patients with MPN and 18 patients with ST were evaluated with *RCo(Agg)* and *RCo(IL)* assays. VWF antigen level (VWF:Ag), distribution of VWF multimers by electrophoresis and VWF binding capacity to collagen (CB) were also analysed for each patient.

Results: In T-MPN and ST patients, there was a significant difference between the two techniques for RCo and RCo/Ag ratio ($p < 0.0001$ and $p < 0.0001$). A majority of T-MPN patients (22/36) had RCo/Ag ratio ≤ 0.6 with HemosIL assay but not with aggregometry. In ST patients, only 6/18 patients had discordant results. Among T-MPN patients with discordant results, 5 had actually a decrease in HMWM and/or CB ≤ 0.6 versus none among the 6 ST patients with discordant results. There was a correlation between *RCo(IL)/Ag* ratio and platelet count in T-MPN patients but not in ST patients.

Conclusions: HemosIL RCo reagent seems to be more sensitive than aggregometry to detect a change in the profile of HMWM in T-MPN patients

PB1529 | The Super Glue of Ultra-Large Multimers - Characterisation of Recombinant and Platelet Derived von Willebrand Factor

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Background: The multimeric composition of recombinant von Willebrand factor (rVWF) concentrate as well as platelet VWF (plateletVWF) is different compared to plasma-derived VWF (pdVWF). rVWF as well as plateletVWF retain full-spectrum multimers with a higher proportion of ultra-large multimers (ULM).
Aims: We aimed to characterize and compare structural and functional properties of plateletVWF, pdVWF, and rVWF.
Methods: pdVWF was obtained from whole blood of 10 healthy volunteers and plateletVWF was isolated after centrifugation, two washing steps and application of detergent. Two approved and commercially available plasma derived VWF concentrates and a recombinant VWF were tested. Standard assays (IL Instrumentation Laboratory, Bedford, MA, USA) for 1) VWF:Ag, 2) VWF:GP1bM, and VWF:GP1bR, 3) VWF:CBA were used. Gel electrophoresis served for multimeric analysis.
Results: For optimized comparability VWF measurements were standardized for VWF:Ag (=100%). Results for VWF:Ag and activity parameters are shown in table 1 and 2. Multimer analysis showed the previously described structural differences with both rVWF and plateletVWF containing a high portion of ultra-large multimers (plateletVWF >rVWF>pdVWF) and the absence of triplet bands compared to pdVWF or pdVWF concentrates.
Conclusions: Significant functional and structural differences between plasmatic, platelet and recombinant VWF could be demonstrated. Importantly, the rVWF preparation clinically used as an equivalent of the plasmatic VWF, shows partial structural and functional similarities to plateletVWF with comparable values of activity parameters and multimeric structure potentially implying improved hemostatic efficacy compared to pdVWF.

TABLE 1 Analysis of commercial VWF products (measured in duplicates)

	VWF: Ag	VWF: GP1bM	VWF: GP1bR	VWF: CBA
pdVWF concentrate 1	100	71	82	58
pdVWF concentrate 2	100	70	78	50
rVWF concentrate	100	120	105	110

TABLE 2 Analyses of pVWF and pdVWF from healthy donors (n=10; mean ± SD). * denotes statistically significant difference to pdVWF

	VWF: Ag	VWF: GP1bM	VWF: GP1bR	VWF: CBA
plateletVWF	100 ± 21	142 ± 44*	46 ± 8*	72 ± 15*
pdVWF	100 ± 36	90 ± 39	81 ± 34	99 ± 29

PB1531 | Investigating Pathomolecular Mechanisms of Type 2A VWD Variants Located in D1 and A Domains of the VWF

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Background: Von Willebrand disease (VWD) type 2A is characterized by decreased von Willebrand factor (VWF)-dependent platelet adhesion and a deficiency of VWF multimers.
Aims: Mutation analysis of the VWF gene revealed missense substitutions p.G55E and p.W191R as compound heterozygous in two patients with an ambiguous phenotype of type 2A/2B VWD as well as p.L1288R and V1524G as heterozygous in two type 2A VWD patients, respectively. This study aimed to determine the pathomolecular mechanisms by which these variants cause deficiency in multimers assembly and VWF-mediated platelet adhesion.
Methods: The full-length wild-type (wt) or mutant VWF cDNA were expressed in HEK293/T cells. The VWF antigen levels secreted into the medium were measured. The VWF binding to GPIbα and multimer analysis was performed. The comparative degree of co-localization of VWF and ER in HEK293 cells was calculated after acquiring z-stack images with Zeiss Apotome.2. Biogenesis of the Weibel-Palade bodies (WPBs) was explored by the arivis Vision4D program.
Results: Transfection of the p.G55E, p.W191R and p.V1288R demonstrated impaired VWF secretion, 9.8%, 10.9%, and 38.2% of wt, respectively. The co-transfections of the p.G55E and p.W191R with the wt improved the expression but still showed reduced secretion and loss of large multimers. Co-transfection of the p.L1288R with wt corrected secretion, but still with diminished binding to GPIbα. Interestingly, the V1524G did not affect VWF secretion in both single and co-expression studies but disrupted binding to GPIbα. Immunostaining studies showed the accumulation of the p.G55E and p.W191R in the ER, leading to no formation and reduction in the number of WPBs in single and co-transfected cells, respectively.
Conclusions: Missense variants p.G55E and p.W191R, in the VWF D1 domain, interfere with ER-Golgi VWF anterograde transport, leading to impaired multimerization and secretion. While the p.L1288R

and V1524G mutants in A1 and A2 domains affect proper folding for multimer assembly or binding to GPIbα, respectively.

PB1532 | Reduced Cleavage of Circulating Ultra-large von Willebrand Factor by ADAMTS13 Exacerbates Microvascular Disease Following Traumatic Injury

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Background: Increases in plasma von Willebrand Factor (vWF) and decreases in its respective metalloprotease, ADAMTS13, have been demonstrated in diseases of microvascular injury such as acute kidney injury (AKI).
Aims: We hypothesized that following severe trauma, ultra-large vWF (UL-vWF) is released into the bloodstream by damaged endothelium. We further hypothesized that traumatic injury would lead to a deficit of ADAMTS13. The deficient pool of circulating ADAMTS13 would worsen the accumulation of UL-vWF forms and, ultimately, increase the risk of AKI.
Methods: Plasma from a cohort of patients with trauma induced AKI, collected at 0-, 24-, and 72-hours after admission, was evaluated against plasma from trauma patients matched for injury characteristics but without AKI. VWF and ADAMTS13 activity and antigen levels were evaluated. Circulating vWF multimeric composition was determined by vertical agarose gel electrophoresis.
Results: VWF antigen was increased in trauma patients when compared to healthy controls at all time points (314% (253-349) vs. 100% (87-117)). VWF levels were strongly correlated with vWF type 3 collagen binding activity (CBA) (r=0.76; p< 0.0001). Trauma patient ADAMTS13 activity was decreased at all time points when compared to healthy patients (36.0% (30.1-44.7) vs. 100.0% (83.1-121.0)). Trauma patient CBA:vWF antigen was inversely related to ADAMTS13 activity (r=-0.45; p=0.001) and was significantly elevated when compared to controls (0.21 (.19-.25) vs. 0.16 (.15-.20) p=0.001). While trauma patients, as a whole, demonstrated increased high molecular weight multimeric vWF when compared to healthy controls (27.7 (24.6-31.00) vs. 15.9 (14.5-16.8)), the subset whom developed AKI showed significantly elevated levels at 72-hours when compared to non-AKI counterparts (32.9 (30.4-35.3) vs. 27.8 (24.6-30.8) p=0.02).
Conclusions: Following injury, increases in vWF and decreases in ADAMTS13 support life-saving hemostasis. However, as these changes extend into the following days, the early hemostatic benefit shifts to burden that may contribute to AKI.

PB1533 | Lyophilized Human Platelets Show Hemostatic Function Independent of von Willebrand Factor

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Background: Von Willebrand Disease (VWD) is the most prevalent bleeding disorder, causing excessive bleeding and bruising in 1% of the population. vWF mediates platelet collagen interactions under high shear by binding to CD42b. Murine vWF does not interact with human CD42b. Thrombosomes®, or lyophilized human platelets (LHP), are a stabilized platelet derived hemostatic agent currently under clinical development.
Aims: To determine the role, if any, that vWF plays in the promotion of hemostasis by LHP.
Methods: Surface expression of vWF binding sites on LHP and fresh platelets was measured by flow cytometry using the CD42b function blocking antibody clone AN51. The ristocetin cofactor assay was performed to measure ristocetin induced binding of vWF and subsequent agglutination of LHP relative to fresh and fixed platelets. Ristocetin induced platelet aggregation (RIPA) was performed in PRP and in plasma supplemented with LHP. The Total Thrombus-formation Analysis System (T-TAS) was used to assess thrombus formation by LHP and fresh platelets under shear in thromboplastin-collagen coated microcapillaries using normal and VWD Type 3 plasma. The *in vivo* hemostatic capability of LHP was assessed via tail bleeding times in NOD/SCID mice.
Results: Binding of αCD42b was reduced by 55.6% in LHP relative to fresh platelets as shown by mean fluorescent intensity. The average agglutination for LHP in the ristocetin cofactor assay was reduced by 83.5% compared to fixed platelet controls. Fresh platelets but not LHP supported RIPA. Occlusion to collagen under flow by LHP occurred similarly in normal and Type 3 VWD plasma. Average bleeding time in mice receiving LHP was reduced to 4.2 minutes relative to > 9 minutes in untreated mice and mice administered fresh platelets.
Conclusions: LHP may work independently of vWF *in vitro* yet support thrombus formation and hemostasis *in vivo*, suggesting that LHP may therapeutically promote hemostasis in patients with VWD.

PB1534 | Type 2B Mutations Differentially Alter Conformational Dynamics of VWF A1 Domain

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Background: Type 2B VWD mutations share a common feature of enhanced association of VWF to platelet GPIbα, but lead to disparate extent of thrombocytopenia in patients. Concurrent homogeneous and heterogeneous effects dictate that a type 2B mutation affect at least two separable properties of VWF. Previous studies have posited that the flanking residues of the VWF A1 domain function as an autoinhibitory regulator of its accessibility, but whether they adopt inherent structural elements or function by steric hindrance has been contested.
Aims: We aim to characterize how type 2B mutations alter conformational dynamics of A1 and flanking residues, with the broader goal of defining additional alterable properties of VWF.
Methods: Stability and dynamics of mammalian A1 fragments (residues 1238-1493) bearing type 2B mutations were analyzed using thermal shift assays, hydrogen deuterium exchange mass spectrometry, and analytical ultracentrifugation. Binding and functional activity of these variants were assessed via biolayer interferometry and platelet aggregometry.
Results: Of the seven mutant A1 fragments that are being studied, all are primarily monomers and have unfolding temperatures significantly lower than WT. Interestingly, most mutants including H1268D and R1341Q, but not R1306W, undergo two thermal transitions. All mutants exhibit slower dissociation rates from immobilized recombinant GPIbα (Figure 1) and spontaneously aggregate washed platelets at 60 nM concentration albeit to various degrees. Lastly, compared to WT, some mutants such as H1268D has increased deuterium uptake in flanking residues beyond Asp1261 and Asp1472 and in residues of α1β2, β3α2, and α3β4 loops during 10-30s exchange time frame.
Conclusions: Type 2B mutations reduce global stability of the A1 domain (including flanking residues) and, in some cases, enhance exposure of the secondary GPIbα binding site. The increased deuterium uptake of flanking residues beyond Asp1261 and Asp1472 in some type 2B mutants suggest that these flanking residues adopt certain structural elements that may contribution to VWF autoinhibition.

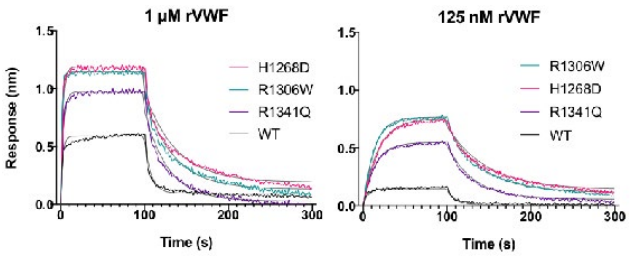

FIGURE 1 Binding Kinetics of A1 Variants

VWF AND VON WILLEBRAND FACTOR DISORDERS - CLINICAL CONDITIONS

PB1538 | Clinical Presentation of Von Willebrand Disease in a Developing Country-A Cross Sectional Study

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Background: Von Willebrand's disease (VWD) is a common inherited bleeding disorder.
Aims: Our study aims to access the information regarding clinical presentation and frequency of types of Von Willebrand Disease in Pakistan.
Methods: A cross sectional study was carried out in Sir GangaRam Hospital Lahore from Dec 2017 to Nov 2018. 50 patients were included in the study. The present study was carried out to find the frequency of VWD, its types, and clinical features of the disease. Informed consent was obtained.
A detailed personal and family history of bleeding manifestations including onset, signs, and symptoms were noted. Complete Blood Count (CBC), bleeding time (BT), Prothrombin time (PT), Activated Partial Thromboplastin Time (APTT), fibrinogen and blood grouping were the laboratory tests performed who met inclusion criteria.
Results: 23 (46%) patients were male and 27 (54%) were female. Type3 VWD (92.12%) was found to be the commonest type. Two (5.92%) cases of type-2 VWD and only one (1.96%) case of type-1 VWD were identified. Easy bruising was the most commonly observed clinical presentation, 23 (46.13%) patients, followed by menorrhagia (20.40%), epistaxis (11.68%), bleeding after surgery (9.68%), bleeding after circumcision (6.45%), umbilical cord bleeding (4.92). Consanguineous marriages were reported in parents of (84.6%) patients.
Conclusions: Type-3 VWD was found to be the commonest type which can be attributed to the fact that type-3 VWD is transmitted through autosomal recessive pattern of inheritance and consanguineous marriages are highly practiced in our society leading to high frequency of this form of VWD. Easy bruising, menorrhagia and epistaxis were concluded to be the most common clinical presentation.

PB1541 | Population Pharmacokinetics and Pharmacodynamics of Recombinant von Willebrand Factor

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Background: Recombinant von Willebrand factor (rVWF; vonicog alfa; Baxalta US Inc., Lexington, MA, USA) replacement therapy for

von Willebrand disease (VWD) corrects VWF activity (measured by VWF:ristocetin cofactor [RCo]) and increases factor VIII (FVIII) activity to hemostatically effective levels. Increased understanding of the effects of rVWF dosing on VWF:RCo and FVIII activities could optimize outcomes for individual patient needs.

Aims: To develop population pharmacokinetic (PK)/pharmacodynamic (PD) models that describe VWF:RCo and endogenous FVIII activity-time profiles following administration of rVWF.

Methods: A population PK model for rVWF was developed from individual patient data (1664 samples; n=79) from 3 rVWF studies in patients with different VWD types (phase 1: NCT00816660; phase 3: NCT01410227, NCT02283268) and a phase 1 study in patients with severe hemophilia A. A population PK/PD model was subsequently developed using FVIII activity data from both phase 3 VWD studies (686 FVIII samples; n=41, different VWD types). The rVWF trials were approved by the institutional review boards or independent ethics committees of all participating sites, and all patients provided written informed consent.

Results: Standard methods including goodness-of-fit plots and visual predictive checks verified that the rVWF population PK/PD model captures the variability in VWF:RCo and FVIII activity after single and repeated rVWF doses. This model allows the prediction of the VWF:RCo-versus-time curves as well as the FVIII-activity-versus-time curves following single and multiple doses of rVWF. For example, simulation of repeated 50 IU/kg rVWF doses every 72 hours suggested that FVIII activity is maintained at highly hemostatically effective levels (trough level of ≥40 IU/dL) in a considerable proportion of patients with type 3 VWD.

Conclusions: This population PK/PD model describes VWF:RCo and FVIII activity in rVWF-treated patients with different VWD types, and could assist in the optimization of rVWF personalized dosing strategies.

PB1542 | Symptomatic Venous Aneurysm of the Internal Jugular Vein Presenting with Small Thrombotic Inclusions in von Willebrand Disease Type I

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Background: Venous aneurysms present a rare condition, and only a few case reports exist about venous aneurysms located in the area of the jugular veins. Even if venous aneurysms are classified benign, they may cause severe complications such as local thrombosis, rupturing and pulmonary embolism.

Aims: This case reports a venous aneurysm of the right internal jugular vein (IJV) showing small thrombotic inclusions in a 42 year-old woman with bleeding tendency due to von Willebrand disease (vWD) type I.

Methods: The patient presented to our vascular medicine outpatient clinic at the Medical University of Graz because of a painful swelling in the right neck area with an increase in size within the past few months. A previously performed CT-scan assumed venous thrombosis of the right jugular veins. Further diagnostic workup was performed by color-encoded duplex sonography and MRI.

Results: Color-encoded duplex sonography revealed a hypoechogenic lesion with small intraluminal hyperechogenic spots connected to the IJV. The MRI showed a T2-hyperintense lesion with intraluminal inhomogeneity, consistent with a venous aneurysm. Anticoagulation with Edoxaban 60 mg o.d. was preexisting because of the suspicion of jugular vein thrombosis. The patient reported a significant decrease of symptoms with this therapy, but also common episodes of nose bleeding, which may result from a combination of a bleeding tendency due to vWD with intake of oral anticoagulation. Surgical as well as endovascular treatment for the aneurysm were supplied, but both options were refused because the patient did not feel capable of these procedures with regard to a severe panic disorder.

Conclusions: In spite of lack for evidence-based recommendations, we decided for a resumption of anticoagulation with Edoxaban in a reduced dose of 30 mg o.d. and for strict observance of bleeding complications for this rare case of IJV aneurysm with thrombotic lesions in concomitant vWD type I.

PB1543 | The Utility of ISTH-BAT in Iranian Patients with von Willebrand Disease

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Background: The ISTH bleeding assessment tool (ISTH-BAT) was developed to aid the evaluation of patients who are suspected for bleeding disorders.

Aims: To assess the utility of ISTH-BAT in the diagnosis and determining the severity of the bleeding in Iranian patients with VWD.

Methods: In this prospective study the ISTH-BAT as well as PT, aPTT, FVIII: C, VWF: Ag, VWF: RCo, VWF: CB, RIPA and blood group typing were assessed for 65 patients with VWD(VWF: Ag or/ and VWF: RCo< 40 IU/dl) and 25 adult healthy controls with no significant bleeding history or a known coagulation disorder. Results are expressed as medians and interquartile ranges (IQR).

Results: Demographic data are summarized in Table 1. In the healthy controls the range of BS was 0-6 in females and 0-5 in males, however since a large majority of scores were < 2 (~68%), IQR was used for normalizing the data. Ultimately the ISTH-BAT normal range was found to be 1-4 in females and 0-2 in males. BS was significantly

TABLE 1 Baseline characteristics of the study population

Subjects	N	Age (y)	Sex M/F	Blood group O/Non O	Total BS
VWD type1	31	24 (1-62)	17/14	16/10	5.78 (1-15)
VWD type 2	28	27 (0.5-55)	16/12	12/16	8.42 (1-21)
Healthy Control	25	35 (24-59)	12/13	9/16	1.96 (0-6)

higher in VWD patients as compared to controls (P< 0.05). After excluding 6 patients with incomplete ISTH-BAT result, median bleeding score in patients with VWD type 1 and 2 was 5.78 (range 1-15) and 8.42 (1-21) respectively. The most common clinical symptoms in patients with VWD were cutaneous bleeding (77%), epistaxis (61.5%) and tooth exaction (52%). Women showed a higher BS than men but no significant difference was observed (median: 7.16 VS 6.95). 71% of men and 55% of women had significant clinical symptoms (BS≥4 and BS≥6 respectively).

Conclusions: Our result showed that the ISTH BAT can help the identification of subjects requiring laboratory evaluation for VWD thus will improve the decision making and management of patients.

PB1544 | Investigating the Relationship between VWF Multimeric Pattern and VWF: RCo Activity through Population PK Modeling in Patients with Severe VWD Treated with Recombinant VWF

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Background: Recombinant von Willebrand factor (rVWF, vonicog alfa; Baxalta US Inc., a Takeda company, Lexington, MA, USA) is a VWF replacement therapy for von Willebrand disease (VWD). Unlike plasma-derived (pd)VWF, rVWF is not exposed to enzymatic proteolysis and has an intact multimer pattern that contains higher proportions of large and ultra-large (L/UL) multimers than pdVWF. Modelling pharmacokinetic (PK) profiles of small (S), medium (M), and L/UL multimers following rVWF dosing could help understand how the contributions of these multimers affect VWF:ristocetin cofactor (RCo) activity, a functional measure of VWF platelet binding activity.

Aims: To develop population PK models describing activity-time profiles of S, M, and L/UL multimers following intravenous rVWF dosing and compare multimer kinetics with those of VWF:RCo.

Methods: Population PK models were developed using non-linear mixed-effects modeling based on multimer distribution data from a phase 3 study in patients with severe VWD receiving 50 or 80 IU/kg

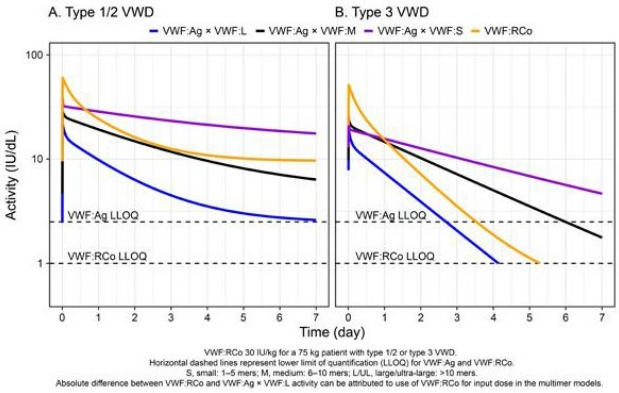

FIGURE 1 Population predictions of VWF:RCo, VWF:Ag x VWF:S, VWF:Ag x VWF:M and VWF:Ag x VWF:L activity over time after rVWF in patients with VWD

VWF:RCo rVWF (n=36) (NCT01410227; ethics committee approval and patients' informed consent were obtained). Multimer PK profiles were derived through scaling the proportion of S, M, and L/UL multimers by total VWF antigen (VWF:Ag) levels, calculated as VWF:Ag x VWF:S, VWF:Ag x VWF:M and VWF:Ag x VWF:L, respectively.

Results: Disposition kinetics of VWF:Ag x VWF:S, VWF:Ag x VWF:M and VWF:Ag x VWF:L were best described by two-compartment models. Endogenous levels were accounted for as a constant background. PK model predictions showed that L/UL multimers had the highest degree of similarity in terms of disposition kinetics, indicated by a near-parallel PK curve to VWF:RCo, whereas S and M multimer fractions were characterized by a slower PK profile (Figure 1).

Conclusions: This model-based analysis provides supporting evidence that the VWF:RCo activity in patients with severe VWD is associated with the persistence of L/UL VWF multimers in systemic circulation.

PB1545 | Recombinant Human von Willebrand Factor (rVWF) Differs in Structure and Function from Plasma Derived Concentrates

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Background: VWF-containing concentrates from human plasma (pdVWF) vary in the content: and composition of VWF, FVIII and other constituents. rVWF (vonicog alfa, Baxalta US Inc., a Takeda company, Lexington, MA, USA) is a highly purified VWF protein produced in CHO cells. In contrast to any pdVWF, rVWF maintains an intact multimer pattern because it is never exposed to proteolytic degradation, particularly not by the VWF cleaving protease ADAMTS13. Although some comparisons of pdVWF and rVWF are

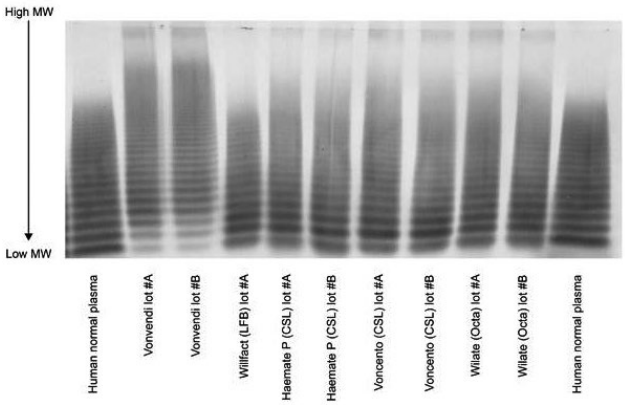

FIGURE 1 Low resolution (1%) agarose electrophoresis of rVWF and pdVWF concentrates

published, a comprehensive analysis of multimers and functional characteristics of VWF products is lacking.

Aims: Structural and functional comparison of clinically used pd and rVWF concentrates::

- pdFVIII/VWF: HAEMATE P/HUMATE-P, VONCENTO (CSL Behring, King of Prussia, USA); WILATE/EQWILATE (Octapharma, Lachen, Switzerland)
- pdVWF: WILFACTIN/WILLFACT (LFB, Courtaboeuf, France):
- rVWF: VONVENDI/VEYVONDI:

Methods: Cross-product comparisons included VWF content by determination of VWF:Ag, and biological function assessed by VWF:RCo, VWF:CB, and VWF:GpIbM (VWF activity assay Innovance; Siemens) using routine commercial test kits. VWF multimeric structure was determined by low (1%) and high (2.5%) resolution agarose electrophoresis.

Results: In all pdVWF concentrates the ratio between measurements of biological function of VWF and VWF:Ag content was below 1, indicating the presence of not fully active VWF between 5 and 30%. Corresponding ratios for rVWF were above 1. Thus, rVWF has higher specific activity by all biological measurements. Only rVWF contained the full spectrum of ultra-large and high molecular weight multimers (Figure 1). While pdVWF showed varying satellite band structures indicative for different degrees of proteolysis, rVWF had an intact multimeric pattern. VWF:GpIbM measurements were, for all products, higher than those obtained with the VWF:RCo assay.

Conclusions: VWF products differ in their contents, multimer size and structure of functional VWF. These differences may translate into improved biological activity of rVWF in clinical settings and influence the treatment regimen of individuals with VWD.

PB1546 | von Willebrand Disease Combined with Other Coagulation Defects: Experience at a Single Reference Coagulation Laboratory in Iran

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Background: Von Willebrand disease (VWD) is known as the most common inherited bleeding disorder however, well-documented cases of combined VWD with other coagulation defects have been infrequently reported.

Aims: To describe a series of patients with combined VWD and coagulation defects and also evaluate the incidence of combined factor deficiencies in Iran.

Methods: All subjects with VWD panel test requests from June 2018 - May 2019 were included in this prospective monocentric study. For all subjects, demographic data, PT, PTT, VWF: Ag, VWF: RCo, FVIII: C were evaluated by standard methods. Patients with prolonged PT and/or PTT were further investigated for other factor assays and lupus anticoagulant (LA) panel. Also ISTH- BAT, RIPA, and VWF: CB for patients with confirmed VWD results were performed. The patients receiving FVIII or VWF concentrate in 10 days before sampling and pregnant women were excluded.

Results: 1107 subjects were evaluated which results of 70 cases showing VWF: Ag or/and VWF: RCo ≤ 40% and VWD diagnosis was

TABLE 1 Clinical presentation and von Willebrand disease screening in patients with combined coagulation defects

Case	Clinical presentation	FVIII:C (IU/mL)	VWF:Ag (IU/mL)	VWF:RCo (IU/mL)	VWF:CB (IU/mL)	BS	coagulation defect(s)
1/5 year/M	Postoperative bleeding	54	34	29	36	21	LA: Positive
2/4 year/F	Epistaxis, Oral cavity, GI bleeding	64	61	35	36	20	FVII: 34, LA: Positive
3/5 year/M	Postoperative bleeding	78	42	34	28	10	FVII: 42
4 /27 year/F	Epistaxis, Menorrhagia	75	43	35	55	7	FXI: 2
5/31 year/F	Mucocutaneous bleeding, Tooth extraction	43	50	36	53	7	FVII: 11
6/32 year/M	Mucocutaneous bleeding, Tooth extraction	70	45	32	41	4	FVII: 52
7/5 year/M	Epistaxis, Mucocutaneous bleeding	74	40	30	39	2	FXII:42

confirmed. After further investigation, we identified seven patients with a combination of coagulation defects and VWD (Table1). All patients except one had significant clinical symptoms (median: 10 and range: 4-21) with at least three hemorrhagic symptoms that might be a result of combined deficiencies. Interestingly in two patients LA was positive. This study providing an estimated incidence of 8.5% for combined deficiencies in our patient population with the almost same prevalence in men and women.

Conclusions: We can conclude that combined factor deficiencies incidence in our patient population is higher than other records due to consanguineous marriages and it seems to be underdiagnosed. As Co-inheritance of VWD and factor deficiencies can alter the treatment approach for the management of patients, it worth attention.

PB1547 | Harnessing Social Media to Improve Awareness of Bleeding Disorders

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Background: Public awareness of bleeding disorders (BD) is low, resulting in delays in diagnosis. For women with BD, heavy menstrual bleeding (HMB) is frequently underestimated with 40% of women with low Von Willebrand Factor and HMB self-reporting their periods as "normal". Improved public understanding of HMB may increase recognition, referral and diagnosis of BDs.

Aims: We sought to heighten awareness of HMB amongst females (13-33 years old, yo) utilising a social media (SoMe) approach. The reach and influence of digital marketing in health promotion was analysed as part of the "KnowYourFlow" campaign.

Methods: A video asset explaining the signs of HMB and BD was distributed through a SoMe campaign. Pre-marketing research identified high Instagram use (85% of teenagers) and the video was targeted to both Instagram Stories and Newsfeed. Results over 4 weeks were analysed (budget €400), with engagement determined by impressions (times content shown to users) and reach (unique users), age category and user location. Affordability was assessed by the cost per post reaction (CPPR).

Results: Over 4 weeks, the Instagram digital media marketing campaign recorded 695,147 impressions with a reach of 150,048 unique Irish female users. From these users, 7,159 thru plays (^{3}15 seconds) were recorded. At a 2 week interim analysis, increased engagement was noted via Stories rather than Newsfeed and among 13-18yo; the subsequent campaign focused on this approach and cohort.

Overall, 21.3% of 13-33yo Irish females viewed this campaign, highlighting the success of this approach. High levels of affordability were seen with a CPPR of €0.40.

Conclusions: SoMe marketing of health promotion campaigns can successfully target selected cohorts at low cost. Dynamic responses to adapt campaigns based on interim analysis help to maximise cost effectiveness. Using this approach, KnowYourFlow successfully reached 1 in 5 young Irish females, helping to improve awareness of both HMB and BD.

PB1548 | Characterization of Type 2B VWD in the US Zimmerman Program

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Background: Type 2B VWD is primarily characterized by increased binding of VWF to the GPIbα receptor. Sequence variants (SV) in type 2B are found in the *VWF* A1 domain. These cause gain-of-function VWF binding to platelets and subsequent proteolysis by ADAMTS13, resulting in the loss of HMW multimers. However, not all type 2B patients have reduced VWF activity and abnormal multimers.

Aims: To examine this further, 52 type 2B subjects enrolled in the Zimmerman Program were analyzed to determine the causative *VWF* SV and correlation with subject laboratory and bleeding phenotypes.

Methods: Analysis included *VWF* exonic sequencing, VWF laboratory assays, and expression of recombinant VWF in HEK293T cells.

Results: Five amino acids in the *VWF* A1 domain were responsible for our type 2B cases: p.R1306W/Q, p.V1316M, p.R1341Q/W, p.R1308C and p.H1268D/N/Y. The most common were p.R1306W (35%), p.V1316M (27%), p.R1341Q (15%) and p.R1308C (8%). Most had consistent laboratory phenotype including reduced VWF:RCo/VWF:Ag, absent HMW multimers, increased platelet-VWF binding and abnormal ISTH-BAT, except for those with p.R1341W who had normal VWF:RCo/VWF:Ag, VWF:CB/VWF:Ag and multimers. p.R1341W subjects had a good response to DDAVP/Stimate and no thrombocytopenia. Twelve affected family members with p.R1341W had a heterogeneous phenotype including a spectrum of normal to absent HMW multimers, however all had normal VWF:RCo/VWF:Ag. Index cases with p.R1341W had greater enhanced VWF binding to GPIb with low-dose ristocetin compared to those with p.R1341Q, which were corroborated by expression studies. Recombinant R1341Q and R1341W (heterozygous and homozygous) had similar VWF:Ag, VWF:CB and multimers compared to wild type, but an increase of VWF:GPIbM and VWF:GPIbM/VWF:Ag.

Conclusions: While the majority of type 2B VWD is caused by recurrent SV in the *VWF* A1 domain, patients with p.R1341W present with a heterogeneous phenotype and mild bleeding symptoms and

may be more challenging to diagnose using VWF:RCo/VWF:Ag and multimers alone.

PB1549 | Response to Treatment for Gastro-Intestinal Bleeding in von Willebrand Disease

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Background: Gastro-intestinal (GI) bleeding is a characteristic feature of severe Von Willebrand disease (VWD), that arises generally in older patients, requiring in most cases blood transfusion and hospitalization. The presence of arteriovenous malformations is often described. Patients with congenital type 3, 2A and 2B are those most frequently affected by this symptom, which could be due to the loss of high-molecular-weight multimers (HMWM).

Aims: To evaluate the response to treatment in case of GI bleeding in patients with severe VWD.

Methods: Case-series. All charts from patients with severe VWD (defined as any type of VWD with VWF:RCo < 6 IU/dL) evaluated at the A. Bianchi Bonomi Hemophilia and Thrombosis Center in Milano between 2015 and 2019 were reviewed. GI bleeding was defined as occult (positive stool test) or evident (hematemesis or melena).

Results: A total of 116 patients were evaluated, finding 35 (30%) with at least one episode of GI bleeding during their life (occult in 4); 2 patients with severe GI bleeding (not responsive to treatment with FVIII/VWF concentrate) were excluded as they presented a bleeding comorbidity (Idiopathic Thrombocytopenia and Hereditary hemorrhagic telangiectasia), 1 patient was excluded because bleeding was due to hemorrhoids inflammation and was resolved by surgery. A blood transfusion was required in 62.5% (20/32 patients) and a local lesion (ulcer, gastric or intestinal polyps or mucosal inflammation) was associated to GI bleeding in 41% of patients (13/32). Recurrent bleeding was present in 56% of patients (18/32). Tables 1 and 2 present patients and GI bleeding characteristics.

TABLE 1 Patients characteristics

			All patients, N=116	Patients with GI bleeding, N=32
Female sex		*number (%)*	58 (50)	15 (47)
Age at diagnosis, years		*median (range)*	19 (0-69)	34 (3-53)
Age at last follow-up, years		*median (range)*	49 (6-88)	61 (7-87)
FVIII:C, IU/dL		*median (range)*	34 (1-110)	29 (1-76)
VWD type	with loss of HMWM (type 3, 2A and 2B)	*number (%)*	68 (59)	20 (62.5)
	without loss of HMWM (type 2M and 1, not classified)	*number (%)*	48 (41)	12 (37.5)
Age at last follow-up, years	with loss of HMWM (type 3, 2A and 2B),	*median (range)*	45 (6-87)	54 (7-87)
	without loss of HMWM (type 2M and 1, not classified),	*median (range)*	57 (7-86)	65 (38-86)

TABLE 2 Characteristics of GI bleeding events

		Patients with GI bleeding N=32	VWD with loss of HMWM N=20	VWD without loss of HMWM N=12
Age at first GI bleeding, years	*median (range)*	45 (3-81)	43 (3-75)	49 (6-81)
GI bleeding event before diagnosis	*number (%)*	12 (37.5)	7	5
Local lesion causing GI bleeding	*number (%)*	13 (41)	5	8
Recurrent GI bleeding	*number (%)*	18 (56)	12	6
Presence of angiodysplasia	*number (%)*	8 (25)	5	3
No treatment with FVIII/VWF concentrate	*number (%)*	8 (25)	5	3
Treatment (FVIII/VWF concentrate) with complete response	*number (%)*	14 (44)	5	6
Long term prophylaxis (FVIII/VWF concentrate) with complete response	*number (%)*	3 (9)	3	-
Need of rescue therapy associated with prophylaxis	*number (%)*	4 (13)	4	-

Conclusions: One-third of the patients with severe VWD presented at least 1 episode of GI bleeding during their life. A fifth of the patients with GI bleeding (n=7, 22%), all characterized by loss of HMWM, needed long term prophylaxis or some rescue therapy (surgery, atorvastatin, octreotide, lenalinomide).

PB1550 | Real-life Management of Digestive Endoscopies: Is There a Bleeding Risk for Patients with von Willebrand Disease?

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Background: Gastrointestinal (GI) bleedings are well-recognised complications of von Willebrand disease (VWD), requiring many endoscopic procedures.

Aims: To report our real-life experience of digestive endoscopies' management in patients with VWD and identify a potential post-examination hemorrhagic risk.

Methods: A retrospective, non interventional, multicentric study was carried out over 48 months, from the BERHLINGO database, in 5 hemophilia centers in Western France. Types of endoscopies, VWD and treatments were assessed.

Results: *The analysis phase is currently going on and data presented here are for the coordinating centre cohort only.* So far, among a cohort of 481 patients, 207 were hospitalized during 439 in-stays. Notably, 92 GI in-stays were identified: 48 involve digestive endoscopies, for 37 patients (Cf table 1).

TABLE 1 patients' characteristics

	Patients: n (%)	In-stays: n (%)	Age: mean [Min;Max]	Mean basal VWF Activity (IU.L^{-1})	Mean basal FVIII:C (IU.L^{-1})
Type non classified	1 (2.7%)	1 (2.1%)	46.9 [46.9;46.9]	19.0	27.0
Type 1	5 (13.5%)	5 (10.4%)	47.9 [29.0;63.7]	25.0	52.0
Type 2 (Total)	26 (70.3%)	33 (68.7%)	56.0 [18.9;78.4]	22.5	38.3
Sub-type 2 unclassified	2 (5.4%)	3 (6.2%)	61.7 [58.6;63.1]	8.3	28.7
Sub-type 2A	5 (13.5%)	5 (10.4%)	60.3 [56.2;66.9]	20.4	50.0
Sub-type 2B	5 (13.5%)	9 (18.8%)	56.7 [44.6;63.0]	20.7	45.2
Sub-type 2M	11 (29.8%)	13 (27.1%)	50.7 [18.9;78.4]	11.8	34.7
Sub-type 2N	3 (8.1%)	3 (6.2%)	64.6 [57.9;71.4]	89.0	21.7
Type 3	5 (13.5%)	9 (18.8%)	50.7 [21.7;68.0]	< 1.9	1.5

TABLE 2 Patients' VWF treatments

	Patients: n	Treatments (in-stays, n)	Mean basal VWF Activity (IU.L^{-1}) [Min; Max]	Mean basal FVIII:C (IU.L^{-1}) [Min; Max]	Pre-endoscopy VWF (IU/kg/inj) [Min; Max]	Pre-endoscopy FVIII (IU/kg/inj) [Min; Max]	Mean VWF dosage (IU/kg/ED) [Min; Max]	Mean FVIII dosage (IU/kg/ED) [Min; Max]
Type 1	1	VWF+FVIII, n=1	14 [14; 14]	41 [41; 41]	47.4 [47.4; 47.4]	19.7 [19.7; 19.7]	36.8 [36.8; 36.8]	15.4 [15.4; 15.4]
Type 2 (Total)	13	/	15.0 [4.0; 38.0]	41.0 [17.0; 80.0]	30.9 [0.0; 62.1]	11.7 [0.0; 25.9]	37.9 [21.2; 62.1]	14.0 [0.0; 25.9]
Sub-type 2A	1	VWF+FVIII, n=1	24.0 [24.0; 24.0]	50.0 [50.0; 50.0]	0.0 [0.0; 0.0]	0.0 [0.0; 0.0]	37.9 [37.9; 37.9]	15.8 [15.8; 15.8]
Sub-type 2B	3	VWF only, n=2 VWF+FVIII, n=4	20.0 [11.0; 38.0]	46.2 [34.0; 80.0]	34.4 [0.0; 44.1]	11.3 [0.0; 18.2]	40.2 [23.4; 54.5]	12.0 [0.0; 22.7]
Sub-type 2M	9	VWF+FVIII, n=10	10.8 [4.0; 19.0]	36.5 [17.0; 50.0]	32.0 [0.0; 62.1]	13.3 [0.0; 25.9]	36.4 [21.2; 62.1]	15.2 [8.8; 25.9]
Type 3	5	VWF+FVIII, n=9	<1.9 [<1.0; <3.0]	1.5 [1.0; 2.0]	43.1 [0.0; 60.0]	15.2 [0.0; 30.0]	52.4 [33.3; 84.0]	24.3 [5.0; 80.0]

An ileocolonoscopy alone was performed in 54.2% of cases (n = 26), a upper endoscopy alone in 20.8% of cases (n = 10) and a combination of several procedures in 25.0% of cases (n = 12).

DDAVp (desmopressin) was administered in 27.1% of cases (n = 13), mainly for types 1 and 2M vWD. Without biopsies (n = 6), the mean DDAVp dosage was 0.25 µg/kg/injection during 1.0 day, whereas it was 0.26 µg/kg/injection with 1.19 injection/day during 2.4 days in case of biopsies (n=7). von Willebrand Factor (VWF) was administered in 56.2% of cases (n = 27), essentially for type 2 (63.0%) and type 3 (33.3%) VWD (Cf table 2). Without biopsies (n = 17), the mean VWF dosage was 44.1 IU/kg/ED (exposure day) during 2.3 ED whereas it was 45.9 IU/kg/ED during 4.1 ED in case of biopsies (n = 10).

Concomitantly, all patient were prescribed per os tranexamic acid. No patient was readmitted within 15 days of the endoscopy.

Conclusions: The implementation of registries with real-life data could assess clinical practices and safety in patients with VWD undergoing endoscopy, and ensure compliance with guidelines.

PB1551 | A Retrospective Study to Evaluate the Efficacy and Safety of a Plasma-Derived, von Willebrand Factor-Containing FVIII Concentrate, in Patients with Von Willebrand Disease (VWD) in Spain: Interim Analysis Results

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Background: Positive results on VWD therapy with Fanhdi® (Grifols) a plasma-derived, von Willebrand factor-containing FVIII concentrate (pdFVIII/VWF) have been reported in clinical trials. However, studies on use of Fanhdi® in standard clinical practice are scarce.

Aims: The main objective of this observational study was to retrospectively evaluate in an interim analysis the clinical efficacy and safety of Fanhdi® in standard clinical practice in patients with VWD in Spain.

Methods: Patients diagnosed with congenital VWD and treated with Fanhdi® for bleeding episodes or during surgical prophylaxis, when desmopressin is ineffective or contraindicated, were included in the study, which was granted the approval from the hospital Ethics Committee. Medical records of patients followed up from January 2011 to December 2017 were retrospectively collected. Efficacy of Fanhdi® achieving haemostasis was evaluated in bleeding episodes (mean dose ± SD: 45.33±8.24 IU/Kg) and in the prevention of surgical bleeding (mean dose ± SD: 36.66±11.28 IU/Kg) according to the following criteria: Excellent, Good, Poor, and No Response. For safety endpoints, related adverse events were assessed.

Results: Thirty-six patients (male [n=14]; female, [n=22]) with VWD type 1 (n=10), type 2 (n=17), and type 3 (n=9) were analysed. Median (P25;P75) age was 34.5 (19.5;50.0) years old (Table 1). A total of 61 bleeding episodes and 28 surgical procedures were reported. Overall, replacement therapy with Fanhdi® showed an Excellent to Good clinical efficacy in 96.7% of bleeding episodes and in 100% for the prevention of excessive bleeding during surgical procedures (Table 2). No related adverse events occurred during 318 infusions.

Conclusions: Awaiting the final study results, this interim analysis suggests that Fanhdi® can be efficacious and safe in the management of bleeding episodes and in the prevention of excessive bleeding during surgery in patients with VWD.

TABLE 2

Clinical situations	n (%) episodes reported	Efficacy of treatment with Fanhdi®	
Bleeding episodes	**61 (61.6%)**	**Excellent**	**41 (67.2%)**
		Good	18 (29.5%)
		Poor	2 (3.3%)
		No response	0 (0.0%)
Prevention of surgical bleeding	28 (28.3%)	Excellent	19 (67.9%)
		Good	9 (32.1%)
		Poor	0 (0.0%)
		No response	0 (0.0%)

TABLE 1

	TYPE OF VWD DIAGNOSED						
	All types	Type 1	Type 2A	Type 2B	Type 2M	Type 2N	Type 3
Parameter	n=36 (100.0%)[1]	n =10, (27.7%)	n =11, (30.5%)	n=4, (11.1%)	n=0, (0%)	n =1 (2.7%)	n=9 (25.0%)
Male/female, n (%)	14 (38.9)/ 22 (61.1)	5 (50.0)/ 5 (50.0)	4 (36.4)/ 7 (63.6)	2 (50.0)/ 2 (50.0)	–	0 (0.0)/ 1 (100.0)	3 (33.3)/ 6 (66.7)
Age, years, Median (P25;P75)	34.5 (19.5;50.0)	35.5 (20.0;69.0)	35.0 (22.0;62.0)	33.5 (21.0;45.5)	–	36.0 (36.0;36.0)	34.0 (15.0;39.0)

[1]One of the patients with VWF Type 2 was not subclassified due to lack of information in the eCRD at the time of the interim analysis. This information will be included in the final report of the study.

PB1552 | von Willebrand Factor Propeptide: Useful Diagnostic Add-On to Differentiate von Willebrand Disease Based upon Increased von Willebrand Factor Clearance

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Background: In 2006 Haberichter et al. pointed out the clinical utility of von Willebrand factor propeptide (vWFpp) in identifying patients with type 1 von Willebrand disease (vWD) with decreased von Willebrand factor (vWF) survival. In 2015 Sanders et al. reported, that vWFpp identified severe type 1 vWD with very low vWF levels in patients who had previously been classified as type 3 vWD underlining the diagnostic significance of vWFpp in classifying vWD patients. In 2018 O'Sullivan emphasized that enhanced vWF clearance may play an important role in the pathogenesis of vWD with direct therapeutic relevance.

Aims: Aim of the study was the evaluation of selected samples from patients with different types of vWD - Low Willebrand factor (LWF), type 1, 1C, 3, 2A, 2M as well as Acquired vWD (AQ-vWD) - with a new, rapid and specific vWFpp ELISA available since 2019.

Methods: Recently a CE-labeled vWFpp-assay (Senova GmbH, Erfurt, Germany) became available. The sandwich type ELISA employs precoated ready-to-use strips and requires a total of about 90 min for completion. It was applied on specimen from 34 patients.

	von Willebrand disease				
vWFpp/vWF:Ag-ratio	LWF	type 1C	type 1	type 2A	AQ-vWD
>2	-	4	4	2	6
<2	6	-	8	1	-

FIGURE 1 Distribution of vWFpp/vWF:Ag-ratios in remaining vWD types

Results: vWFpp was below the limit of detection in vWD type 3 cases (n=2). The vWD type 2M case disclosed a vWFpp/vWF:Ag-ratio close to 2.

Conclusions: The vWFpp/vWF:Ag-ratio proves to be helpful in discriminating patients with a markedly increased vWF clearance (vWD type 1C, AQ-vWD and specific type 1 and type 2 cases). Identification of vWD patients with enhanced vWF clearance may contribute to optimize therapeutic regimens, especially considering the treatment-option with DDAVP.

PB1553 | WISH-QoL Study: Assessment of Health-Related Quality of Life and Health-Economic Aspects in Patients with von Willebrand Disease in France: Results from the 2nd Interim Analysis on the 355 Enrolled Patients

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Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder with around 0,01% prevalence for the most symptomatic forms where bleeding requires replacement treatment and/or hospitalization.

TABLE 1 Patient's characteristics at inclusion (n = number of subjects)

n=355	Type 1 75 (21%)	Type 2 226 (64%)	Type 3 27 (8%)	Unknown 27 (8%)	Total 355
Female	54 (72%)	141 (62%)	14 (52%	18 (67%)	227 (64%)
Age category (years) <18	25	76	12	12	125
Age category (years) ≥18	50	150	15	15	230
Familial VWF deficiency n (%)	65 (87%)	178 (79%)	11 (42%)	19 (70%)	273 (77%)
Median [range] VWF:RCo (%)	20 [1; 40]	13.5 [0.5; 128]	4 [0; 15]	15.5 [2.5; 42	14 [0; 128]
Median [range]VWF:Ag (%)	22 [3; 55]	33 [3; 144]	2 [0.5; 24]	20.5 [5; 66]	27 [0.5; 144]
Median [range] FVIII (%)	41 [5; 100]	42 [2; 202]	2.5 [1; 57]	44 [5; 97]	40 [1; 202]
Median [range]Tosetto hemorrhage score	4 [0; 19]	7 [-1; 25]	15.5 [3; 31]	3 [0; 19]	6 [-1; 31]
On-demand treatment n (%)	73 (97%)	222 (98%)	14 (52%)	27 (100%)	336 (95%)

VWD affects both genders with a higher prevalence and more frequent symptomatic forms in females.

Aims: The aim of the WiSH-QoL study is to characterize the quality of life (QoL) and the impact of the disease and its treatment on daily-life, in VWD patients and their families.

Methods: WiSH-QoL is an observational, ongoing, descriptive, prospective, multicenter study conducted on patients whatever the VWD phenotype, in 28 French centers.

Primary objective is to evaluate VWD patients QoL, using scoring from generic and disease-specific questionnaires, at baseline and at 24 months.

Patients fill QoL questionnaires according to their age: patients ≥18 filled the generic SF-36 and the VWD-QoL; patients aged 4-17 the Disabkids and the VWD-QoL (I or II) questionnaires and their parents filled the corresponding VWD-QoL and the FABEL. For patients < 4, the VWD-QoL 0 and the FABEL questionnaires were filled by parents.

Clinical phenotype such as bleeding Tosetto score is documented.

Results: In this second interim analysis, data at enrolment are presented for 355 patients with a median Tosetto score of 6 [-1; 31].

At inclusion, the SF36 questionnaire's Physical (PCS) and Mental Component Scores (MCS) tended to be more impaired in females: PCS 54.04 vs 55.22; MCS 44.71 vs. 47.85 in females and males respectively.

Conclusions: WiSH-QoL, the first prospective Quality of life study in Von Willebrand disease conducted in France, will provide important insights into a large cohort of type 2 (226/355) patients' daily-life with their perception of well-being and their specific health care.

These results emphasize too, that all VWD females need a specific healthcare throughout their fertile life.

PB1554 | The Role of the von Willebrand Factor Propeptide (VWFpp) in the Evaluation of Desmopressin Response in a Patient with Acquired von Willebrand Syndrome (AVWS)

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Background: AVWS is a rare disease characterized by clinical and laboratory findings similar to von Willebrand disease, but occurring later in life. The main pathogenic mechanism (when associated with heart valve disease, autoimmune diseases, monoclonal gammopathies, myeloproliferative disorders and cancer) is due to an accelerated VWF clearance from plasma.

Aims: The role of VWFpp in the evaluation of desmopressin response in a patient with AVWS.

Methods: Case-report. Patient plasma and platelet VWF were evaluated as recommended by ISTH-SSC guidelines. Biochemical, clinical and other medical evaluations were performed to identify the associated disease causing AVWS.

Results: A 40 years old male was referred to our A.B.B. Center for repeated epistaxis associated to anemia. Mild nose bleeding was present since childhood, but without need of medical attention. Surgery had been performed in the past without bleeding complications. An AVWS associated to monoclonal gammopathy (IgG kappa, 0.1 g/dL) was diagnosed, based on low plasma levels of VWF and FVIII, associated to an elevated VWFpp/VWF:Ag and normal VWF platelet levels *(Table)*. ELISA evaluation of anti-VWF IgG was positive and mixing tests with the Bethesda method using VWF:RCo yield 1BU of inhibitor (results were negative using VWF:CB, VWF:Ag and FVIII:C assays). Patient desmopressin response test was performed twice *(Figure A/B)* with different detection times. The second desmopressin test showed increased levels of FVIII and VWF after 20 minutes and their complete clearance after 1 hour, associated with increasing levels of VWFpp.

Conclusions: In this AVWS patient, the measurement of VWFpp along with VWF levels after desmopressin infusion showed a rapid clearance of secreted VWF. This finding may suggest that elevated

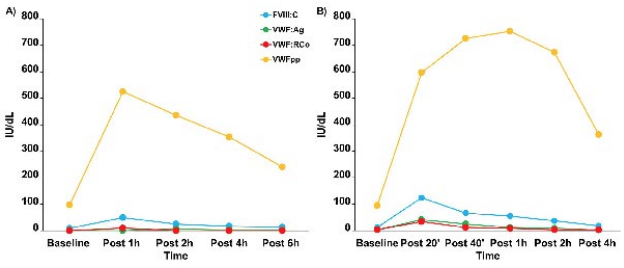

FIGURE 1 A) First desmopressin infusion test, B) Second desmopressin infusion test]

TABLE 1 Biochemical data of the patient

	FVIII:C (IU/dL)	VWF:Ag (IU/dL)	VWF:RCo (IU/dL)	VWF:CB (IU/dL)	VWFpp/VWF:Ag	Multimer analysis	Intra-platelet VWF:Ag (IU/10^9 plts)	Intra-platelet VWF:RCo (IU/10^9 plts)	Intra-platelet Multimer analysis
Patient	5	5	< 6	< 1	19	Faint trace of a full multimeric pattern	0.25	0.24	Normal multimeric pattern
Normal range	50-150	40-169	41-168	45-174	0.6-1.6	Normal	0.20-0.54	0.16-0.57	Normal

VWFpp may be predictive showing lack of efficacy of desmopressin treatment due to very rapid clearance of VWF, released after its administration. Such enhanced clearance of endogenous VWF, could also predict a poor response to exogenous VWF concentrate.

PB1555 | Retrospective Chart Review of Gastrointestinal Bleeding in Patients with von Willebrand Disease: Study Design and Initial Demographic Results

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Background: Specific etiology and treatment of gastrointestinal (GI) bleeds in patients with von Willebrand disease (VWD) is challenging and compounded by limited published data. Despite von Willebrand Factor (VWF) replacement therapy, GI bleeding may be refractory resulting in various treatment approaches with no consensus regarding the most effective therapeutic approach.

Aims: To describe etiology and management of GI bleeds, and outcomes following VWF replacement products and adjuvant therapy, including recombinant VWF (rVWF).

Methods: This retrospective, multicenter, observational chart review included patients with confirmed congenital VWD with ≥1 GI bleed within last 5 years (abstraction initiated 2019 and ongoing). The target sample size is 20 patients from 6 US centers. Demographics and clinical information, including treatment regimens, will be abstracted from patient records on all recorded GI bleeds within the last 5 years. Clinical effectiveness will be defined by treatment response, change in duration of treatment or time to bleed resolution across treatment cohorts (e.g. prophylaxis, on-demand; rVWF, plasma-derived VWF) at the time of a GI bleed and for any subsequent period of prophylactic treatment to prevent GI bleed recurrence. Data will be analyzed descriptively.

Results: To date, data on 22 bleeds in 7 patients with type 1 (14%), type 2 (43%) or type 3 (43%) have been abstracted. 57% were female, mean (±SD) age was 55.71 (18.86) years, and all patients had ≥1 recorded GI-specific morbidity. Treatments used for GI bleeding included aminocaproic acid, tranexamic acid, plasma-derived VWF-factor VIII concentrates, antihemophilic factor (recombinant), rVWF, corticosteroids, polypectomy, and thalidomide.

Conclusions: This retrospective chart review will describe real world experience to identify etiology and management of GI bleeds in VWD patients, and provide insights into the use and effectiveness of current treatments for GI bleeding in individuals with VWD. Results from this study may identify additional therapeutic approaches for the treatment of GI bleeding in VWD.

PB1556 | Treatment with Emicizumab Leads to Clinical Improvement in a Type 3 von Willebrand Disease Patient with Inhibitor Suffering from a Left Ankle Arthropathy

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Background: The occurrence of anti-von Willebrand Factor inhibitor (anti-VWF) in patients with type 3 von Willebrand disease (3VWD) is a rare but severe complication of the treatment with VWF. It leads to an almost complete therapeutic inefficiency and sometimes to severe allergic reactions.

We report here the off-label use of emicizumab in a 48-year old patient with 3VWD and persistent anti-VWF.

She developed a left ankle arthropathy in 2000 and had a tibiotalar arthrodesis in 2005. She began to experience pain and joint blockings in 2015, which worsened in 2019.

She was currently treated on demand with recombinant Factor VIII (FVIII) infusions (simoctocog alfa, 4,000 IU i.e. 67 IU/kg b.w. per infusion once or twice 2 to 4 hours later due to the short half-life of FVIII in 3VWD patients), but it was less and less effective.

Aims: As emicizumab showed great efficacy on hemophiliac arthropathies, we started an off-label treatment with emicizumab at the end of August 2019 following the recommended dosage.

Methods: Case report.

Results: She experienced a complete relief from pain by the end of the first month of treatment: she has been on emicizumab for 6 months and walks now freely.

We performed Thrombin Generation Assays that showed significant improvement. We also measured the plasmatic concentration of emicizumab: there was no biological sign of neutralizing anti-drug antibody.

Conclusions: Emicizumab showed great efficacy in this patient with left ankle arthropathy. Thus it might be considered in patients with 3VWD and anti-VWF having arthropathies.

PB1557 | Clinically Relevant Differences between Two New Generation VWF: GPIbR Assays in a Patient with Acquired von Willebrand Syndrome - Case Report

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Background: Acquired von Willebrand Syndrome (AVWS) is a rare bleeding disorder that is considered 'under-recognised' and 'misdiagnosed'. It commonly occurs secondary to other conditions and the pathogenic mechanisms are heterogeneous. Here we describe a case of AVWS in a 63-year old female diagnosed with a breast cancer who was referred to a haemophilia consultant for pre-operative assessment prior to lumpectomy. The patient had an established diagnosis of essential thrombocythemia (ET) and presented with extensive bruising but with no previous history of bleeding.
Aims: This is a first case report highlighting clinically relevant differences in VWF:GPIbR results obtained using different assay principles in a patient with AVWS.
Methods: Immunoturbidimetric VWF activity assay involves binding of plasma VWF to wild-type recombinant GpIbα fragments attached to latex particles in a ristocetin-dependent manner. The chemiluminescent method utilises magnetic particles instead of latex. The VWF inhibitor screen was performed using immunoturbidimetric VWF:GPIbR activity on patient plasma, normal pooled plasma (NPP) and a 50:50 mixture of patient plasma and NPP. Platelet function assay (PFA) and multimer analysis were also performed.

TABLE 1 VWF parameters generated by both methods

Test	Results		Units
	Immunoturbidimetric	Chemiluminescent	
VWF Antigen	110.5 (RI=67-124)	71.3 (RI=37-146)	IU/dL
VWF: GPIbR	15.1 (RI=58-107)	41.5 (RI=34-119)	IU/dL
VWF: CBA	68.5 (RI=42-147)	48.5 (RI=35-147)	IU/dL
RI - reference interval			

TABLE 2 Inhibitor screen results

Samples	VWF:GPIbR (immunoturbidimetric)	Units
Patient	15.4	IU/dL
Normal Pooled Plasma (NPP)	86.7	IU/dL
Theoretical mix value	51.1	IU/dL
50:50 mix	36.3	IU/dL
Inhibition	29.0	%

Results :
Conclusions: Typically in ET, AVWS occurs due to loss of HMW and intermediate VWF multimers to the platelet surface. However, in this case the abnormalities in VWF:GPIbR activity and PFA but normal multimer profile is a consequence of an inhibitory antibody disrupting VWF-GPIb interaction, which was confirmed in mixing studies. While the immunoturbidimetric method allowed the detection of the functional anti-GPIb inhibitor, the chemiluminescent method did not mirror this. Patient's clinical symptoms have improved following the treatment and VWF parameters for both methods are expected to be normal. The investigation is ongoing.

PB1558 | Recombinant von Willebrand Factor (rVWF) in Patients with von Willebrand Disease (VWD) Undergoing Elective or Emergency Surgery in Real World Setting - The Dijon University Hospital Experience from March 2019 to January 2020

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Background: Few publications are available on the use of rVWF in medical practice as the drug has only recently become available. We report ten surgical procedures in 8 patients (5 males, 3 females, 36 to 73 yo).
All type 1 patients had baseline VWF parameters < 30% and are included in the FranceCoag Network.
Aims: rVWF efficiency assessment in elective and emergency surgery.
Methods: Patients were hospitalised on the day of surgery. rVWF was administered at 32-48 $IUkg^{-1}$, generally 1-2 hours before procedure.

TABLE 1 Table of surgical procedures

Minor/ major procedure	Type 1 VWD (n=6) Elective	Type 1 VWD (n=1) Emergency	Type 3 VWD (n=1) Elective
Minor	Shoulder infiltration	Dental abscess drainage	Hyaluronic acid knee therapy
Minor	Double knee infusion with platelet rich plasma		
Minor	Gastroscopy and recto-sigmoidoscopy with biopsies		
Minor	Endometrial ablation		
Minor	Endovenous thermal ablation with phlebectomy		
Minor	PICC line insertion		
Minor	Prostate Biopsy		
Major	Gastric bypass in morbidly obese male (BMI = 42)		

VWF antigen (VWF:Ag) and GPIbM activity (INNOVANCE® Siemens) were measured 24 hours post-surgery and once daily, until discharge (hospitalisation 0-6 days). The patient undergoing gastric bypass received 34.8 $IUkg^{-1}$ rVWF before surgery and 12 $IUkg^{-1}$ daily infusion for the following 13 days. All minor surgeries were managed with one rVWF infusion (average dose 30.2 $IUkg^{-1}$). No FVIII infusion was needed in any patient; all type 1 patients had baseline FVIII activity (FVIII:C) ≥49%; the type 3 patient received one infusion of rVWF 12 hours before procedure, allowing endogenous FVIII:C to rise > 50%.

Results: VWF parameters measured 24 hours post-surgery were consistent with expectations: VWF:Ag 60-109%, VWF:GPIbM activity 57-97%, FVIII:C 77-164%. Tolerance was overall excellent, no allergic reactions, bleeding or thromboembolic events were reported. Low molecular weight heparin was administered during 10 days after gastric bypass, with an uneventful follow-up.

Conclusions: Our data support the efficiency, safety and good tolerance of rVWF administered alone across multiple surgical procedures, with an excellent haemostatic response, warranting further clinical evaluation.

PB1559 | Inhibition of ADAMTS13 Rescues Acquired von Willebrand Syndrome in a Preclinical Mechanical Circulatory Support Animal Model

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Background: Bleeding is the most frequent adverse event in patients with continuous flow mechanical circulatory support (CF-MCS) and has been linked to the occurrence of acquired von Willebrand syndrome (aVWS). MCS devices cause an increased shear-induced proteolysis of von Willebrand factor (VWF) by ADAMTS13, leading to aVWS. We previously showed that blocking human ADAMTS13 is an efficient way to prevent aVWS in in vitro CF-MCS experiments. The efficacy of this therapy in a preclinical animal model remains to be determined.

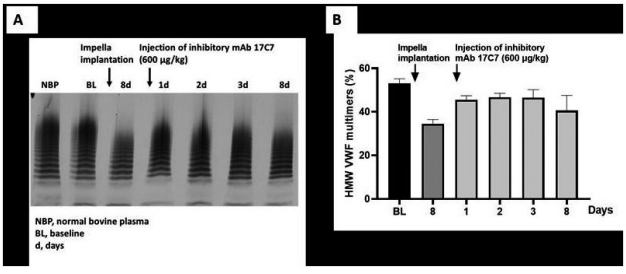

FIGURE 1 Blocking ADAMTS13 rescues the loss of HMW VWF multimers in a preclinical mechanical circulatory support calf model

Aims: To investigate if blocking ADAMTS13 using an in-house inhibitory anti-ADAMTS13 monoclonal antibody (mAb) 17C7 rescues the loss of high molecular weight (HMW) VWF multimers in a preclinical CF-MCS calf model.

Methods: Bovine blood was circulated through an in vitro Impella 5.5 heart-pump with mAb 17C7 or PBS and VWF multimers were analyzed. Next, Impella 5.5 pumps were implanted in calves and VWF multimers were determined. One dose of mAb 17C7 (600 µg/kg) was injected eight days after Impella implantation and plasma was reanalyzed for VWF multimers.

Results: Blocking bovine ADAMTS13 using mAb 17C7 could prevent the loss of HMW VWF multimers in the in vitro Impella system, showing that the calf is a good preclinical animal model to study the in vivo effect of this novel therapy. Impella implantation led to a decrease in HMW VWF multimers (Figure A and B), similar to what is seen in patients with an Impella device. Hence, this preclinical animal model represents the VWF laboratory features of MCS-induced aVWS. Interestingly, the loss of HMW VWF multimers after pump implantation could be rescued after injection of mAb 17C7 (Figure A and B), demonstrating the therapeutic potential of 17C7 to rescue the loss of HMW VWF multimers.

Conclusions: Blocking ADAMTS13 rescues MCS-induced VWF proteolysis in calves and could become a promising therapeutic strategy to rescue aVWS-induced bleeding in MCS patients.

PB1560 | Quality of Peri-Procedural Care in Patients with von Willebrand Disease

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Background: Hemophilia Treatment Centers (HTC) are specialized centers that provide comprehensive care for patients with chronic bleeding disorders. While it has been shown that hemophilia patients receiving care at HTCs experience decreased morbidity and mortality, little research has been done on the outcomes of patients with von Willebrand disease (VWD).

Aims: To compare the quality of peri-procedural care received by patients with VWD at HTCs vs non-HTCs.

Methods: Patients with a diagnosis of VWD meeting inclusion and exclusion criteria were abstracted from the electronic medical record. Comparisons were performed according to the setting of care, HTC vs non-HTC, at the time of the invasive procedure. Outcomes included frequency of post-procedure bleeding, procedural estimated blood loss (EBL), and peri-procedural VWD-specific therapy use per National Heart, Lung, and Blood Institute (NHLBI) guidelines. Mixed-effects models incorporated within-person dependencies.

TABLE 1

[1]Per procedure	HTC (N=61)	Non-HTC (N=620)	p-value
Age, mean (SD)	43.8 (14.6)	52.6 (17.0)	0.033
Sex - Male, Female (%)	16 (14.8), 45 (73.8)	123 (19.8), 497 (80.2)	0.66
[2]Race - White, Black, Asian (%)	53 (89.8), 6 (10.2), 0 (0.0)	565 (91.7), 45 (7.3), 6 (1.0)	[3]0.22
[4]Ethnicity - Non-Hispanic, Hispanic (%)	57 (100.0), 0 (0.0)	607 (99.4), 4 (0.6)	[5]-
[6]VWD type - 1, 2A, 2B, 2M, 2N, 3 (%)	43 (70.5), 1 (1.6), 0 (0.0), 0 (0.0), 1 (1.6), 16 (26.2)	421 (98.6), 3 (0.7), 0 (0.0), 3 (0.7), 0 (0.0), 0 (0.0)	[7] <0.001
[8]VWF antigen and ristocetin cofactor (IU/dL), mean (SD)	0.61 (0.44), 0.39 (0.31)	0.91 (0.50), 0.61 (0.40)	0.049, 0.032
Major invasive procedure, Minor invasive procedure (%)	14 (23.0), 47 (77.0)	142 (22.9), 478 (77.1)	0.34
Comorbidities - Alcohol abuse, Chronic liver disease, Chronic kidney disease, Malignancy (%)	13 (21.3), 0 (0.0), 3 (4.9), 5 (8.2)	14 (2.3), 25 (4.0), 25 (4.0), 46 (7.4)	<.001, [5]-, 0.56, 0.96

[1] All patient characteristics are per procedure except VWF antigen and ristocetin cofactor.
[2] Race was not available in 2 HTC and 4 non-HTC cases.
[3] P-value tested white vs. non-white.
[4] Ethnicity was not available in 4 HTC and 9 non-HTC cases.
[5] Statistical comparison not appropriate.
[6] VWD type was not available in 193 non-HTC cases.
[7] P-value tested type 1 VWD vs non-type 1 VWD.
[8] VWF antigen and ristocetin cofactor are based on lowest recorded values available.

TABLE 2

	HTC (N=61)	Non-HTC (N=621)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
[1]Post procedure bleeding (%)	2 (3.3)	22 (3.5)	1.03 (0.22-4.90)	0.98 (0.17-5.72)
[2]Estimated blood loss >=200 mL (%)	7 (11.5)	30 (4.8)	2.53 (1.00-6.38)	2.14 (0.76-6.04)
[3]VWD-specific therapy use per NHLBI guidelines (%)	61 (100.0)	66 (10.6)	[4]-	[4]-

[1] Adjusted for age, sex, VWF antigen and ristocetin cofactor, alcohol abuse, chronic liver disease, and chronic kidney disease.
[2] Adjusted for age, sex, VWF antigen and ristocetin cofactor, alcohol abuse, and malignancy.
[3] VWD-specific therapy refers to DDAVP, VWF concentrates, and antifibrinolytics.
[4] Statistical comparison not appropriate.

Results: There were 681 invasive procedures performed on 310 patients with 9% performed at HTCs. Cases at HTCs were younger with more severe VWD (Table 1). VWD-specific therapy was used per NHLBI guidelines in 100% of HTC cases compared with 10.6% of non-HTC cases. DDAVP, 96.0%, and Humate-P, 78.7%, were the primary therapies of choice in non-HTC and HTC cases, respectively. In 66.6% of non-HTC cases, "no treatment" was the principal reason for not following NHLBI guidelines. There was no difference in frequency of post-procedure bleeding or procedural EBL (Table 2).
Conclusions: Despite the widespread peri-procedural use of VWD-specific therapy outside established guidelines at non-HTCs, there was no difference in frequency of post-procedure bleeding or procedural EBL between HTCs and non-HTCs. Possible explanations include fewer patients with severe VWD and higher, even normal, VWF levels at non-HTCs. In conclusion, compared to VWD patients at non-HTCs, those receiving care at HTCs are more severe and more likely to receive peri-procedural VWD-specific therapy per recommended guidelines, and with no greater bleed risk.

PB1561 | Clinical and Phenotypic Evaluation of Patients with Low von Willebrand Factor

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Background: The term low von Willebrand factor (VWF) is referred to patients with VWF levels of 30-50 IU/dL. Diagnosis and management of patients with low VWF levels is clinically challenging.
Aims: To determine the clinical and phenotypic features of patients with low VWF.

TABLE 1 characteristics of the study population

Subjects	N	Median age years	Blood group (O/non-O)	Gender (male/female)
Healthy control	25	35 (24-59)	9/16	13/12
Low VWF	29	27 (1-54)	16/13	10/19
VWD	36	20 (5-62)	10/12	17/19

TABLE 2 Median (range) of laboratory results

Variable	Low VWF (n: 29)	VWD (n:36)	Healthy control (n:25)
FVIII:C (IU/dl)	64 (55-120)	42 (8-78)	114 (66-158)
VWF:Ag (IU/dl)	48 (39-110)	32 (2-70)	116 (70-196)
VWF:RCo (IU/dl)	34 (30-38)	14 (1-36)	81 (55-135)
VWF:CB (IU/dl)	49 (30-120)	19 (2-76)	92 (57-133)
FVIII:C/VWF:Ag	1.3 (0.86-1.95)	1.3 (0.25-5.56)	0.97 (0.65-1.2)
Bleeding Score	6.5 (1-20)	7.5 (1-21)	1.96 (0-6)

Methods: We evaluated 29 patients with low VWF levels (VWF:Ag and/or VWF:RCo in the range of 30-50 IU/dL) and compared them with 36 VWD patients and 25 healthy control. All subjects underwent a panel of VWF tests, including VWF activity (VWF:RCo and VWF:CB), VWF:Ag, FVIII:C, RIPA and blood group typing. The ISTH Bleeding Assessment Tool (BAT) was assessed for each subject and was considered abnormal if BS was >3 for males or >5 for females.

Results: Demographic characteristics of the study population are summarized in Table 1. In patients with low VWF the mean of VWF:Ag was 47 IU/dL and VWF:RCo was 33 IU/dL and were significantly lower than control (114 IU/dL; P < .0001 and 116 IU/dL; P < .0001 respectively). In comparison to healthy control the median of BS in patients with low VWF was significantly higher (6.5 vs. 1.96; P < .0001) but was not significantly different from VWD patients (Table 2). 73% of female patients with low VWF had the experience of menorrhagia. Albeit significant bleeding in low VWF patients and reduced FVIII:C levels compared with controls (mean 65 IU/dL vs. 114 IU/dL; P < .0002), FVIII:C were normal in 83% of our low VWF cohort. FVIII:C/VWF:Ag ratios were significantly increased in low VWF levels patients as well as VWD patients compared with controls (P < .0002) that represent decreased VWF synthesis and/or secretion.

Conclusions: Our findings showed that low VWF levels can be associated with significant bleeding and patients should be considered as having a mild bleeding disorder rather than having a risk factor for bleeding.

PB1562 | The Compound Heterozygosity with Two in Cis Mutations p.R924Q and p.A2178S in the D'D3 and D4 Domain Causes a Type 2B-Like von Willebrand Disease

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Background: According to the ISTH classification, the type 2B VWD forms arise from dominantly inherited missense mutations in the exon 28 of the VWF gene, with expression of mutated aminoacids in the A1 domain (AA 1266-1461) of VWF. These mutations alter the VWF conformation, facilitating spontaneous interaction with the platelet receptor GpIb.

Aims: Investigation of the genotype-phenotype correlation in a 55-year-old male patient with a mucocutaneous hemorrhagic syndrome, low levels of VWF with a strong decrease of the VWF:Act/VWF:Ag ratio and increased VWF interaction with platelets.

Methods: VWF-antigen (VWF:Ag) and VWF:activity (VWF:RCo) were measured by chemiluminescence assays (Werfen). FVIII binding to immobilized VWF was performed with the Asserachrom® VWF:VIIIB assay (Stago). VWF:pp was measured by an ELISA assay (Sanquin). VWF multimers were studied with SDS-agarose (0.8-1.5%) gel electrophoresis. Ristocetin-induced platelet aggregation (RIPA) was performed with AggRAM aggregometer (Helena Biosciences). Ready Reaction kit and an ABI 3130 DNA sequencer (Applied Biosystems) was used to identify the genetic mutations in leukocyte-derived DNA.

Results: VWF:Ag and VWF:Act levels were 25U/dl and 8U/dl, respectively. A deficiency of high molecular weight multimers and increased presence of "triplets" were found in electrophoretic analysis. The VWF:pp/VWF:Ag ratio was heightened (=2.7). Hyperresponsiveness was found in the RIPA at low ristocetin doses ($Aggr_{max}$>50% at 0.3 and 0.6 mg/ml). The VWF gene sequencing in the patients revealed the presence of a compound heterozygous *in cis* mutation,, causing a c.2771G>A (rs33978901) in the exon 21 (p.R924Q in D'D3-domain), and a c.6532G>T substitution (rs34230288) in the exon 37 (p.A2178S in D4-domain).

Conclusions: This case shows that, although the type 2B VWD forms mostly derive from dominantly inherited missense mutations in the VWF A1 domain, type 2B-like forms may also stem from mutations occurring in different domains that, likely affecting the conformational status of the protein, facilitate the interaction with platelets.

PB1563 | Increased von Willebrand Factor Levels in Polycythemia Vera and Phenotypic Differences with Essential Thrombocythemia

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Background: An acquired von Willebrand factor (VWF) deficiency had been described in Philadelphia-negative myeloproliferative neoplasms (MPNs) in association with severe thrombocytosis, especially in Essential Thrombocythemia (ET) patients with high platelet count. VWF pattern in patients with Polycythemia Vera (PV) diagnosed and treated according to current criteria and guidelines remains less explored.

Aims: To evaluate VWF phenotype in PV patients as compared to healthy and ET subjects matched with PV patients for age, sex and blood group; to identify the determinants of VWF phenotype in PV.

Methods: Forty-eight PV patients diagnosed according to current WHO diagnostic criteria, treated to keep hematocrit ≤45%, on low-dose aspirin prophylaxis were enrolled. Forty-eight healthy subjects and forty-one ET patients, were studied. VWF antigen and activity were measured by standard chemiluminescence assays, ADAMTS-13 and Factor VIII (FVIII) by standard ELISA.

Results: In PV patients, VWF antigen and activity were significantly higher than in healthy subjects (119 [96-137] and 114 [95-128] IU/dL, respectively, p< 0.0001) and not significantly correlated with blood group. Likewise, FVIII levels were higher in PV versus healthy subjects (141 [118.8-168.8] vs 97.5 [88.0-123.0] IU/dL, respectively). ADAMTS-13 levels were in PV patients were comparable to healthy subjects. In multivariable analysis, JAK2-p.V617F allelic burden, erythrocytes (inversely) and male sex significantly predicted VWF levels. As compared to ET, PV patients showed similar VWF antigen, but approximately 30% higher VWF activity levels.

Conclusions: PV patients show increased VWF and FVIII levels, predicted by JAK2-p.V617F burden and erythrocyte count. At variance with ET, acquired VWF defect was not observed in PV. High VWF/FVIII levels may sustain the thrombotic diathesis of PV and tested as biomarkers for risk stratification.

PB1564 | Prevalence and Diagnostic Rate of von Willebrand Disease in an Argentine Medical Care Program

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Background: Von Willebrand disease (VWD) is the most common inherited bleeding. Few epidemiological studies have been published in Latin America.

Aims: Determine the prevalence in 2019 and the diagnosis rate of VWD from 2000 to 2019 in members of the medical care program of the Hospital Italiano de Buenos Aires (HIBA).

Methods: Retrospective descriptive observational study. The studied population were members of the Medical Care Program, which are treated only in the HIBA. Information was collected from electronic medical records.

The point prevalence at 31 of december 2019 was calculated for the following categories in 10000 members: VWD patients confirmed (VWF ristocetin cofactor activity -VWF:RCo- and/or VWF antigen -VWF:Ag- less than 30%), "low VWF levels" (VWF:Ag and/or VWF:RCo between 30-50%) and "VWF levels below the normal range" (VWF:Ag and/or VWF:RCo less than 50). This last category includes the previous two Diagnostic rates were calculated for the periods (2000-2004, 2005-2009, 2010-2014, 2015-2019) per 10000 members (95%CI) in patients with "VWF levels below the normal range".

Results: On December 31, 2019 the medical care program of the HIBA had 187222 members, 256 (80% women) diagnosed with VWD. Table 1 shows the prevalences.

In the group with confirmed VWD disease, 80% were type 1, 20% type 2 and there were no cases of type 3. In the total cases, the median (IQR) of VWF and CoR were 50 (43-52) and 45 (37-60), respectively.

TABLE 1 Prevalence of VWD disease in 2019

	Prevalence per 10000 (95% CI)
"VWF levels below the normal range"	13.68 (12.05-15.45)
VWD patients confirmed	3.95 (3.10 - 4.96)
"low VWF levels"	8.65 (7.37- 10.09)

TABLE 2 Diagnosis rate in patients with 'VWF levels below the normal range'

Period	Diagnosis rate per 10000 (95% CI)	Age-adjusted diagnosis rate per 10000 (95% CI)
2000-2004	1.03 (0.77 - 1.34)	0.9 (0.65-1.14)
2005-2009	1.13 (0.9 - 1.39)	1.05 (0.82-1.28)
2010-2014	0.5 (0.35 - 0.67)	0.49 (0.34-0.63)
2015-2019	0.3 (0.19 - 0.43)	0.29 (0.18-0.41)

Table 2 represents the diagnostic rates of patients with "VWF levels below the normal range" in periods of 5 years. There is a marked decrease in the most recent periods.

Conclusions: Like other authors, we agree that the prevalence of clinically significant cases is markedly lower than that estimated for the general population (0.6-1%).

In our series we observed a decrease in the diagnostic rate in recent years, probably related to changes in diagnostic methods.

PB1565 | Evaluation of Carriers of Type 3 von Willebrand Disease with PFA200

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Background: Type 3 Von Willebrand disease (VWD) is a rare autosomal recessive disorder characterised by undetectable levels of Von Willebrand Antigen (vWF:Ag) and reduced levels of Factor VIII. Patients with type 3 VWD are homozygous or compound heterozygous for two null alleles causing virtually complete deficiency of Von Willebrand factor (VWF). Carriers of type 3 VWD are usually asymptomatic with VWF 50% of normal. PFA100/200 (platelet function analyser; Dade-Behring, Marburg, Germany) has been found to have high sensitivity to VWF and VWD. However, there is no data available for carriers of type 3 VWD with PFA200.

Aims: To evaluate carriers of type 3 VWD with PFA200.

Methods: This is a retrospective study. Data was collected from patients who presented to a tertiary hospital in South India from January 2016 to December 2019. Parents were evaluated as part of the diagnostic workup and following tests were performed - complete blood count, closure time (CT) in PFA200 with COL/ADP and COL/EPI cartridges, blood group, APTT, Factor VIII, vWF:RCo and vWF:Ag. Carriers with haematocrit < 30% and platelet count < 100000/cumm were excluded from PFA analysis. Reference range for CT for COL/ADP and COL/EPI was 68-142 secs and 89-167 secs respectively.

Results: 98 carriers from 63 type 3 VWD patients were included in the study.The summary of the results are shown in Tables 1 & 2. 68% of the carriers had prolonged CT with either COL/ADP or COL/EPI, which was more pronounced in O blood group carriers. 96% of the carriers with vWF:RCo < 50% had prolonged CT. Surprisingly, 55% of carriers had prolonged CT even with vWF:RCo >50%.

Conclusions: PFA100/200 assesses primary hemostatic function under high shear conditions and is very sensitive to alterations in VWF. The significance of abnormal PFA200 in type 3 VWD carriers needs to be validated by further studies and molecular analysis.

PB1566 | Prophylaxis with Recombinant von Willebrand Factor in a Case of Concomitant von Willebrand's Disease and CREST Syndrome

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Background: Von Willebrand's syndrome (VWS) is an inherited bleeding disorder due to quantitative or qualitative defects of the von Willebrand factor (VWF). There is also an acquired form secondary to various underlying conditions, e.g. autoimmune diseases. CREST syndrome (calcinosis cutis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia) is a subtype of systemic sclerosis. The angiodysplasias are mainly localized in the skin but also throughout the mucosal tract.

Aims: We report on a patient (54 y) with CREST syndrome since 2001. Despite immunosuppressive treatment she developed disseminated

TABLE 1 Summary of carriers of type 3 Von Willebrand disease

	Factor VIII (IU/dL) Median (IQR)	vWF:RCo (%) Median (IQR)	vWF:Ag (U/dL) Median (IQR)	COL/ADP in PFA200 in (secs) Median (IQR)	COL/EPI in PFA200 (secs) Median (IQR)
All carriers	125.8 (105.4 - 166.9)	60 (39.2 - 79.4)	60.5 (45.6 - 79.3)	145 (113 - 186)	193 (151 - 281)
O blood group carriers	109.9 (93.9 - 129.2)	44 (32.3 - 62.3)	47.1 (37.8 - 129.2)	166.5 (120 - 220)	221 (181 - 299)
Non O blood group carriers	149.9 (111.5 - 187.5)	68.6 (54.3 - 83.3)	69.7 (54.3 - 87.6)	128 (111 - 156)	171 (145 - 238)

TABLE 2 Percentage of carriers of type 3 Von Willebrand disease with prolonged closure time in PFA200

	Prolonged COL/EPI (%)	Prolonged COL/ADP (%)	Either COL/ADP or COL/EPI prolonged (%)	Both COL/ADP and COL/EPI prolonged (%)
All carriers (85)	54/85 (63.5%)	42/83 (50.6%)	58/85 (68.2%)	38/85 (44.7%)
O blood group carriers (35)	27/35 (77.1%)	24/35 (70.5%)	29/35 (82.8%)	21/35 (60%)
Non O blood group Carriers (48)	28/48 (52%)	18/47 (38.2%)	27/48 (56.2%)	16/48 (33.3%)

mucosal angiodysplasias and recurrent gastro-intestinal bleeding, eventually leading to constant need of blood transfusions. With a hemoglobin level of 7.0 g/dl the patient was referred to our clinic in May 2019.

Methods: We newly diagnosed a VWS type 2a with normal factor VIII:C activity and concentration of VWF:Ag., diminished VWF:RCo activity (31%) and loss of large VWF multimers. Since the same VWS was detected in the brother, acquired VWS could be ruled out.

Results: Intermittent administration of plasma-derived VWF/FVIII concentrates had no durable effect. Regular prophylaxis with these products - although effective - could not be continued, since FVIII:C plasma levels reached high values, further deteriorating the underlying Raynaud's syndrome with crippling complications. We then opted for the recombinant VWF concentrate Vonicog alfa, containing ultralarge multimers which are most important for hemostasis but also for regulation of angiogenesis. Prophylactic (off lable) administration (3x/w) significantly reduced the number of bleeding episodes and transfusions and stabilized hemoglobin levels without adverse events.

Conclusions: In this special case, the CREST syndrome-typical angiodysplasias were obviously aggravated by the concomitant functional VWF defect, underlining the importance of hemostaseological evaluation. Prophylaxis with Vonicog alfa stabilized hemostasis without undesirable effects from high FVIII:C levels. In the long run, it remains to be clarified whether it will also positively influence the vascular malformations.

PB1567 | Pelvic Haematoma in Women with Bleeding Disorders: A Single Center Experience

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Background: Women with inherited bleeding disorders have significant gynaecological bleeding. Though many have menorrhagia, some suffer more prominent bleeding causing pelvic haematomas. Discovery of haematomas can be daunting as many are initially thought to be malignancies before being diagnosed as haematoma.

Aims: We report 3 cases of pelvic haematomas at our hospital.

Methods: Retrospective review of patients clinic notes.

Results: Case 1: 47-year-old nulliparous lady, with GT and significant menstrual bleeding. She experienced prolonged menstruation in 2017 with abdominal distension. Imaging showed a heterogenously enhancing solid cystic mass at the posterior wall of uterus measuring 13.5 x 1.0 x 13.3 cm with bilateral obstructive uropathy. She was transferred to us for further management of suspected malignant tumour. Multidisciplinary discussion concluded that it was a haematoma. She underwent ultrasound guided culdocentesis and drained 1 liter of infected clots.She received continuous combined OCP (ccOCP), iron supplements and regular tranexamic acid and has been symptom free since.

Case 2: 32 year old, diagnosed with Type 2 vWD when she suffered post partum haemorrhage. in 2018, she developed severe abdominal pain with palpable pelvic mass consistent with a 14 weeks gravid uterus. Ultrasound showed a multiseptated solid-cystic mass measuring 5.4x6.3 cm; concluded to be a haematoma and was started on ccOCP. Despite being advised against it, she wants to get pregnant and not compliant to OCPs. She continues to have abdominal pains.

Case 3: 19 year old, nulliparous diagnosed with type 3 vWD at menarche. In 2017 she had severe abdominal pain and ultrasound revealed an ovarian cyst measuring 5.8 x 5.0 cm. She received ccOCP and serial ultrasound reveals slightly smaller cyst at 5.0 cm x 5.0 cm.

Conclusions: Pelvic haematoma in women with bleeding disorders is usually due to bleeding during ovulation. The use of continuous combined hormonal therapy ensures anovulation preventing further bleeding into the pelvis.

PB1569 | The Impacts of Genetic Polymorphisms on the Von Willebrand Factor Level in Type 1 Von Willebrand Disease

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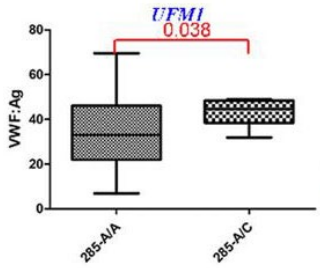
Background: In type 1 von Willebrand disease (VWD), there are only 53-81% patient with found mutations of VWF gene. There were 7 genetic variants of single nucleotide polymorphism (SNP) reported to be associated with the variation of VWF levels, including CLEC4M, STXBP5, SCARA5, ABO, VWF, STAB2 and UFM1.

Aims: This study were to explore the frequency and impacts of the 7 genes SNPs on VWF level in type 1 VWD patients.

Methods: There were 62 patients (M/F: 1) with type 1 VWD enrolled in this study, including 32 (51.6%) patients with found mutations of VWF gene. The patients' VWF levels of both VWF:Ag and VWF activity (VWF:ACL or VWF:RCo) and FVIII: C were retrospectively collected and analyzed. Each enrolled patient's SNPs of the 7 genes were targeted by PCR followed by direct sequencing.

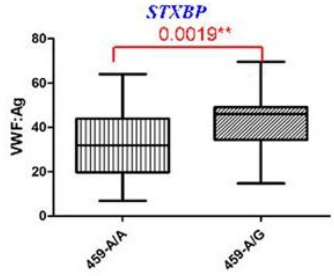
Results: The patients without variant (A/A) of UFM1 has significantly lower median VWF:Ag than variant (A/C) type (33% vs 44.7%, p = 0.038, Figure 1A). Patients without variant (A/A) of STXBP gene had both significantly lower median value of VWF:Ag (31.9% vs 46.1%, p = 0.019, Figure 1B) and VWF activity (26.55% vs 30.4%, p = 0.0419, Figure 1C) than variant (A/G). Four alleles substitution of SNP of UFM1(C>A) and STXBP genes (G>A) variant has remarkably and significantly lowest median VWF antigen level as compared to three and two substitutions (Figure 2).

Conclusions: Our study demonstrates that both C to A variant of UFM1 gene and G to A variant of STXBP5 gene were associated with



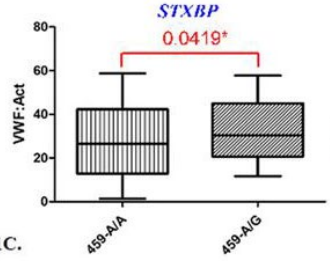
Median	33.00	44.70

Figure 1A



Median	31.90	46.10

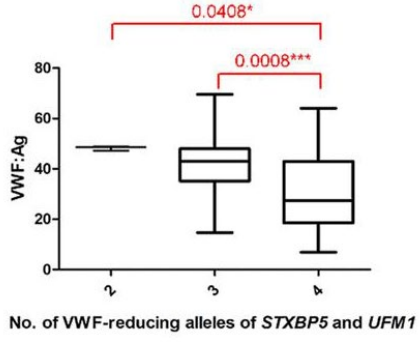
Figure 1B.



Median	26.55	30.40

Figure 1C.

FIGURE 1



Median	48.60	43.05	27.40

Figure 2. Numbers of von Willebrand factor (VWF)-reducing alleles of *STXBP5* and *UFM1* in type 1 VWD. The boxplots indicate the median, 25–75% interquartile range, and 1–99 percentile.

FIGURE 2

lower VWF: Ag level than those without variant, respectively. The combination of four allelic variant of UFM1 and STXBP5 genes had the most reducing effect on VWF: Ag level.

PB1570 | Adolescent Menorrhagia and Von Willebrand Disease

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Background: Adolescent metrorrhagia most often results from hormonal dysfunction, 10 to 20% related to a constitutional abnormality of hemostasis including 70% of Willebrand Diseases
As no gynecological etiology is found in half of the cases, the possibility of abnormalities of hemostasis is often raised. By enabling targeted treatment, the identification of a hemostasis disorder contributes to improving the quality of life of women
Menorrhagia can cause anemia, fatigue and absenteeism from school or work:
Aims: Hemostasis disorders should always be investigated in case of adolescent meno-metrorrhagia
Methods: We proceeded to a meticulous interrogation, personal and family history of bleeding, the realization of a blood assessment, a hemogram, a prothrombin time, an aPTT, fibrinogen and evaluation of the platelet function According to the first results, additional examinations were carried out; measurement of FvW activity and antigen complemented by measurement of factor VIII. Particular account was taken of the blood group, and the measurement of the FvW was made on D5-D7 of the menstrual cycle
Results: This is a prospective study from September 2016 to September 2019 9 cases of von willebrand disease were diagnosed with hemorrhagic symptoms like menorrhagia The average age is 17 years, 4 patients in groups O positive, two A positive and three group B (2 rhesus positive and 1 rhesus negative), the first menstruation was hemorrhagic with an abnormal duration in the 9 patients (100%) there were 3 cases of von willebrand disease typeIIB and 6cases of type 3.The prevalence of the decline in FvW in menorrhagia is 10 to 20% (Kirtava A et al,) and that 50 to 75% of women with von Willebrand disease have menorrhagia (Woo YL, and al).
Conclusions: Coagulopathies constitute 20% of cases of pubertal metrorrhagia. It is imperative to look for hemostasis disorders in the case of adolescent menometrorrhagia

PB1571 | Response to Desmopressin Acetate in Von Willebrand Disease Type 2

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Background: We hypothesized that the response to desmopressin acetate (DDAVP) in type 2 VWD might be related to genotype. If

this is the case, it may be possible to individualize the treatment of these individuals.

Aims: To evaluate the response to DDAVP response and its relationship with genotype in type 2 VWD (except subtype 2B).

Methods: The study was cross-sectional. We collected data of type 2 VWD patients registered in two hemophilia treatment centers in Brazil after informed consent. The project was approved by the Ethics Committee of each institution. We used the Bleeding Assessment Tool (BAT) from International Society on Thrombosis and Haemostasis (ISTH). DDAVP challenge test was performed in all participants, except in individuals with subtype 2B. The identification of FVW gene variants was performed in all probands by Sanger sequencing of exons 17, 18, 20 and 28. We consulted the ISTH database to identify VWD gene variants associated with type 2 VWD.

Results: Twenty unrelated probands were evaluated, with a median age of 36 years (interquartile range [IQR], 16-49), 50% female. VWD was classified into subtypes 2A and 2M in 30% and 20% of participants, respectively. Among the probands, the median BAT was 7.5 (IQR, 4.5-11.0), 40% had a history of consanguinity and 40% responded to DDAVP test. Twelve variants were identified of which 9 (75%) were located in exon 28 and 8 (67%) were potentially pathogenic. Among the potentially pathogenic variants, individuals carrying Met740Ile and Arg1315Cys responded to DDAVP, while those carrying Val1360Ala, Arg1374Cys and Leu1580Val did not.

Conclusions: We showed that 40% of patients with VWD classified as subtypes 2A and 2M responded to DDAVP and that this response might be influenced by genotype. We are currently working on the collection of a larger sample of type 2 VWD patients to further study this association.

PB1572 | Gastrointestinal Bleeding from Angiodysplasia in Constitutional von Willebrand Disease: Improved Diagnosis Yield and Risk Stratification when Using Video Capsule on Top of Conventional Endoscopy

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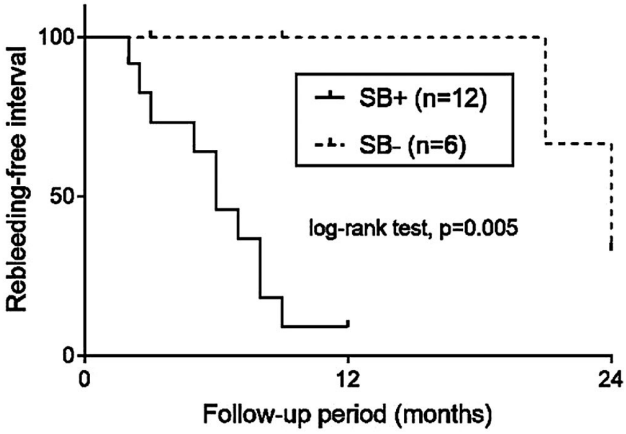

FIGURE 1 Comparison of the rebleeding free interval after first GIB event in VWD-patients with (SB+, n=12) or without small bowel angiodysplasia (n=6) on VCE

Background: Video capsule endoscopy (VCE) is the gold standard endoscopic investigation to look for small-bowel angiodysplasia when no gastrointestinal bleeding (GIB) source is identified on conventional endoscopy. We hypothesized that VCE could improve the diagnosis yield of GIB in patients with constitutional von Willebrand disease (VWD), a population prone to angiodysplasia.

Aims: To assess the diagnostic yield and prognosis value of VCE on top of conventional endoscopy in constitutional VWD-patients with GIB.

Methods: A survey was sent to the centers of the French MHEMO-network to identify constitutional VWD-patients referred for endoscopic exploration of at least one GIB episode from 1 January 2015 to 31 December 2017. We analyzed patient characteristics (including VWD phenotype), GIB pattern, the modalities and results of each endoscopic exploration and the medical management applied (including endoscopic therapy with argon plasma coagulation). We assessed the re-bleeding-free interval after first GIB according to the underlying VWD or to the result of endoscopic exploration (categorized as "angiodyplasia", "no-angiodysplasia" or "occult GIB") by Kaplan-Meier method. In VWD-patients with angiodysplasia, another survival analysis was performed to assess the rate of GIB-recurrence according to the presence of small-bowel angiodysplasia on VCE.

Results: 129 GIB-episodes were reported in 50 VWD-patients. Angiodysplasia was the most frequent GIB-etiology. Angiodysplasia diagnosis was significantly improved when using VCE on top of conventional endoscopy (all cases p=0.001, incident cases p=0.03). VWD-patients with angiodysplasia had a higher number of GIB recurrences (p=0.008) and a lower rebleeding-free interval after first GIB-episode (log rank test, p=0.02). Accordingly, the re-bleeding rate was significantly higher in VWD-patients with small-bowel angiodysplasias (log rank test, p=0.005; Figure).

Conclusions: The use of VCE in VWD-patients with GIB improves the diagnostic yield for angiodysplasia and the risk stratification for bleeding recurrence.

PB1573 | Successful Surgical Interventions In Children with von Willebrand Disease Type 3

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Background: Von Willebrand disease (VWD) is a hereditary bleeding disorder which arises from deficiency or qualitative defects of von Willebrand factor (VWF). VWD occurs in 0.5-1% of the population. The complete deficiency of VWF or type 3 VWD occurs in 1:500000 of population.

Aims: To demonstrate our experience in managing patients with type 3 VWD.

Methods: In 2018-2019 2 patients (A and B) with severe forms of VWD went to the surgery department of Saint Petersburg State Pediatric Medical University clinic. Another patient (C) with type 3 VWD and infantile nephrotic syndrome went to the nephrology department for peritoneal dialysis and hydrocele correction. VWF activity level of all three patients was up to 1%, factor VIII activity was up to 5%. The combined medication of FVIII and VWF (1:2,4) was used to prevent massive bleeding during the treatment.

Results: The activities of VWF and FVIII were tested in 30 minutes after the combined medication of facor VIII and VWF injection. Patient A: VWF activity was 58.0% and FVIII activity - 54.6%. Patient B: VWF activity - 62.0%, FVIII activity - 48.9%. Patient C: VWF activity - 142.4%, FVIII activity - 166.2%. The activity level depended on medication dose: patients got 20 ME/kg in cases A and B, 40 ME/kg in case C. The received levels gave us an opportunity to make a surgery without any complications for 2 patients, peritoneal dialysis and hydrocele correction for the third one. There were no toxic, thrombogenic, allergic reactions and no cumulative effects of FVIII during the treatment.

Conclusions: Type 3 is the most severe and rare form of VWD. Difficulties can occur in concominant disease treatment, especially when surgery is required. We demonstrated our experience of using the combined medication of factor VIII and VWF (1:2,4) as one of the options of the successful treatment for patients with VWD.

PB1574 | Establishing Reference Intervals for von Willebrand Factor Multimers

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Background: von Willebrand factor multimers (VWF:MM) methodologies are technically difficult, laborious, time consuming, non-standardized and results vary between labs. A first step towards standardization was made by introducing a semi-automated VWF:MM assay by Sebia (Lisses, France). In addition to qualitative interpretation of multimer patterns, the Sebia PHORESIS software allows quantification of VWF:MM band patterns, thus the percentage of each molecular weight multimer fraction can be calculated. Due to lack of reference values for VWF:MM fractions, results interpretation can be challenging in some cases.

Aims: The aim of this study was to determine reference intervals for low molecular weight (LMW), intermediate molecular weight (IMW) and high molecular weight (HMW) multimers.

Methods: To collect a larger sample size an international cooperation was initiated. We analyzed data from 134 participants (51 males and 83 non-pregnant females) aged 17-69 years. Acceptance criteria: no history of hemorrhagic episodes; no usage of any medication for at least 10 days before blood collection; normal coagulation screening tests; normal VWF screening assay results; written consent provided.

Reference intervals (with 90% confidence intervals) were calculated using a robust method according to the CLSI C28-A3 standard. The study was performed according to the Declaration of Helsinki and was approved by national ethical committees.

Results: After outlier exclusion (n=3), done by Grubs double sided and Tukey methods, a total of 131 samples were analyzed statistically.

TABLE 1 Proposed reference intervals for VWF:MM

	Low Molecular Weight	Intermediate Molecular Weight	High Molecular Weight
Lower limit, % [90% CI]	10.42 [9.94 - 10.95]	22.55 [21.81 - 23.32]	45.58 [44.54 - 46.71]
Upper limit, % [90% CI]	22.52 [21.52 - 23.51]	37.57 [36.44 - 38.72]	66.59 [65.10 - 68.02]

Results were normally distributed. No significant differences were found between males and females. The proposed reference intervals for VWF:MM are presented in table 1.

Conclusions: Quantification of VWF:MM fractions, in addition to qualitative assessment of VWF:MM patterns, has potential value to aid in differential diagnosis of VWD sub-types. The reference values calculated in this study can be used in future research to establish clinical decision liimits.

PB1575 | Inhibitor Development in Patients with Type 3 von Willebrand Disease, a Comprehensive Study on a Large Number of Iranian Patients

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Background: Alloantibody formation is a rare but serious complication of replacement therapy that can be accompanied by anaphylaxis reaction following re-injection of von Willebrand factor (VWF) concentrate in patients with type 3 von Willebrand disease (VWD).

Aims: This study is aimed to evaluate the presence of inhibitor in patients with type 3 VWD.

Methods: This study conducted on 99 patients with type 3 VWD. Laboratory diagnosis was performed by factor (F) VIII coagulant activity (FVIII: C), VWF antigen, and VWF: ristocetin cofactor. VWF and FVIII inhibitor titers were determined by a modified Nijmegen-Bethesda assay.

Results: Of 99 patients, 19 (~19.5%,) were positive for inhibitor against VWF ranged from 2 to 80 Bethesda Unit (BU); most were high-titer, high responder inhibitor (n: 10, 58%). The mean age of diagnosis and the mean age at inhibitor detection were 3.7 and 20.5 years old, respectively. Hemarthrosis was observed in 41% of patients after inhibitor development. Before inhibitor formation, the majority of the patients (94%) received on demand regimen (Haemate®, *CSL Behring*, Marburg, *Germany)*, while one patient received a prophylactic regimen (Wilate®, Octapharma, Lachen, Switzerland). Twelve patients (70.5%) with inhibitor showed an anaphylactic reaction following > 12 to 300 times exposure to Haemate® and 5 cases (29.5%) showed less severe allergic reactions to Haemate®. These 5 cases were still treated with Haemate®. Three, five, and one cases were switched to on-demand regimen with Wilate®, AryoSeven™ *(AryoGen*, Tehran, *Iran)*, and FEIBA® (Baxalta Inc, Lexington, USA), respectively; none of them were effective. One case refused to receive treatment. Three cases were successfully managed by prophylaxis regimen with Haemate® (25 u/kg/ twice a week), AryoSeven™ (50 µg/kg/a day), and FEIBA® (50 u/kg/twice a month).

Conclusions: Inhibitor formation can significantly exacerbate the clinical presentations, therefore, early detection of major risk factors of inhibitor formation should be considered as a preventative program.

PB1576 | The Homozygous Variant p.Gln1311* in Exon 28 of VWF Is Causing Type 3 von Willebrand Disease and Is at High Risk of Allo-Immunisation

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Background: Type 3 von Willebrand disease (VWD) is the most severe form of VWD but fortunately the less frequent (< 5% of cases). It's an autosomal recessive disease, characterized by a near-total absence of von Willebrand factor (VWF) antigen and activity, a secondary factor VIII (FVIII) deficiency and bleeding symptoms. Replacement therapy is the main treatment but there is around 10% risk of developing an alloantibody against VWF, rendering the treatment ineffective.

Aims: To identify *VWF* variants that could be associated to a higher risk to develop alloantibodies.

Methods: We studied the database from the French Reference Center for VWD to identify patients that developed an alloantibody and had the same pathogenic variant in *VWF*.

Results: Among the 6 patients with type 3 VWD who developed anti-VWF alloantibodies, 3 had the same homozygous variant c.3931C>T, p.Gln1311* in exon 28. A prophylaxis treatment has been initiated with plasma-derived VWF and FVIII (Wilfactin® ± Factane®) for all patients after their diagnosis of type 3 VWD because of severe bleeding symptoms (ISTH-BAT score: 14-24). The 3 patients developed an anti-VWF alloantibody. One of the patient also has mild haemophilia A (c.2113+466_2113+473del in intron 13) leading to an undetectable FVIII level (FVIII:C < 1%) in combination with his type 3 VWD. He developed an additional anti-FVIII alloantibody. In the literature, the homozygous p.Gln1311* variant has been described in Spain, Portugal and Iran in 17 type 3 VWD patients in total. An anti-VWF alloantibody was described for 1 on 5 patients identified in Portugal. A founder effect was hypothesized with a gypsy origin in Spain, that could be the same origin for 2 of our patients.

Conclusions: We identified the p.Gln1311* variant as a type 3 VWD variant associated with a high risk of allo-immunisation. This information must be considered for future patients in the discussion of prophylaxis instauration.

PB1577 | High Variability of Laboratory Phenotype in Genetically Proven von Willebrand Disease Type 2B

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Background: A wide heterogeneity of laboratory phenotypes is evident in von Willebrand disease (vWD) type 2B. An increased response to low concentrations of ristocetin in platelet agglutination assay (LD-RIPA) is considered as unifying hallmark for all mutations causative for vWD type 2B.

Aims: We describe the laboratory phenotype including bleeding symptoms in 3 index patients with genetically proven vWD type 2B.

Methods: vWF-Antigen (vWF:Ag), vWF-Collagen-binding-activity (vWF:CB) and vWF-Ristocetin-induced-binding (vWF:GP1bR) were determined applying HemosIL AcuStar-Assays (Instrumentation Laboratory, Werfen, Germany) including the calculation of vWF:GP1bR /Ag- and vWF:CB/Ag-ratio. LD-RIPA was performed with a final concentration of 0.5 mg/ml.

Results: Patient 1 is a male adult who presented in childhood with epistaxis and extensive bleeding after tooth extraction. Diagnostics revealed vWD type 2A with no thrombocytopenia and normal LD-RIPA. Genetically a heterozygous c.4272G>A mutation was detected proving a vWD type 2B. His son displayed identical laboratory findings.

Patient 2 is a 3 years old boy with an extensive hematoma tendency. Except LD-RIPA, Willebrand diagnostics showed normal results including platelet count. Genetic diagnostics revealed heterozygous c.3789G>A and c.3797C>T mutations which were also detected in his mother and sister who suffered from menorrhagia. Interestingly, the patient also suffers from a vascular Ehlers-Danlos-syndrome.

Patient 3 is a 2 years old boy who was investigated before undergoing adenotomy because of a suspicious bleeding history of his mother who suffered from surgery related bleedings and postpartum hemorrhage. Willebrand diagnostics showed the typical patterns of VWD type 2A or 2M including a normal platelet count and LD-RIPA but a VWD type 2 B was diagnosed by detection of a heterozygous c.4021C>T mutation.

Conclusions: vWD type 2B represents a rare bleeding disorder. It displays a wide heterogeneity of laboratory phenotypes which might clearly differ from genotyping data - including a negative LD-RIPA.

PB1578 | Increasing Levels of von Willebrand Factor (VWF) and Factor VIII (FVIII:C) with Age in Patients Affected by von Willebrand Disease (VWD)

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Background: Increasing levels of von Willebrand Factor (VWF) and factor VIII (FVIII:C) were associated with age in patients affected by VWD type 1.

Aims: To evaluate the increase of FVIII:C and VWF with age in a large group of patients affected by VWD, in whom levels were repeatedly measured.

Methods: Clinical charts from all patients evaluated at the A. Bianchi Bonomi Hemophilia and Thrombosis Center between 1970 and 2018 were reviewed and data on VWF, FVIII:C, cancer, diabetes,

TABLE 1 Clinical characteristics

	All (N=714)	Type 1 (N=293)	Type 2 (N=314)
Sex female, n (%)	412 (58)	185 (63)	163 (52)
Blood group 0, n (%), missing	296 (61), 229	153 (72), 81	109 (53), 107
Age at first measurement, years median, IQ range), missing	28 (13-42), 1	27 (16-39), 2	28 (11-46), 1
VWF activity at 1st measurement, IU/dLmedian (IQ range), missing	21 (6-40), 19	35 (20-44), 5	9 (3-22), 8
FVIII:C at 1st measurement, IU/dLmedian (IQ range), missing	53 (37-70), 19	58 (44-72), 5	49 (37-64), 10
Time between first and last measurement, yearsmedian (IQ range), missing	15 (6-24), 1	15 (7-21)	15 (7-25), 1
Number of repeated measurements, median (IQ range)	3 (1-6)	2 (1-5)	3 (1-7)
Comorbidities (cancer, diabetes, high blood pressure)n (%), missing	21 (5), 287	6 (3.5), 121	13 (6), 105

TABLE 2 Increment of VWF:activity and FVIII:C with age

	All patients(N=714)	Type 1(N=293)	Type 2(N=314)
VWF activity, IU/dL			
Crude Beta coefficient per decade (95%CI)	2.24 (1.63-2.84)	2.67 (1.67-3.66)	1.30 (0.64-1.97)
Adjusted# Beta coefficient per decade (95% CI)	1.81 (0.33-3.28)	5.13 (2.70-7.56)	-0.040 (-2.13-1.33)
FVIII:C, IU/dL			
Crude Beta coefficient per decade (95%CI)	2.50 (1.68-3.33)	2.14 (0.77-3.52)	2.18 (1.16-3.20)
Adjusted# Beta coefficient per decade (95%CI)	2.86 (1.66-4.06)	2.94 (1.08-4.79)	3.84 (2.25-5.44)

adjusted for age at first measurement, sex, blood group, cancer, diabetes, hypertension (and test type for VWF).

hypertension collected. VWF activity was measured with 4 different assays depending on the date of the assay (VWF:Rco, measured by aggregometry, and subsequently with an automated coagulometer; VWF:Ab, and VWF:GpIbR). FVIII:C was measured by one-stage assay. The repeated measurements were evaluated by linear mixed effects models, adjusting for age at first measurement, sex, blood group (O or non-O), presence of comorbidities, and type of test for VWF activity.

Results: In total 714 patients were included (23 excluded for missing data and 11 for VWD affecting only collagen binding or RIPA assays): 39 (5%) were affected by VWD type 3 or severe type 1, 314 (44%) by type 2, 293 (41%) by type 1 or 1 Vicenza, 68 by others (2N, not classified, type 3 carriers, type 2N carriers). Repeated measurements were available for 429 (60%) patients (172 type 1 and 211 type 2) for a total of 2844 tests. After adjustments, VWF activity and FVIII:C were positively associated with age (1.81 IU/dL and 2.86 IU/dL increase per decade respectively). Stratification on VWD type confirmed the increment of VWF activity in type 1 and of FVIII:C in type 1 and type 2 patients.

Conclusions: Our results indicate a mild increase of FVIII with age in patients with VWD. Only patients affected by type 1 VWD show a mild increase of VWF activity with age.

PB1579 | Desmopressin in Patients with Type 2B von Willebrand Disease: Differential Impact on VWF and Platelet Function

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Background: Von Willebrand disease type 2B (VWD-2B), caused by gain-of-function mutations in von Willebrand factor (VWF) gene, is characterized by increased VWF affinity for GPIbα platelet receptor associated with variable loss of high-molecular-weight VWF multimers (HMW-VWF), variable thrombocytopenia and, in severe defects, a thrombopathy. Desmopressin is classically contraindicated in VWD-2B patients due to the risk of thrombocytopenia after abnormal VWF release. However, this contraindication is based on small patients' series, not always taking into account the mutations. Moreover its impact on thrombopathy associated with VWD-2B is not known.

Aims: To evaluate in VWD-2B patients without basal thrombocytopenia the impact of desmopressin administration on VWF and platelet function.

Methods: We assessed platelet count, plasma VWF activity (VWF:Act) and PFA-100 closure times (collagen/epinephrine-CEPI and collagen/adenosine diphosphate-CADP) before and 30 minutes, 1, 2 and 4 hours after desmopressin injection (0.3 μg/kg) to VWD-2B patients.

Results: Fourteen patients (from 7 different families) were included; 8 with R1341Q mutation and 6 with R1306Q mutation, 2 of the most frequently observed mutations in VWD-2B. Before injection, all patients have a platelet count>150G/L, a partial or total HMW-VWF reduction, a VWF:Act< 50% and CEPI and CADP>300sec. Two hours after desmopressin administration, the platelet count decreased (p=0.002) without thrombocytopenia except for one patient (at 143G/L). Moreover, despite a VWF:Act correction (>50%) in all patients, CEPI remain >300sec while CADP shorten (p=0,01).

Conclusions: In conclusion, this study shows that the use of desmopressin in VWD-2B patients allows VWF:Act correction and does not often lead to thrombocytopenia. These results contradict a systematic contraindication of desmopressin in VWD-2B patients with R1341Q or R1306Q and without basal thrombocytopenia. In addition, the persistence of a major prolongation of CEPI in these patients, after VWF:Act correction and in absence of thrombocytopenia, suggests an associated thrombopathy whose impact on hemorrhagic phenotype and involved molecular mechanisms remain to be described.

PB1580 | Comparison of von Willebrand Factor (VWF) Multimeric Profile Obtained by a Commercial Electrophoretic Test (Hydragel 5 von Willebrand Multimers) and Classical Home-made with a Discontinuous 1% Agarose Gel Electrophoresis

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Background: VWF multimers testing is an integral part of the diagnostic process on VWD. By this way, low (LMWM), intermediate (IMWM), high (HMWM) and ultra-high (UHMWM) molecular weight multimers can be estimated.

Aims: To determine if VWF multimeric profile obtained by agarose electrophoresis with gel immunofixation in semi-automatic system (Hydragel 5) and agarose gel electrophoresis reference methods are comparable.

Methods: Samples (n=24): 4 acquired VW syndrome (AVWS) from myeloproliferative syndrome with thrombocytosis (MPS-T), 6 VWD type 1, 9 type 2 (confirmed by molecular biology), 1 probably type 2N, 2 not classified type 2, and 2 from Thrombotic Thrombocytopenic Purpura (TTP).

TABLE 1 results of samples of VWD type 1, AVWS, TTP and a probable VWD type 2N

Type of sample	Patient	VWF:Ag (%)	VWF:RCo (%)	VWF:RCo/ VWF:Ag ratio	Multimeric Profile	
					Reference method	Hydragel 5
AVWS MPS-T	n=4	94-114	32-55	0.3-0.48	-	Slightly decreased HMWM Slightly increased LMWM and IMWM
VWD type 1	n=6	33-58	32-45	0.6-0.85	Normal	Normal
Probably VWD type 2N	n=1	95 (FVIII: 33%)	77	0.77	-	Normal
TTP	BT	56	79	1.4	52% of UHMWM	UHMWM present
TTP	FM	156	157	1	31% of UHMWM	UHMWM present

TABLE 2 Results of 9 samples of VWD type 2 previously classified

Type of sample	Patient	VWF: Ag (%)	VWF: RCo (%)	VWF:RCo/ VWF:Ag ratio	Mutation	Multimeric Profile Reference method	Multimeric Profile Hydragel 5
VWD type 2A	SC	70	<5	0.06	Heterozygous p.Arg1597Trp	Absence HMWM Very decreased IMWM Increased LMWM	Absence HMWM Very decreased IMWM Increased LMWM
VWD type 2A	EC	44	<10	0.20	Heterozygous p.Gly1631Asp	Absence HMWM Very decreased IMWM Increased LMWM	Almost absence HMWM Very decreased IMWM Increased LMWM
VWD type 2M	ZC	17	<10	0.53	Heterozygous p.Arg1374Cys	All multimers present but slightly decreased	Moderate decrease HMWM Slightly decreased IMWM Increased LMWM
VWD type 2M	PMC	21	<10	0.43	Homozygous p.Glu1549Lys	Decreased HMWM Slightly decreased IMWM Increased LMWM	Very decreased HMWM Slightly decreased IMWM Increased LMWM
VWD type 2M	SA	73	10	0.14	Heterozygous p.Thr1468Ile	Normal but Slightly increased LMWM	Slightly decreased HMWM Slightly increased LMWM
VWD type 2M	AB	21	<5	0.19	Heterozygous p.Arg1374Cys	All multimers present with low definition between mers	All multimers present with low definition
VWD type 2B	MC	71	26	0.37	Heterozygous p.Arg1306Trp	Absence of HMWM	Decreased HMWM Increased LMWM and IMWM
VWD type 2B	MK	41	57	1.39	Heterozygous p.Pro1266Leu	Normal	Normal
VWD type 2B	GA	75	38	0.51	Homozygous p.Val1316Met	Absence of HMWM	Very decreased HMWM Slightly increased LMWM

Semiautomatic method: Electrophoresis followed by immunofixation using Hydragel 5 von Willebrand Multimers kit in HYDRASYS 2 instrument (Sebia).

Standard method: Discontinuous SDS-electrophoresis in agarose gels followed by inmunofixation using a polyclonal rabbit anti-Human VWF and polyclonal swine anti-rabbit inmunoglobulins biotinylated, revealed with 4Cl-1-Naftol. Multimeric profile: analyzed by ImageQuant TL 8.1 (GE Healthcare).

Results: Results are shown in tables 1 and 2.

Each gel processing was completed in one working day and its visualization was clear. AVWS showed decreased HMWM. VWD type 1 showed normal multimeric profile. UHMWM were detected in TTP. Results of VWD type 2A, 2B and 2M are shown in table 2. The unclassified VWD type 2 samples showed presence of all multimers in one and slightly decreased HMWM in the other.

Conclusions: All MPS-T AVWS presented decreased HMWM. Samples from VWD type 1, 2A, 2B, and TTP presented full agreement of multimeric profile between methods.

In VWD 2M samples there was also agreement, even in the sample PMC with an unusual multimeric profile in which only semi quantitative estimation of HMWM showed slightly differences. The semiautomatic method seems to be a useful and appropriate method for VWF multimeric profile screening in Hemostasis clinical laboratories.

PB1581 | Gynaecological Problems, Management and Outcomes in Women with Moderate and Severe von Willebrand Disease (VWD)

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Background: Women with VWD have increased risk of gynaecological complications during haemostatic challenges of ovulation and menstruation.

Aims: Review bleeding symptoms and their management in women with moderate/severe VWD.

Methods: Retrospective cohort analysis.

All patients registered at Katharine Dormandy Haemophilia and Thrombosis Centre, Royal Free Hospital.

Information collected from electronic patient records.

Results: 67 women were identified; all reported heavy menstrual bleeding (HMB). 38% experienced periods lasting 7-14 days and 25% bled up-to 2-6 weeks, with post-treatment improvement to «7 days in 72%.

Baseline PBAC scores were 1056 and 393 for severe and moderate cohorts, respectively, with post-treatment improvement to 393 and 115.

All underwent pelvic ultrasound and further 25% had hysteroscopic assessment, revealing 3 cases of submucosal fibroids and 3 cases of endometrial polyps.

TABLE 1 Management of heavy menstrual bleeding

FIRST LINE TREATMENT	Number treated (n=)	Satisfactory outcome (n=)
Non-hormonal - TXA/ DDAVP	16 / 2	1 TXA case
Hormonal - CHC + TXA	46 (11 had Mirena coil inserted on a later date)	28 All Mirena cases
Clotting factor concentrate (CFC) - CHC+TXA+CFC	3	2
SECOND LINE TREATMENT		
Non-hormonal - TXA+DDAVP - TXA+CFC	DDAVP=6 CFC= 4	4 CFC cases
Hormonal - Extended CHC (2-6 months) - Mirena coil	5 8	3 All Mirena coil cases
Surgical - Endometrial ablation + Mirena coil	2	2
THIRD LINE TREATMENT		
SURGICAL - Endeometrial ablation - Hysterectomy	3 3	3 3

11% improvement in haemoglobin and 69% in ferritin levels observed post-treatment.

Table 1 outlines HMB treatment:

TXA/ DDAVP first-line treatment (FLT) in adolsecents did not control period-length. In those with suboptimal response to FLT, 50% reported shortened periods with addition of CHC but had persistent HMB. Other 50% reported excessive, unscheduled breakthrough bleeding associated with history of non-compliance.

TXA/ DDAVP use in women had no effect in reducing period-length.

Addition of combined hormonal contraception (CHC) yielded 79% symptom-control; however relapse was observed with prolonged use (>3 years) or climacteric changes in 22%.

Mirena-coil with 6 months CHC+TXA cover provided 100% long-term symptom-control (n=19).

69% of second-line treatment failures were associated with non-compliance due to concerns regarding long-term hormonal therapy impacting fertility, causing hair loss or weight gain.

There was no significant difference in haemostatic parameters between responders and non-responders to FLT (Table 2.)

Haemorrhagic ovarian cysts encountered in 64% severe and 12% moderate cases. All managed conservatively with TXA and CHC to suppress ovulation; 38% required clotting-factor concentrate.

Conclusions: Management of bleeding complications requires combination of hormonal and haemostatic treatment.

TABLE 2 Mean haemostatic parameter levels

	RESPONDERS	NON-RESPONDERS
VWF:Ag	25.6	28.3
FVIII	45.8	51.7
RiCOF	12	13.3

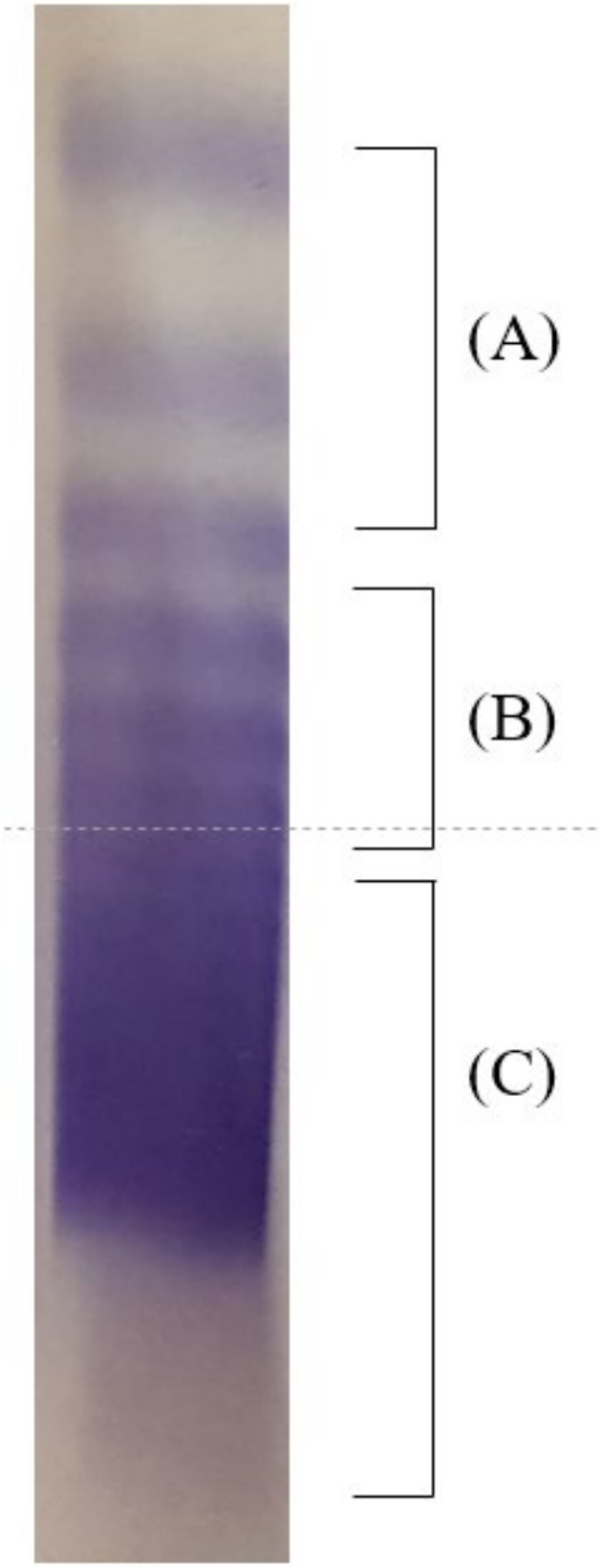

FIGURE 1 The pattern of a normal plasma for von Willebrand factor (vWF) multimers (A) low molecular weight multimer (B) intermediate molecular weight multimer

PB1582 | Degradation of High Molecular Weight von Willebrand Factor Multimers during Extracorporeal Membrane Oxygenation-Treatment

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Background: The Extracorporeal membrane oxygenation (ECMO)-treatment could increase the degradation of the high molecular weight (HMW) of the von Willebrand factor (vWF) due to shear stress. Loss of the HMW of vWF multimers could affect primary haemostasis. Therefore, the bleeding tendency might increase on ECMO-treatment.

Aims: We evaluated the degradation of vWF multimer band on ECMO-treatment.

Methods: We analyzed the vWF multimers in 29 plasma specimens by agarose gel electrophoresis and immunofixation, with semi-automated HYDRASYS-2 instrument in 12 ICU patients. We measured vWF Antigen and observed vWF multimer bands. We collected 10 cases before ECMO-treatment, 15 cases during ECMO-treatment, 4 cases after 24 hours on termination of ECMO-treatment. The normal vWF triplet structure contains low molecular weight (in the 1-3 bands), intermediate molecular weight (in the 4-8 bands), and HMW vWF multimeric (all bands in after 9th band) components (fig 1). The results of vWF multimer bands were estimated in comparison with normal vWF multimer bands.

Results: The mean vWF Ag percent was not changed significantly before and during ECMO treatment (Mean, Before, 347.5%; during ECMO-treatment, 339.1%; after ECMO-treatment, 268.3%, P=0.240) Ten cases collected before ECMO-treatment presented normal vWF multimer band pattern. The 12 cases of vWF multimers band during ECMO-treatment showed the loss of HMW vWF multimer. But 3 cases in two patient who were issued cryoprecipitate during ECMO-treatment revealed a normal pattern. Four cases collected after termination ECMO-treatment also showed a normal pattern (fig 2).

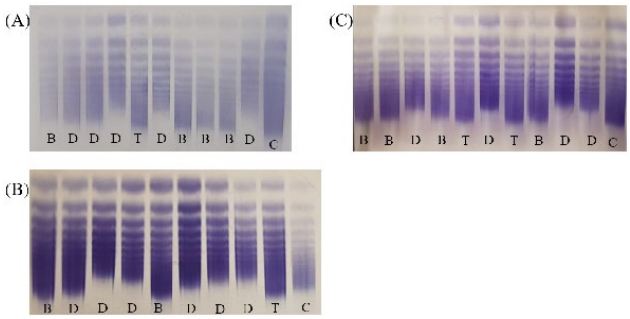

FIGURE 2 The patterns of vWF multimer (A) The 2 and (B) one samples during ECMO showed a normal d/t cryoprecipitate. C, Control; B, before; D, during; T, termination

Conclusions: HMW vWF multimer bands missing occurred during ECMO-treatment and was resolved immediately after completion of ECMO-treatment. This suggests that HMW vWF multimer bands help to detect bleeding tendency during ECMO-treatment. Nevertheless, further evaluation is needed for cryoprecipitate and/or vWF concentrations as treatment of coagulation disorder on ECMO-treatment.

PB1583 | Acquired von-Willebrand-Disease (aVWD) in ECMO Patients: a 3-Year Cohort Study

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Background: Approximately 100% of patients undergoing ECMO develop laboratory aVWD during their hospital stay, in the majority of cases without clinical bleeding.
Aims: Study aim was to collect more relevant data possibly associated with *i)* development of clinically relevant aVWD and/or *ii)* death during the follow-up.
Methods: Within a 3-year period 338 white patients aged 18 to 88 years (median: 60) were enrolled (male 64.5%).
Results: The yearly incidence of patients with aVWD with clinical relevant bleeding symptoms in this high risk cohort was 23% (78/338) with a death rate of 74% within a median time of 9 days (1 - 229) following ECMO start. Of note, 15 of 78 subjects with symptomatic aVWD received aspirin or clopidogrel, vitamin-K-antagonists, heparins, argatroban and/ or anti-factor-Xa inhibitors. As treatment for bleeding episodes blood product administration was performed concomitant with coagulation factor concentrates. The 78 subjects with symptomatic aVWD received von-Willebrand-factor concentrates, in median 6.000 IU (min - max: 1.000 - 157.000 IU) per patient affected. Logistic regression analysis adjusted for age and gender revealed that *i)* the presence of blood group 0 versus non-0 (Odds ratio (OR)/ 95% Confidence interval (CI) 1.76/ 1.005 - 3.11) and the overall need for blood products administered per unit (OR/ CI 1.1/ 1.01 - 1.02) were independently associated with the development of clinically relevant aVWD. *ii)* In the entire ECMO cohort older age (increase per year) at ECMO start (OR/ CI 1.01/ 1.01 - 1.03) and the amount of blood product units necessitated were related with death (OR/ CI 1.01/ 1.002 - 1.014) but not associated with the development of clinically relevant aVWD.
Conclusions: In the present cohort study we found a clinical relevant bleeding incidence of 23% in subjects with aVWD. The latter, however, was not associated with death following the ECMO procedure.

PB1584 | Prothrombotic Gain-of-Function Variants of von Willebrand Factor

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Background: Von Willebrand factor (VWF) is essential to primary hemostasis by mediating platelet recruitment to sites of vascular injury. A wide range of genetic VWF variants were identified leading to the bleeding disorder von Willebrand Disease (VWD). In contrast, high levels of plasmatic wildtype VWF have been shown to be associated with prothrombotic events and we recently identified the frequent polymorphism p.Phe2561Tyr as a VWF gain-of-function variant, which can increase the risk of myocardial infarction.
Aims: We identified a second VWF variant that directly enhances VWF's hemostatic activity and strived to characterize the underlying gain-of-function effect.
Methods: The variant with a missense mutation in the C4 domain (harboring the platelet GPIIb/IIIa binding site) was functionally and structurally analyzed. GPIIb/IIIa binding was investigated in the full-length multimeric protein by static assays as well as under flow conditions. To assess the impact of the mutation on the secondary and tertiary structure of the monomeric single C4-domain and the dimeric domain construct C1-CTCK, far and near UV circular dichroism (CD) spectra were recorded.
Results: The rare mutation p.Pro2555Arg showed normal GPIIb/IIIa binding under static conditions and CD spectra revealed that the mutation has no detectable effect on secondary and tertiary structure within the isolated C4 domain. In the context of a dimeric construct, however, enhanced local disorder was found. In cone and plate assays, p.Pro2555Arg showed increased aggregate size. Importantly, microfluidic assays revealed that, while p.Phe2561Tyr lowers the critical shear rate for VWF-platelet complex formation, p.Pro255Arg forms aggregates at the same shear rate as wtVWF but results in dramatically increased aggregate sizes.
Conclusions: Missense mutations can induce a gain-of-function in VWF, which results in increased platelet aggregation via different mechanisms. The GOF properties of these variants further underline the prothrombotic character of VWF as a potential target for antithrombotic therapy.

PB1585 | Type 2N von Willebrand Disease (VWD2N): Is it Always a Recessive Trait?

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Background: VWD2N is a rare form of VWD, described as a recessive disease due to disease-causing variant (DCV) in homozygous or double/compound-heterozygous state. Heterozygotes individuals are considered carriers, and mostly asymptomatic; those homozygotes and double/compound-heterozygotes have clinical manifestations and laboratory phenotype frequently mistaken for those of mild hemophilia A.

Aims: To show the variable inheritance of VWD2N according to the DCV in a selected group of patients phenotypic and genotypically diagnosed.

Methods: We studied the *F8* in five symptomatic patients with heterozygous DCV in *VWF* associated to VWD2N (Table 1). The severity of bleeding symptoms was evaluated by the ISTH/SSC bleeding assessment tool (ISTH/SSC BAT).

Patients were studied after signing the written-informed consent. The study was approved by local Ethics Committee.

Laboratory tests: FVIII:C (one-stage method), VWF:Ag (ELISA), VWF:RCo (aggregometry). Mixing studies of the patient/control plasmas to evaluate the presence of inhibitors were undertaken by APTT and VWF:RCo. FVIII:C/VWF:Ag (normal value [nv]>0.8) and VWF:RCo/VWF:Ag (nv>0.6) were calculated in each patient.

Genomic DNA was extracted from peripheral blood. Exons 17-27 of the *VWF* and all 26 exons of *F8* were PCR-amplified and directly sequenced (Sanger method). *F8* exons were previously heteroduplex-analysed by conformation sensitive gel electrophoresis.

Results: Both normal VWF:Ag and VWF:RCo levels, and normal VWF:RCo/VWF:Ag were observed in all patients. No DCV were observed in *F8* (Table 1).

Patients with p.Arg816Trp had very low FVIII (< 12 IU/dL) and FVIII/VWF:Ag< 0.11, abnormal BAT; 2 out of 3 patients had major bleeding episodes.

Patients with p.Arg854Gln had low FVIII levels (40-45 IU/dL) and FVIII/VWF:Ag< 0.7, and abnormal BAT. One patient had a major bleeding episode.

Conclusions: According to the FVIII levels and FVIII/VWF:Ag values of the patients, heterozygous p.Arg816Trp appears to cause a clearly dominant VWD2N.

Patients with p.Arg854Gln should be considered as VWD2N in spite of their heterozygous state in *VWF* given their bleeding tendency.

PB1586 | Severe Thrombocytopenia after DDAVP Test (DDAVPt) in a Girl with a p.Cys1272Gly Variant of the VWF Gen

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Background: Thrombocytopenia following DDAVP is reported in patients with von Willebrand disease (VWD) type 2B. We have already described two patients with variants affecting disulfide bridges in the A1 loop of the von Willebrand factor (VWF). Those patients presented thrombocytopenia during DDAVPt.

Aims: To report severe thrombocytopenia after DDAVPt in a girl with type 2A VWD phenotype and a VWF p.Cys1272Gly variant.

Methods: A seven-year-old girl with a VWD type 2A phenotype presented with low VWF:Ag (25%), VWF:RCo and VWF:CB< 5% and no history of thrombocytopenia or hyper-responsiveness to low dose ristocetin (LDR). PCR-sequencing detected a p.Cys1272Gly variant. DDAVPt was performed. Platelet count (PC) and morphology, PT, aPTT,FVIII:C, VWF:Ag, VWF:RCo, VWF:CB were evaluated at 0,30,60,120 and 240min post-DDAVP infusion onset.

Results: At 30min we observed:PC decrease from $206x10^9L^{-1}$ to $3x10^9L^{-1}$, with no full recovery at 24 hours,giant platelet clumps (>100 platelets) in blood smears, slight amelioration of VWF:CB (21%) and of VWF:RCo (23%), spontaneous aggregation and enhanced response to LDR in platelet aggregation mixing studies.

Conclusions: These findings confirm that variants affecting disulfide bridge in the A1 loop of the VWF behaves like type 2B VWD patients,

TABLE 1 Phenotypic and genotypic parameters in patients with VWD2N

Patient	Gender/age	DCV in *VWF*	DCV in *F8*	FVIII	VWF:Ag	FVIII/VWF:Ag	BAT	Major bleeding
VJM	Female/63	p.Arg816Trp	none	12	110	0.11	15	yes
BS	Male/4	p.Arg816Trp	none	8	73	0.11	6	yes
BN	Female/2	p.Arg816Trp	none	4	76	0.05	2	no
RC	Female/11	p.Arg854Gln	none	45	72	0.63	7	yes
BMC	Female/16	p.Arg854Gln	none	40	82	0.49	3	no

as we have already reported. With this knowledge, thrombocytopenia was expected during DDAVPt. Molecular biology evaluation in type 2A VWD patients should be considered before DDAVPt.

PB1587 | Measurement of VWF:FVIII Binding (VWF:FVIIIB) in a Cohort of Patients with Suspected Type 2N VWD and Correlation with Genetic Analysis of the *VWF* Gene

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Background: Type 2N Von Willebrand Disease (VWD) is characterised by a decreased capacity of Von Willebrand Factor (VWF) to bind Factor VIII (FVIII) [1]. The differential diagnosis to exclude mild Haemophilia A includes calculation of FVIII:VWF ratio, assessment of the FVIII binding capacity of VWF, and analysis of the *VWF* gene. Classification is essential for appropriate treatment and genetic counselling.
Aims: The aim of the study was to investigate the relationship between phenotype and genotype for patients with suspected Type 2N VWD.
Methods: VWF:FVIIIB assesses the ability of plasma VWF at a standard concentration (0.10 IU/ml) to bind recombinant FVIII (rFVIII) using an ELISA assay[3]. Results are expressed as percent VWF:FVIIIB by correlation with a standard curve. Analysis was performed on plasma from 31 patients. Patients were genotyped for the common *VWF* variant associated with 2N VWD (p.R854Q) [2].
Results: A local VWF:FVIIIB reference interval was established previously (74-97%). 16/31 patient results were within this reference interval; median FVIII:VWF Ag ratio 0.68 and *VWF* p.R854Q absent on genotyping. Remaining patient results (15/31) were subdivided into two categories based on the local reference interval and kit manufacturers recommendations (patients with VWF:FVIIIB capacity of < /= 20% are considered to have Type 2N VWD). 4/15 patient results were within the range of 20-74% VWF:FVIIIB; median FVIII:VWF Ag ratio 0.72 and *VWF* p.R854Q heterozygous on genotyping. 11/15 patient results were less than 20% VWF:FVIIIB; median FVIII:VWF Ag ratio 0.38. Of these; eight were homozygous for *VWF* p.R854Q, one compound heterozygous for *VWF* p.R854Q and two heterozygous for *VWF* p.R854Q with an additional likely null variant (not further analysed).
Conclusions: This study demonstrates a relationship between phenotype and genotype for patients with suspected Type 2N VWD. A reduction in VWF:FVIIIB is predictive of the presence of *VWF* p.R854Q. Furthermore < 20% VWF:FVIIIB is suggestive of homozygosity for *VWF* p.R854Q.

PB1588 | Uncommon Types of von Willebrand Disease

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Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder caused by lack or impaired function of von Willebrand factor (VWF). Some forms can be misdiagnosed. For example subtype 2N (false diagnosis of haemophilia) and subtype 2b (wrong diagnosis of ITP).
Aims: We present four children from two families with proven rare forms of VWD.
Methods: VWF antigen and activity were measured by imunoturbidimetry using latex particles. For detection of defect of factor VIII based on activated partial thromboplastin time was used human deficient plasma as substrate. Causative mutations were detected by sequencing of exons of VWF gene.
Results: Girl examined for recurrent bruising on lower limbs. After the fall on her knee major bleeding into femoral muscle occurred. Her mother a has history of heavy menstrual bleeding and mother and sister of father have epistaxis. APTT 42.7 s, factor VIII activity 9.4%, VWF antigen 44% and VWF activity 39% were found with no factor VIII inhibitor. Genetic testing confirmed VWD subtype 2N. Heterozygous mutations: c.2401A>C in exon18 (from mother) and c.374_387del in exon 5 (from father) were proven.
3 siblings (2 girls and 1 boy) from the second family were examined for recurrent clinically important bleeding (recurrent epistaxis with a need of tamponade, heavy menstrual bleeding, hemorrhagic ovarian cyst with hemoperitoneum, hematemesis with posthemorrhagic anemia and need of transfusion of red blood cells). Mother and grandmother were treated with ITP in past. Prolonged APTT, VWF antigen 30%, VWF activity 6%, thrombocytopenia 53-119x10^9/l and normal platelet aggregation were found. Genetic testing confirmed VWD subtype 2b. Mutation: R1306w/3916C:T (gain of function) was proven.
Conclusions: Although mild forms of VWD are the most common, there are forms with severe bleeding. In this cases, precise diagnostics including molecular genetic testing is very important to clarify the diagnosis and to prevent inappropriate treatment if misdiagnosis.

PB1589 | Diagnostic Performances Evaluation of LIAPHEN vWF: Ag on CS-2500

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Background: Von Willebrand disease (VWD) is one of the most common inherited bleeding disorders, caused by quantitative and/or qualitative defect of von Willebrand factor (vWF), a multimeric

glycoprotein essential for primary and secondary hemostasis. Conversely, recent studies showed that very high vWF levels might predict in-hospital survival in acute-on-chronic liver failure patients. Diagnosing vWD, based on bleeding history and specific laboratory investigation, may be difficult due to lack of standardization. Numerous assays are available, but measurement of vWF function and plasma concentration is the current practice. A fully automated, immunoturbidimetric vWF:Antigen (vWF:Ag) assay is developed for use on any coagulation analyzer.

Aims: To report diagnostic performance characteristics of this vWF:Ag latex immunoassay on CS-2500.

Methods: LIAPHEN vWF: Ag is an immunoturbidimetric method: vWF:Ag in tested sample reacts with latex particles coupled with rabbit anti-vWF affinity-purified polyclonal antibodies, leading to latex particles agglutination. Performances are evaluated using commercial lyophilized plasma calibrator and controls, and a comparison study with another device is performed on CS-2500 using 100 plasma samples from healthy subjects (n=50), vWD patients (n=25) or liver disease, DIC or sepsis patients (n=25).

Results: Between-run precision coefficient of variation (CV%, n=20), evaluated on lyophilized plasmas with normal and pathological vWF levels, are < 5%. An excellent correlation is obtained with STA-Liatest VWF:Ag on STA-R: y=1.01x-6.61, r=0.993, p< 0.0001. vWF:Ag is well correlated for pathological samples in low range: r=0.971, p< 0.0001 and high range: r=0.983, p< 0.0001.

Conclusions: This vWF:Ag assay is highly sensitive, specific, reproducible and standardized to rapidly assess vWF:Ag concentrations in human citrated plasma. The extended dynamic range is of high interest for the diagnosis of vWD (low levels), as well as for high to very high vWF concentrations, especially for inflammatory diseases and prediction of survival in acute-on-chronic liver failure patients.

PB1590 | Diagnosis and Management of von Willebrand Disease in a Portuguese Reference Center of Congenital Coagulopathies

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Background: The diagnosis of von Willebrand disease (VWD), the most common inherited bleeding disorder, is characterised by a variable bleeding tendency and heterogeneous laboratory phenotype. The diagnosis of VWD is based on the nature of the VWF deficiency: partial or complete VWF quantitative deficiencies (types 1 and 3, respectively) and qualitative deficiency (type 2).

Aims: Our aim is to evaluate how molecular studies can improve the establishment of the correct diagnosis in patients who attended our Center with suspicion of VWD.

Methods: The study included 118 patients (105 families), adults and children with a median age of 38 years (1-80), 70F:48M, with personal/family history of significant haemorrhage identified through the BAT and ISTH guidelines. The functional VWF studies were carried out in all patients followed by next generation sequencing (NGS) workflow using a panel of Thrombosis and Haemostasis (43 genes). The genotype/phenotype was compared and a final diagnosis was established.

Results: VWD was confirmed in 83 patients (70%) with the distribution as follows: type 1 VWD (n = 33), type 3 VWD (n = 12), type 2A (n = 6), type 2B (n = 4), type 2M (n = 23) and type 2N (n = 5). Four patients had variants in different genes (*F8*,*NBEAL2*,*GGCX*,*ITGA2B*) with evidence of oligogenic inheritance. The remaining 30 patients were considered with diminished VWF levels and increased bleeding risk.

A total of 29 different *VWF* pathogenic variants were found. Twenty five probands had two or more *VWF* variants. In addition, variants with pleiotropic effects were detected and explained the clinical variability, which is found even in the same family.

Conclusions: These findings illustrate that improving VWD diagnosis strategy with NGS molecular studies has permitted in our Centre the most correct treatment for each patient and genetic counseling.

PB1591 | Real World Experience of Major Surgery and Delivery in Von Willebrand Disease

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Background: Von Willebrand Disease (VWD) is the most common heritable bleeding disorder. VWD commonly leads to bruising mucocutaneous bleeding and increased bleeding from invasive procedures. Prophylactic treatment to prevent bleeding is often administered.

Aims: To ascertain whether real life practice of factor replacement in VWD patients reflects what is recommended in the guidelines and whether this results in acceptable outcomes with respect to postoperative bleeding and/or thrombosis.

Methods: A single-centre, retrospective, cohort study spanning 13 years. Patients who underwent major surgery or childbirth were required to have at least pre-operative and post initial dose VWF and FVIII levels to be included in this study. The timing of dosing of

TABLE 1 Patient cohort

	Type 1	Type 2A	Type 2B	Type 2M	Type 2N	Type 3
No. of patients	13	3	1	11	0	2

TABLE 2 Summary of procedures

Major procedure- Orthopedic procedures	Major procedure- Intra-abdominal procedures	Major procedure- Neurological procedures	Deliveries- Caesarean Section	Deliveries- Vaginal
8	17	1	9	6

the factor concentrates relative to the previous dose was collected as well as the duration of factor replacement as well as adjunctive therapies (DDAVP, tranexamic acid, or platelet transfusions) were recorded. Surgical complications (bleeding or thrombosis) were also collected.

Results: Majority of the patients achieved the levels recommended by national guidelines; in 88% and 98% of cases patients were at or above target levels for FVIII and VWF:RCo respectively at the time of procedure. 8.3% of FVIII levels measured were over 270 IU/dL (7.5% in those undergoing surgery and 9.6% in those giving birth) similarly to levels reported. Despite these levels, there were no episodes of thrombosis.

Conclusions: Close adherence to national guidelines for FVIII and VWF:RCof replacement in VWD patients undergoing major surgery or delivery appears to reduce the risk of major bleeding to an acceptable level (2.4%). Greater improvement of current guidelines can be achieved by continuous dosing, greater knowledge of the pharmacokinetics of the factor replacements between different VWD patients, particularly in relation to clearance to avoid accumulation, and also the use of population based pharmacokinetic based models.

PB1592 | Von Willebrand's Disease: A Diagnostic Evaluation Using Current Guidelines

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Background: Von Willebrand's disease (vWD) is the most common congenital bleeding disorder. The revised 2014 British Journal of Haematology (BJH) guidelines provide a new framework by which to classify vWD subtypes. This includes a new category for patients with a milder reduction in vWF level who are now classified as 'low vWF'.

Aims: To perform a diagnostic evaluation of patients with a vWD diagnosis using the 2014 BJH guidelines and to correlate their revised diagnosis with bleeding patterns and management.

Methods: A retrospective observational study involving analysis of 227 vWD patients at Addenbrooke's Hospital. Each diagnosis was reclassified according to the 2014 BJH guidelines. Further data was collected from electronic patient records including symptoms and management.

Results: Based on the 2004 BJH guidelines, 70% of patients were diagnosed with type 1, 29% with type 2 and 1% with type 3 vWD. After reclassification, the proportion of patients diagnosed with types 2 and 3 vWD remained largely unchanged at 28% and 1% respectively. However, of the 70% of patients originally diagnosed with type 1 vWD, 16% retained this subtype. The remainder were reclassified as 'low vWF' (42%) or with a normal vWF level (13%).

The pattern of bleeding symptoms for patients with types 1 and 2 vWD were similar. In those with type 1, the proportion with bleeding symptoms reduced compared to those with type 2 after reclassification.

Desmopressin and tranexamic acid were commonly used treatments across all subtypes. Notably, vWF concentrate was increasingly used for more severe phenotypes (types 2 and 3). This remained unchanged following reclassification.

Conclusions: In our cohort, fewer patients were diagnosed with vWD after reclassification. A large proportion of patients are now classified with 'low vWF'. These stricter criteria allow a greater diagnostic accuracy, thus avoiding unnecessary diagnosis in those with a milder reduction in vWF.

PB1593 | Laboratory Monitoring of Perioperative von Willebrand Factor Replacement

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Background: Perioperative replacement of von Willebrand factor (VWF) can be challenging if only plasma-derived factor VIII/VWF concentrates are available. They present different FVIII/VWF ratios (1:1 and 1:2) that must be taken into account. In order to maintain the desired VWF activity sometimes FVIII (FVIII:C) levels surpass 150%, increasing the risk of thrombosis. Although the Brazilian guidance on von Willebrand disease (VWD) recommends daily monitoring only of coagulation factor VIII:C, some authors abroad have included von Willebrand factor activity (VWF:RCo) as a relevant parameter during the post-operative period.

Aims: To check the relevance of VWF:RCo and FVIII:C to guide VWF replacement and investigate their correlation with bleeding or thrombosis in the perioperative period.

Methods: Data of all patients with the diagnosis of VWD and submitted to an invasive procedure at HCFMUSP between 2008 and 2018 were collected whenever available and complete. VWF was replaced with FVIII/VWF plasma derived concentrates, according to

the Brazilian guidance. Recombinant FvW is not available in Brazil. Results are described.

Results: Data of 10 patients were collected. They were predominantly female (70%) and adults (mean age = 46 years). Only 1 patient presented VWD type 3, the others being equally distributed between types 1 (n=4) and 2 (n=5). Fourteen procedures were studied, most of them major (n=9) and the others, minor. All patients achieved the desired levels of FVIII:C and no thrombosis ocurred, even in 2 patients with FVIII:C higher than 150%. Low levels of VWF had no correlation with bleeding, except in 1 patient with FVIII:C of 111%. Decisions on VWF replacement were based on the presence of bleeding and FVIII:C levels, since results of VWF:RCo were not always available at the time of prescriptions.

Conclusions: Perioperative VWF replacement was guided by FVIII:C levels and the presence of bleeding and was independent of FvW:RCo levels in the procedures studied.

PB1594 | The *VWF* Variant D1472H Affects VWF Binding to Ristocetin *in vitro* in the Platelet Agglutination Assay but Not with Latex Particles (HemosIL® Acustar VWF:GpIbR): The Usefulness to Change Practices to Avoid VWD Genotyping

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Background: The diagnosis of von Willebrand disease (VWD) relies on measurements of von Willebrand factor antigen (VWF:Ag) and functional activity. Until now, the gold standard assay for measuring VWF functional activity is the ristocetin cofactor activity (VWF:RCo),that quantifies the binding of VWF to platelets through GpIb, induced by ristocetin. However, some polymorphisms as the variant D1472H can falsely decrease VWF:RCo by interacting *in vitro* with ristocetin. Other tests are available to measure VWF activity, both using latex particles, as the HemosIL®Acustar VWF:GpIbR assay by chemiluminescence (with ristocetin) or the Innovance®VWF:GpIbM assay (no ristocetin).

Aims: Verify the sensitivity of VWF:GpIbR assay to the D1472H and reevaluate the practices for the biological diagnosis of VWD to avoid useless explorations and especially genotyping.

Methods: By studying the database of the French Reference Center for VWD, we noticed that 137 patients had a VWF:Ag>30% and a VWF:RCo/VWF:Ag ratio< 0.7 with local methods but were finally excluded from congenital VWD as no pathogenic variant was identified in *VWF* after genotyping. Among them, 79 presented the only variant D1472H. We were able to retest VWF:Ag and VWF activity with the VWF:GpIbM and the VWF:GpIbR assays for 30 patients.

Results: The VWF activity was significantly higher in 23/30 patients with the VWF:GpIbM and VWF:GpIbR methods (p< 0,0001), compared to VWF:RCo leading to a normalized ratio (p< 0,0001). Two patients still had a low ratio and 5 were discordant between the 2 methods but the results were very close to the 0.7 threshold.

Conclusions: The HemosIL VWF:GpIbR assay seems not to be sensitive to the D1472H variant, nor the Innovance VWF:GpIbM assay. Genotyping could have been avoided for these patients that don't have VWD. We suggest that in case of low ratio with VWF:Ag>30%, the activity should be checked using VWF:GpIbR or VWF:GpIbM.

PB1595 | Gum Bleeding in Low Von Willebrand Factor- What's the Score?

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Background: Bleeding Assessment Tools (BAT) have standardized phenotypic evaluation for bleeding disorders (BD) incorporating oral cavity (PO) bleeding. PO bleeding was previously reported in 48% (76/159) of adults in the Low Von Willebrand in Ireland Cohort (LoVIC) study.

Aims: We investigated whether PO bleeding was related to Low Von Willebrand Factor (VWF) or underlying gingivitis.

Methods: Patients with Low VWF were enrolled and diagnostic ISTH BAT scores recorded. Dental staff, blinded to the ISTH BAT, used objective dental measures to assess gum bleeding (Modified Papillary Bleeding Index [MPBI]), gingivitis (Gingival index [GI]) and plaque burden (Quigley-Hein Index [QHI]). All received hygienist-led standardized but personalized information session on oral hygiene and reassessed 4-6 weeks later. Data analysis was performed using Prism 8 software.

Results: 30 patients with Low VWF levels enrolled (median age 41 years, 90% female). 80% of patients enrolled reported PO cavity bleeding, with 13% requiring consultation. Median MPBI (6, range 0-29), GI (0.72, range 0.16-2) and QHI scores (1.18, range 0.35-2.38) were elevated at baseline. Objective dental bleeding (MBPI) correlated poorly with either the total ISTH BAT (r^2=0.006) or PO domain (r^2=0.06) scores. In all individuals, a personalised oral hygiene intervention resulted in highly significant reductions in objective bleeding (MBPI median 7 to 1, p< 0.0001; GI 0.72 to 0.25, p< 0.0001) and plaque scores (QHI 1.18 to 0.67 p< 0.0001).

Conclusions: The current oral cavity bleeding component of ISTH BAT lacks specificity for BD and may reinforce perceptions that bleeding is related to BD rather than gingivitis. Gingivitis is common and untreated gingival disease may progress to periodontitis with associated systemic impacts, tooth loss and reduced oral function; delays in recognition should be avoided. The ISTH BAT correlated poorly with objective dental measures of bleeding (MPBI, GI), suggesting limited benefit of this domain for physicians and dentists alike.

PLATELETS AND MEGAKARYOCYTES

MEGAKARYOCYTES AND THROMBOPOIESIS

PB1596 | Megakaryocytes Possess a Functional Necroptosis Pathway

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Background: Necroptosis is a newly discovered pathway of programmed necrosis, with RIPK1 being a critical regulator of the pathway. RIPK1 promotes the sequential activation of two downstream targets, RIPK3 and MLKL. Previously, it has been shown that RIPK3 plays a role in regulating thrombosis and haemostasis (*Zhang et al., 2017 PNAS*), however characterisation of necroptosis signalling and the functional significance in megakaryocytes has not been explored.
Aims: To determine whether necroptosis signalling influences the megakaryocytic lineage.
Methods: Genetic mouse models included WT and MLKL KO counterparts. Western blot was employed on cell lysates from megakaryocytes and platelets to assess the expression of necroptosis signalling proteins. Cell viability and morphology was assessed by the cell titre glow assay and time lapse imaging of WT and MLKL KO megakaryocytes via confocal microscopy. Agonists induced platelet activation was measured via flow cytometry and bleeding times after 3-mm tail amputation were assessed.
Results: Bone marrow derived WT megakaryocytes expressed RIPK1, RIPK3, and MLKL proteins. Phospho MLKL was induced in response to activation of necroptosis (TSI -TNF-a/Smac mimetic/ the caspase-8 inhibitor, Idun). WT megakaryocytes treated with TSI were sensitized to necroptosis, which could be rescued by Necrostatin-1, or genetic deletion of MLKL. Time lapse imaging demonstrated that the necroptotic process could be visualized as early as 1 h post TSI treatment, while TNF/SMAC induced apoptosis was apparent at 4h post treatment. Furthermore, MLKL KO mice exhibited increased re-bleeding indicating unstable thrombus formation. MLKL KO platelets showed reduced degranulation and integrin activation in response to thrombin, suggesting impaired platelet signalling.
Conclusions: For the first time, we demonstrate that bone marrow derived megakaryocytes harbour a functional necroptosis signalling cascade, triggered via TNF receptor signalling. MLKL KO mice have unstable thrombus formation and impaired platelet activation, demonstrating that the necroptosis pathway can influence function of the megakaryocytic lineage.

PB1597 | Combined α4a- and β1-Tubulin Deficiencies Profoundly Affect Platelet Biogenesis and Haemostasis

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Background: Platelet microtubules are arranged into an annular submembranous structure called marginal band. This organization has no equivalent in any other mammalian cell, and the mechanisms responsible for this unique mode of assembly are not fully understood. Previous studies in mice and humans established a central role for β1-tubulin, whose disruption causes macrothrombocytopenia and hinders marginal band formation. Recently, we reported a missense mutation in α4a-tubulin that phenocopied the defects seen in β1-tubulin knockout mice (*Tubb1*$^{-/-}$).
Aims: We investigated the hypothesis of a functional link between α4a- and β1-tubulin in platelet biogenesis and marginal band formation.
Methods: We generated α4a- and β1-tubulin double knockout mice (DKO) and evaluated their platelet parameters,biogenesis and functional properties *in vitro* and *in vivo*.
Results: DKO mice exhibited worsened macrothrombocytopenia in comparison to single knockouts. In addition, their platelets were completely devoid of their marginal band and had lost the typical disc-shape morphology. DKO mice also displayed clear signs of defective megakaryopoiesis, with a decreased proportion of mature megakaryocytes *in situ* and the inability of cultured megakaryocytes to extend proplatelets. Strikingly, DKO mice exhibited prolonged tail bleeding times as well as decreased thrombus formation in a $FeCl_3$-injury model, despite normal platelet aggregation responses. Follow-up work will explore the underlying mechanisms responsible for these haemostatic defects, but preliminary data already point toward a decreased procoagulant activity. Since α4a-tubulin is also expressed in the vessel wall, additional vascular defects in DKO remain to be explored.
Conclusions: The combined deficiency of α4a- and β1-tubulin results in additive defects in marginal band formation and adds more ground to the hypothesis that the marginal band could contribute to platelet haemostatic properties.

PB1598 | Systmetic Study of Biological Properties and Functions of Bone Marrow Mesenchymal Stem Cells from Patients with Essential Thrombocythemia and Explore the Effects of Interferonα-2b on Them

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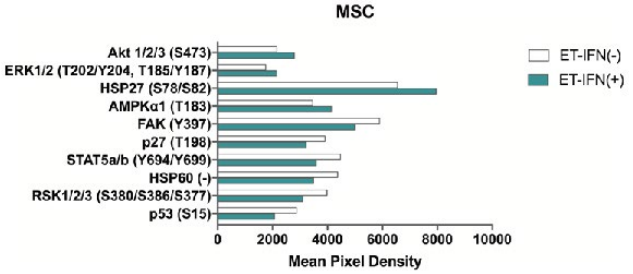

FIGURE 1 Graphic representation of the quantification of 10 proteins with the most significant difference in phosphorylation status between MSCs treated with o

Background: Recent work by our group revealed insufficient stromal support in JAK2V617F-positive ET resulting from molecular and functional deficits of the microenvironment. IFNα-2b has been used to treat ET patients, while the role of IFNα-2b on the microenvironment remains unclear.

Aims: This study aims to explore the functional and molecular effects of IFNα-2b on the microenvironment of ET.

Methods: We collected specimens from clinical patients, and then combined qPCR, western blotting, flow cytometry, immunochemiluminescence, liquid chip and so on to perform functional difference analysis of the microenvironment before and after IFNα-2b treatment. Then, based on the existing literature and database information, the molecular mechanism is explored, and the function of potential target molecules is verified according to Coho′s law.

Results: IFNα-2b had no effect on the morphology, immunophenotype and JAK2 mutant allele of BM-MSCs derived from ET patients. IFNα-2b was capable of suppressing the proliferation of BM-MSCs, but increasing its apoptosis and its function of supporting hematopoiesis. IFNα-2b also ameliorated its defective in inhibiting the proliferation of PBMCs and promotes PBMCs to differentiate into regulatory T cells. IFNα-2b affects the phosphorylation levels of multiple signaling pathways such as AKT and ERK, which may be the molecular mechanism by which it exerts biological effects.

Conclusions: IFNα-2b affects BM-MSCs from ET patients in multiple differentiation capacities, proliferation, apoptosis and the capacity to support long-term hematopoiesis and immune regulation. AKT and ERK signaling pathways may be the molecular mechanism by which it exerts biological effects.

PB1599 | A Novel Image-Based Chemical-Genetics Screen Identifies Inducers of Platelet Release from Immortalized Megakaryocytic Cell Lines, Enabling Clinical-Scale Production of Functional Platelets

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Background: Platelets are critical to hemostasis and platelet transfusion prevents and treats bleeding in thrombocytopenic patients. Limited availability and functional variability inherent to donor platelets may be overcome by transfusion of human induced pluripotent stem cell (hiPSC)-derived platelets. To enable cost-effective megakaryocyte (MK) expansion and differentiation, conditionally immortalized megakaryocytic progenitor cell lines (imMKCLs) were developed (*Cell stem cell*. 2014; 14: 535) that can produce functional platelets. However, like many other *in vitro* platelet production systems, the imMKCL system suffers from low yields (~10 platelets/MK or about $1x10^9$ platelets per 10-liter shaker flask culture volume).

Aims: To identify novel compounds and pathways to improve efficiency of megakaryocytic *in vitro* maturation and platelet production.

Methods: We developed a confocal live-cell imaging platform that allowed us to assess the rate and extent of megakaryocte-maturation and pro-platelet production in high-throughput manner and used it to screen chemical libraries.

Results: We identified two structurally related compounds (targeting the same pathway) that robustly induce *in vitro* (pro)-platelet production. We also identified additional compounds (acting through a distinct pathway) that promote imMKCL-maturation and/or endomitosis. Sequential exposure of imMKCLs to the maturation- and (pro)-platelet formation promoting compounds in a PBS-Mag type bioreactor system enabled generation of $\geq 1x10^{11}$ platelets per 10 liters, satisfying clinical production requirements. These imMKCL-derived platelets are structurally and functionally similar to normal human platelets as judged by 1) size, morphology and ultrastructure (electron microscopy and immunofluorescence), 2) resting and agonist-stimulated surface expression of activation biomarkers (flow cytometry), and 3) platelet aggregation. *In vivo* studies in non-thrombocytopenic mouse indicate that exogenous bioreactor-produced platelets are incorporated into the developing thrombus at a rate similar to that of normal human platelets during laser-induced thrombus formation.

Conclusions: Using a novel image based chemical-genetics screen, inducers of imMKCL-maturation as well as platelet release were identified. This method enables cost-efficient clinical scale production of functional platelets.

PB1601 | Mesenchymal Stromal Cells Display Metabolic Alterations in JAK2-Mutant Myeloproliferative Neoplasms

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Background: Myeloproliferative neoplasms (MPNs) are clonal disorders of hematopoietic stem cells driven by gain-of-function mutations in genes. Three phenotypic manifestations of MPNs can be distinguished: PV, ET and PMF. JAK2V617F mutation is present in about 90% patients with PV and 55% patients with ET. Increased energy requirement and metabolic reprogramming has been recognized as one of the hallmarks of cancers.Recent work by our group revealed multilevel defects in the hematopoietic microenvironment in JAK2V617F-positive MPNs resulting from molecular and functional deficits. Whether the insufficient stromal support is related to metabolic changes of the bone marrow niche remains unclear.

Aims: This study aims to explore the metabolic alterations of mesenchymal stromal cells in JAK2-mutant MPNs.

Methods: We collected the supernatant of bone marrow aspirate from clinical JAK2-mutantMPN patients and measured the glucose, triglyceride and total cholesterol in the bone marrow. Total RNA was isolated from (mesenchymal stromal cells) MSCs at passage four and used for transcriptomic analyses. And GO analysis, KEGG analysis and GSEA computational assays were carried out.

Results: (1) The level of glucose, triglyceride and total cholesterol is obviously higher in the bone marrow of JAK2-mutantMPN patients. (2)A total of 766 upregulated and 429 downregulated genes were detected in the MSCs of JAK2-mutant MPN patients. (3) GO analysis revealed changes in the gene sets related to sterol biosynthetic process, cholesterol biosynthesis, cholesterol biosynthetic process, sterol metabolic process and organic hydroxy compound metabolic process. (4) KEGG analysis showed a prominent abundance of gene signatures associated with glycolysis/gluconeogenesis and steroid biosynthesis. (5) GSEA confirmed significant enrichment of dysregulated genes involved in organic hydroxyl compound metabolic process and fatty acid metabolic process.

Conclusions: The level of glucose, triglyceride and total cholesterol was upregulated in the bone marrow of JAK2-mutant MPNs, which may be related to the metabolic alterations displayed by MSCs in the microenvironment.

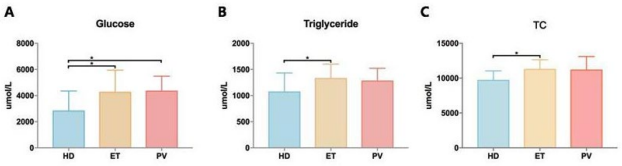

FIGURE 1 The level of glucose, triglyceride and total cholesterol is obviously higher in the bone marrow of JAK2-mutant MPN patients

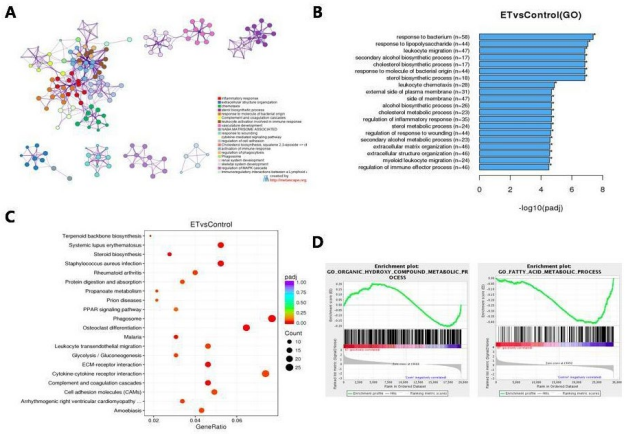

FIGURE 2 Mesenchymal stromal cells display metabolic alterations in JAK2-mutant MPNs

PB1602 | Dysfunctional RBM8A/Y14 Does Not Impair Megakaryopoiesis but Terminal Thrombopoiesis in Thrombocytopenia Absent Radii (TAR) Syndrome

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Background: Thrombocytopenia Absent Radii (TAR) syndrome is a rare genetic disorder comprising radial aplasia and thrombocytopenia. The low platelet count is attributed to a marked decrease of megakaryocytes (MKs). TAR syndrome is based on a microdeletion on Chr.1q21.1 including the RBM8A/Y14 gene and one of two known regulatory, non-coding SNPs in RBM8A. The molecular mechanism for thrombocytopenia is still not understood.

Aims: We used CD34-positive hematopoietic stem and progenitor cell (HSPC) and induced pluripotent cell (iPSC) models to resolve the implication of a partial Y14 loss-of-function mimicking TAR-syndrome in vitro.

Methods: Critical loss of Y14 in HSPCs was investigated by shRNA-mediated knockdown using lentiviral transduction. Decreased transcript levels were validated to an amount modelling TAR syndrome. MK maturation and formation of platelet-like particles (PLPs) was performed in liquid culture and methylcellulose. Moreover, an iPSC line of a TAR syndrome patient was used in combination with an adapted iPSC monolayer protocol producing MKs under specific media conditions. To investigate context-dependent functional relevance of Y14, apoptosis was studied using K562 cells bearing an RBM8A shRNA cassette and treated with BCL-X inhibitors.

Results: Unexpectedly, megakaryopoiesis was unaffected after RBM8A/Y14 knockdown in both HSPCs and TAR-patient-derived

iPSCs. Mature MK fractions were comparable to vector and healthy controls, respectively, by means of CD41a/CD42a expression and proplatelet formation. However, PLP generation after RBM8A/Y14 knockdown was significantly decreased for some donors, characterizing the heterogeneous phenotype of TAR syndrome. We hypothesize that BCL-X splicing mediated by Y14 is the functional cause of thrombocytopenia in TAR patients. For this, we combined RBM8A/Y14 knockdown with BCL-X inhibitor treatment and could indeed observe a direct correlation indicated by enhanced apoptosis capacity and decreased expression.

Conclusions: Thrombocytopenia in TAR syndrome cannot be solely explained by a hypomegakaryocytic condition but rather defective platelet budding during terminal thrombopoiesis, caused by misbalance of context-dependent BCL-X splicing.

PB1603 | Essential Thrombocythemia (ET): A Study of Clinical Features and Treatment Outcome in Pediatric Patients at King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

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Background: Essential thrombocythemia is one of the 'classic' Philadelphia chromosome negative myeloproliferative neoplasm characterized by sustained thrombocytosis, increased megakaryopoiesis and high risk of vascular complications. ET is very rare in childhood. The annual incidence is approximately 1 per 10,000,000 in children less than 14 years. The genetic landscape and clonal features in childhood ET has not been well defined. There is no evidence-based guidance on the diagnosis of childhood ET.

Aims: Characterize the different mutation profiles and to evaluate the treatment modalities used and the potential long-term outcome.

Methods: A retrospective case study was conducted at KFSHRC. Medical records (28) pediatric patients (age ≤ 14 years at diagnosis) with ET from 1987 to 2017, were reviewed and evaluated.

Results: More than half of the patients were found to have positive history of parental consanguinity (57.1%) whereas positive family history was documented for more than a quarter of our patients (28.6%). *JAK2* mutation was positive in 2 of 26 patients (7.7%). Treatment included Low Dose Aspirin (LDA) in 7 patients (50.0%), combination of LDA with Hydroxyurea in 3 patients (21.4%), Hydroxyurea in 2 patients (14.3%), combination of platelets pheresis with LDA and Anagrelide in 1 patient each (7.1%). During the treatment, 2 patients experienced stroke (7.1%),
1 patient developed Budd-chiari syndrome (3.6%) and 1 patient developed azoospermia (3.6%).

Conclusions: The incidence of ET in children is extremely low in Saudi Arabia. Notably, most of the children with ET were asymptomatic and thrombocytosis was often discovered incidentally. *JAK2* mutation has no known impact on the prognosis or on the outcome of the disease in the pediatric age group which in contrast to the adult ET. This cohort of patients must be considered at high risk for complications particularly during acute precipitating infectious episode. The potential complications and clinical course of pediatric ET are unpredictable.

PB1605 | Autoantibody Mediated Changes in Megakaryocytes Glycan Pattern: Potential Impact on Thrombopoiesis in Immune Thrombocytopenia

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Background: Patients with immune thrombocytopenia (ITP) are characterized by low platelet (PLT) count caused by impaired thrombopoiesis and increased PLT destruction. Desialylation, the cleavage of sialic acid on PLTs' glycoproteins, has been suggested to contribute to the enhanced PLT destruction in ITP.

Aims: To investigate the impact of desialylating ITP-autoantibodies (AAbs) on megakaryocytes' (MKs) differentiation and PLT production.

Methods: The sialylation status of CD34-derived MKs after incubation with sera from ITP patients or healthy donors was analysed by flow cytometry. Maturation status and pro-platelet generation were investigated via flow cytometry and microscopy. The MK's adhesion ability on different surface coatings was analysed in vitro.

Results: ITP-AAbs sera induced significantly higher desialylation on the MKs' surface compared to control sera (mean fold-increase (FI) of ß-galactose: 2.18, range: 1.03-3.66, p=0.012; and FI of N-acetylglucosamine: 1.25, range: 1.01-1.7, p=0.027). Although no abnormality in MKs morphology or vitality was observed, a significant decrease in the pro-platelets formation ability of MKs was found in the presence of desialylating-AAbs compared to control (% of pro-platelet forming MKs mean±standard error mean [SEM]: 42±9% vs. 100±0, p=0.004). The pro-platelet generation ability of the MKs was rescued in the presence of desialylating-AAbs preincubated with a sialidase inhibitor compared to buffer (% pro-platelet forming MKs mean±SEM: 42±12% vs. 90±9%, p=0.033). Interestingly, MKs incubated with desialylating-AAbs showed a significant impairment in the adhesion to fibrinogen and von-Willebrand factor compared to non desialylating-AAbs (% adherent MKs/field mean±SEM: 58±8% vs. 88±8%, p=0.049 and 47±4% vs. 79±8%, p=0.023, respectively).

Conclusions: Our findings suggest that AAbs from ITP patients are able to induce cleavage of sialic acid on the MKs' surface causing a reduction of their ability to adhere to different extracellular matrix proteins. Furthermore, we demonstrate that ITP AAbs-mediated desialylation interferes with pro-platelet formation from MKs which is a key step in thrombopoiesis.

PB1606 | Megakaryocyte Mitochondrial Dynamics and Oxidative Metabolism Are Essential to Initiate Platelet Biogenesis

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Background: Increased platelet production is known to occur upon oxygen concentration rise. In several cell types a relation between reactive oxygen species (ROS) and mitochondrial dynamics exists but nothing is known about thrombopoiesis initiation.

Aims: We addressed the role of ROS and mitochondrial (mt) dynamics in the initiation of thrombopoiesis from human mature MK.

Methods: Primary human cord blood CD34+ cells were differentiated for 12-13 days to generate mature MK. These cells were treated for 5hrs with various pro- and anti-oxydants : N-acetylcysteine (NAC), MitoTEMPO, nicotinamide (NAM),deacetylation-null SIRT3 (DN-SIRT3) mutant. ROS levels were quantified in CD42b+ MK using DHE or MitoSOX probes and flow cytometry; MK morphology and mitochondrial dynamics were monitored using phase or immunofluorescent microscopy and MitoSOX, MitoTracker or Drp1 probes.

Results: The anti-oxidant NAC decreased by 1.6-fold, whereas the pro-oxidant NAM enhanced by 1.5-fold platelet biogenesis from MK. Quenching mtROS by MitoTEMPO decreased by 2-fold the % proplatelet-forming MK, whereas enhancing mtROS through DN-SIRT3, increased them by 2-fold. We delineated three stages of thrombopoiesis from mature MK according to roundness index and identified an intermediate stage with small cell deformations assessing thrombopoiesis onset. Analysis of ROS levels showed that thrombopoiesis onset correlated with 1.7-fold increase of mtROS levels and a 2-fold decrease in mitochondrial area in intermediate MK. A higher mitochondrial recruitment of fission activator Drp1 was also identified before detectable thrombopoiesis initiation. Blocking mitochondrial fission by Drp1 self-assembly inhibition through Mdivi, inhibited thrombopoiesis and lowered mtROS, whereas quenching mtROS inhibited mitochondrial recruitment of active Drp1 in round MK.

Conclusions: These observations indicate a role of mtROS and mitochondrial dynamics in platelet biogenesis onset. They provide evidence for a reciprocal relationship between mtROS and mitochondrial fission in MK that contributes to initiate platelet biogenesis.

PB1607 | The Ablation of Collagen VI Leads to the Release of Platelets with Altered Functions

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Background: Hemostatic abnormalities and impaired platelet function have been described in patients affected by connective tissue disorders, however the mechanisms underlying platelet defects have never been investigated. Megakaryocytes, the platelet progenitors, express different extracellular matrix proteins. Mutations of genes encoding for the Collagen VI chains are responsible for a broad spectrum of diseases in humans, including Bethlem myopathy and Ullrich Congenital Muscular Dystrophy (UCMD).

Aims: We observed a mild bleeding tendency in Bethlem myopathy and UCMD patients and investigated the defects in platelet functionality whose mechanisms are unknown.

Methods: We exploited a Collagen VI-null mouse model (*Col6a1*$^{-/-}$) by performing megakaryocyte culture and radiation chimeras using bone marrow Lineage negative cell transplantations. Further, we performed platelet function tests and megakaryocyte culture from Bethlem myopathy and UCMD patient samples.

Results: We found that megakaryocytes express Collagen VI and that platelets produced by *Col6a1*$^{-/-}$ megakaryocytes display significantly increased susceptibility to activation and intracellular calcium signaling. Consistently, *Col6a1*$^{-/-}$megakaryocytes and platelets showed increased expression of Stromal Interaction Molecule 1 (STIM1) and ORAI1, the components of Store-Operated Calcium Entry (SOCE), and activation of the mammalian Target Of Rapamycin (mTOR) signaling pathway. Rapamycin rescued STIM1 and ORAI1 expression, calcium flows and platelet susceptibility to activation in mice *in vivo*. We demonstrated that these defects are cell autonomous as transplantation of Lineage negative cells from *Col6a1*$^{-/-}$mice in wild type lethally irradiated mice showed the same alteration in SOCE and platelet activation as compared to *Col6a1*$^{-/-}$mice. Importantly, platelets of patients affected by Bethlem myopathy and UCMD displayed increased expression of STIM1 and ORAI1 and were more prone to activation.

Conclusions: These data indicate that the absence of Collagen VI in megakaryocytes leads to the production of platelets with altered function. Platelet function tests should be performed in the management of patients affected by Collagen VI-related diseases.

PB1608 | Generation of Megakaryocytes from Human Bone Marrow Aspirates as a Simple Diagnostic and Research Tool: Assay Development and Validation

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Background: Defects in megakaryopoiesis and platelet production are common causes of inherited thrombocytopenias in children. Megakaryocytes (MKs) are relatively rare in bone marrow comprising 0.5 to 5% of the hematopoietic cells, which makes direct isolation impractical. The published methods of human MK production are complicated and require large blood volumes; thus adapted methodology is needed to allow efficient research on primary MKs physiology in humans.

Aims: To develop a method to differentiate MKs from a low volume of bone marrow aspirates obtained routinely for diagnostic procedures.

Methods: small volume of bone marrow (0.5-1 mL) was collected from pediatric patients in clinical remission from acute lymphoid leukemia without any treatment (n=5) and undergoing treatment with cytostatic agents (n=5). Mononuclear cells were isolated by Ficoll-Paque density gradient centrifugation. The mononuclear band was seeded in media supplemented with cytokines and allowed to differentiate into MKs for 14 days. Flow cytometry analyses were performed to estimate CD41+ and CD42b+ percentage and analyze the ploidy of CD41+ cells. Immunofluorescent confocal microscopy and live-cell imaging were performed to visualize differentiated MKs and proplatelet formation.

Results: Extensive proliferation was observed in the first 3-4 days, with an increase in CD34+ event count from 5-7% to 13-15% on average. Subsequently, an increase of large CD41+CD42b+ events to 30-35% of the live cell population was observed. Half of the analyzed CD41+ events had >4N DNA content. Interestingly, samples from treated patients had no significant differences in MK differentiation. One-step BSA gradient centrifugation on day 14 enriched CD41+ cells up to 70%. Immunofluorescent microscopy revealed relatively large CD41+CD62p+ cells with multi-lobular nuclei. Overnight live-cell imaging showed 3-5% MKs undergoing proplatelet formation.

Conclusions: Our work suggests using a low volume of bone marrow aspirates to generate fully functional MKs is a viable option for studying human MK production ex vivo and in vitro.

PB1609 | A Close Relationship between Adipocytes and Megakaryocytes

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Background: Megakaryocytes (MKs) come from a complex hematopoietic progenitor maturation process within the bone marrow (BM) that give rise to *de novo* circulating platelets. BM microenvironment contains a large number of adipocytes which medullar role remains ill-defined. It has been shown that either bone marrow adipocytes in a steady state or under high-fat-diet -induced obesity that increases medullar adiposity content have an impact on general hematopoiesis, lymphopoiesis and myelopoiesis. This make the adipocyte an influent member of the BM niche whereas for a long time adipocytes have been considered as passive BM "space fillers".

Aims: Considering the critical influence of BM niche for a normal hematopoiesis and the susceptibility of BM microenvironment to obesity, we addressed the question whether adipocytes and increased medullar adiposity could influence medullar megakaryopoiesis.

Methods: To that aim, increased medullar adiposity linked to obesity was induced in mouse by a 12 weeks high-fat-diet. In parallel, we set up an *in vitro* co-culture assay where freshly isolated hematopoietic progenitors from mouse BM directed towards megakaryocytic maturation by thrombopoietin were co-cultivated with adipocytes without any direct contact.

Results: We showed for the first time that an increased medullar adiposity *in vivo* caused by high-fat-diet was associated to an enhanced MK nuclear and membrane maturation along with a faster proplatelet formation. *In vitro* co-culture experiments allowed to demonstrate that, in one hand, adipocytes directly support MK maturation by enhancing polyploidization, amplifying demarcation membrane system and accelerating proplatelet formation and that, in another hand, MKs are responsible for adipocyte delipidation and dedifferentiation. Interestingly, we further showed the existence of an unsuspected crosstalk between adipocytes and MKs through a direct adipocyte fatty acid transfer dedicated to hematopoietic progenitors to reinforce MK maturation.

Conclusions: Thus, these findings unveiled a hitherto unrecognized influence of adiposity on MK homeostasis based on a dialogue between adipocytes and MKs.

PB1610 | Thrombopoiesis in Bone Marrow Culture in Norm and in Some Pathological States

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Background: Platelets play an important role not only in hemostasis and thrombosis, but in many physiological or pathological processes including inflammation, immune response and fibrotic tissue formation. Therefore, investigation of thrombopoiesis is remaining to be one of the important problems in modern medicine. The source of thrombocytopoesis are matured megakaryocytes.

Aims: The aim of our study was investigation of megakaryocytes and observation of thrombocytopoesis in healthy persons, in patients with immune thrombocytopenia, acute myeloid leukemia, that sometimes is accompanied by thrombocytopenia, acute megacaryoblastic leukemia and primary myelophibrosis accompanying with prominent fibrosis.

Methods: For this aim we investigated bone marrow cultures of hematologically heathy donors and patients with abovementioned diseases. Thrombocytopoesis was observed in 3-36 - day bone marrow cell cultures. This culture method worked out by Shvelidze T. (1998, 2005) supports hemopoietic cells to maintain their proliferative activity and function.

Results: In patients with immune thrombocytopenia normal megakaryocytes containing mainly 6-7 fragmented nucleus with normal or increased platelet release were observed, in patients with acute myeloid leukemia megakaryocyte with endophagocytosis of myeloid blast cells was mentioned, in cases of primary myelofibrosis megakaryocytes contained 2-3 fragmented nucleus and non-active thrombocytopoesis was revealed. In the cases of acute megakaryoblastic leukemia abundance of magacaryoblasts, promegakaryocytes and misshaped megakaryocytes producing immature platelets was observed.

Conclusions: Results of our study shows that bone marrow cultures can be successfully used for the estimation of thrombocytopoiesis in norm and different pathological states. Besides thrombcytopoiesis following studies can be held to investigate platelet growth factors and other cytokines in vitro.

PB1611 | Short-term Application of Caffeic Acid Specifically Promotes Expansion of Hematopoietic Stem Cells

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Background: Caffeic acid (CFA) is a commonly used medicine in China for treatment of various causes of thrombocytopenia and leukocytopenia, but the mechanism is unclear.

Aims: To investigate the effect of CFA on umbilical cord blood hematopoietic stem cells (UCB-HSCs) *in vitro*, and explore the effect of CFA on cell expansion and differentiation in various tissues *in vivo*.

Methods: $CD34^+$ UCB-HSCs were cultured with or without CFA for 6 days, total cell number and proportion of $CD34^+$ cells were detected. Differentiation ability was analyzed by colony formation assay and megakaryocytes inducing assay. Xenotransplantation of CFA-treated HSC to detect Hematopoietic reconstitution capacity. Additionally, to determine BrdU uptake rate in bone marrow and other tissues, CD-1 mice were administered with CFA or vehicle for 14 days and BrdU injection started from day 7. Finally, mRNA and protein expression levels of c-fos, cyclinD1 and CKD4 were assessed.

Results: In HSC expansion tests, after 6-day culture of CD34+ cells, the total number of cells in CFA group (8.45±4.89*105) was significantly higher than that in control group (6.23±4.24*105; P=0.006). In HSC colony formation assay and megakaryocytes inducing assay, there were no significant difference between CFA groups and control. *In vivo*, the uptake capacity of BrdU by bone marrow mononuclear cells but not any other tissue in CFA group was significantly higher than that in control group, which indicating the tissue specificity of CFA. Elevated mRNA and protein expression level of c-fos, c-jun, cyclinD1 and CKD4 were detected in 15 to 30 minutes after cultured with 10μg/mL CFA in CD34+ cells.

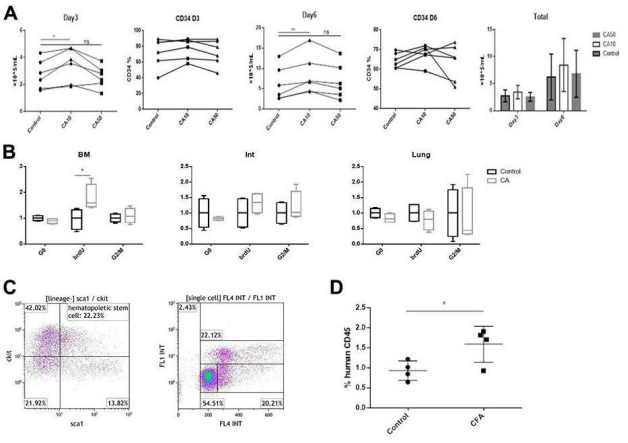

FIGURE 1 A.HSC expansion test B.Uptake rate of Brdu by mouse tissues C.mice HSCs and bone marrow Brdu uptake rate D.CD45 cells in mice after transplantation

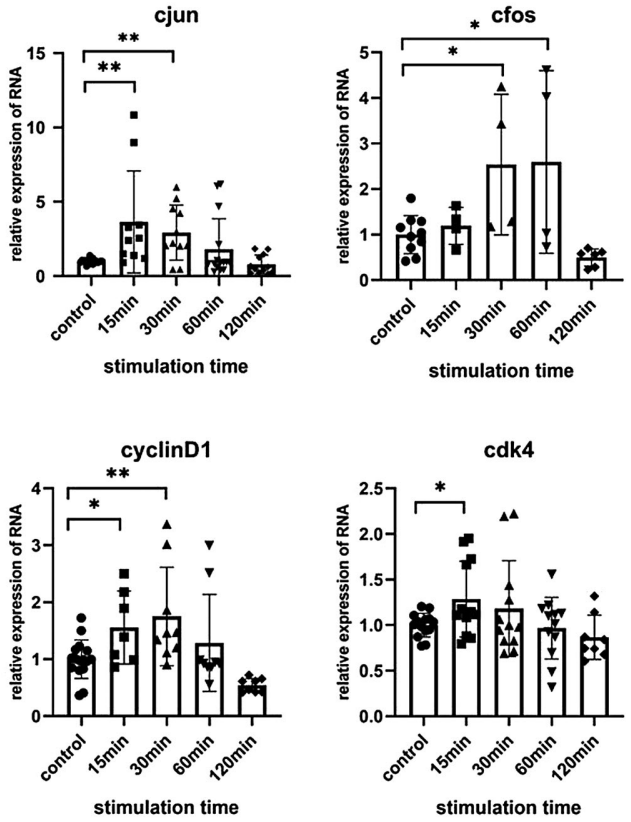

FIGURE 2 mRNA expression level of c-fos, cyclinD1 and CKD4 in CD34+ cells cultured with 10μg/mL CFA

Conclusions: In conclusion, CFA can promote the proliferation of bone marrow and umbilical cord HSCs by stimulating expression of cfos and speeding up cell cycle process through cyclinD1 and CKD4, which further revealed the pharmacological effects of CFA.

PB1613 | TREM-like Transcript-1 Is a Positive Regulator of Platelet Production and Thrombosis

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Background: TREM-like transcript-1 (TLT-1) is an immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor that is highly and exclusively expressed in the megakaryocyte (MK) lineage. TLT-1 is localized in α-granules, and rapidly translocates to the plasma membrane upon activation. Unlike other ITIM-containing receptors, TLT-1 acts as a positive regulator of platelet function and protects against inflammation-mediated hemorrhaging. However, its functional roles in thrombopoiesis and thrombosis remain undefined.

Aims: To determine the physiological function of TLT-1 in platelet production and function in mice, and the molecular mechanism underlying how it regulates these processes.

Methods: MK morphology, proplatelet formation and platelet function were analyzed in TLT-1 knockout (KO) and control mice by biochemical and microscopic assays.

Results: TLT-1 KO mice have a 20% reduction in platelet count and normal platelet size, and reduced proplatelet formation in vitro, despite normal megakaryopoiesis. Platelets from TLT-1-deficient mice expressed normal receptor levels, but exhibited reduced aggregation, secretion and thrombus formation to collagen under flow in vitro. Thrombus formation and stability was also reduced following laser-induced arterial injury in TLT-1 KO mice. Mechanistically, we demonstrate that TLT-1 is marginally tyrosine phosphorylated and associated with the SH2 domain-containing tyrosine phosphatases Shp1 and Shp2 in resting platelets, which increased in response to collagen in suspension. TLT-1 phosphorylation and Shp1, Shp2 interactions were also increased in fibrinogen-adhered platelets, and significantly reduced in thrombin-stimulated platelets pretreated with the alphaIIbbeta3 antagonist lotrafiban. Residual TLT-1 phosphorylation and bound Shp1 and Shp2 was maintained observed in the presence of lotrafiban, suggesting this is a direct consequence of fibrinogen binding to TLT-1.

Conclusions: Findings from this study establish that TLT-1 is a positive regulator of platelet function, proplatelet formation and thrombus formation in mice, and that is signals via Shp1 and Shp2 in platelets in response to collagen and fibrinogen.

PB1614 | Accelerated Death of Wiskott-Aldrich Syndrome Megakaryocytes

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Background: Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder affecting only hematopoietic cells and caused by mutation in *WAS* gene. WAS has wide clinical phenotype range with varying degree of such symptoms as microthrombocytopenia, eczema, combined immunodeficiency, autoimmunity and malignancies. Recent studies reported accelerated death of WAS platelets, T and B lymphocytes but there are few data for megakaryocyte (MK) status in WAS.

Aims: To study WAS MK morphology and viability before or during thrombopoietin receptor agonist romiplostim treatment.

Methods: All procedures were approved by Ethical Committee, donors and patients gave their written consent. Bone marrow aspirate was collected into EDTA-containing tubes. The mononuclear cell fraction was purified using Histopaque 1077 (Sigma), and placed on

anti-CD61 antibody or monafram covered microarray for MK attachment. Confocal microscopy was employed for viability evaluation by phosphatidylserine (PS) externalization and/or mitochondrial membrane potential. Maturation stage and sizes of MK were determined by May-Grünwald-Giemsa staining and light microscopy.

Results: We examined samples of 14 WAS patients and 7 healthy donors. The fraction of dead MK with exposed PS varied in WAS patients from 0 to 83% (25±25% average±SD) and in healthy donors from 0 to 11% (2±4%). The PS+ MK fraction correlated with the disease severity Zhu score (Pearson C=0.73, p< 0.01). MK with exposed PS were with collapsed mitochondria. Analysis of MK size and maturation stages distribution did not provide significant differences between healthy and WAS MK. Romiplostim treatment increased percentage of megakaryoblasts from 20±19% before treatment to 48±20% during treatment and decreased percentage of MK on maturation stage 3 from 36±22% before treatment to 18±16% during treatment. The increase in micromegakaryocyte fraction was only observed when patients had a mutation in the WH1 domain of WAS protein.

Conclusions: WAS megakaryocytes have increased tendency for programmed cell death that could contribute to thrombocytopenia.

PB1615 | Impact of Irradiation on the Bone Marrow Matrix and Megakaryopoiesis

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Background: Mature megakaryocytes (MKs) reside juxtaposed to bone marrow (BM) vessels where they protrude proplatelets across the endothelium to release platelets into the blood stream. MKs are exposed to distinct extracellular matrix (ECM) proteins (e.g. collagens and laminins) that prohibit premature platelet release into the BM cavity. After total body irradiation (TBI) ECM proteins become degraded by members of matrix metalloproteinases (MMPs), especially MMP9, preceding a reduction in MKs and finally leading to thrombocytopenia. The underlying mechanisms of radiation-induced thrombocytopenia are still poorly understood.

Aims: This study aims to analyze the effect of irradiation on alterations of the subendothelial vascular niche and the role of MMPs in BM remodeling.

Methods: Cryosections of mouse bones were subjected to confocal laser scanning immunofluorescence microscopy (IFM) to study expression and localization of sinusoidal vessels, MKs, and ECM proteins. MMP expression was assessed by immunoblot analysis, their activity by gelatin-zymography. We established an *in situ* zymography assay for unfixed, calcified femur sections to localize activity. Studies were performed using MMP9-deficient mice and littermate controls.

Results: MMP9 is present at low levels in BM of non-irradiated mice. MKs become strongly positive on day 3 after 5 Gy irradiation, indicating increased expression, which was confirmed by immunoblot analyses of whole BM lysates. Gelatin-zymography and *in situ* zymography analyses revealed a 45% increase of MMP activity on day 1 after irradiation, which was not seen in MMP9-deficient mice. A cleaved collagen peptide was detected in BM by immunoblot and IFM, as a result of collagenolysis. Despite extensive remodeling of the subendothelial niche, MK counts, MK area and platelet counts are stable during the first four days after sublethal irradiation.

Conclusions: Expression and proteolytic activity of MMP9 in BM MKs is markedly increased after irradiation while collagen is degraded. This hints towards an involvement of MMP9 in the BM matrix remodeling after TBI.

PB1616 | Hyperactive SRC Kinase Results in Inherited Thrombocytopenia: Transcriptomics and Proteomics to Delineate the Defect in Megakaryopoiesis

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Background: SRC is a non-receptor tyrosine kinase protein that phosphorylates specific tyrosine residues in other proteins upon activation. A germline heterozygous gain-of-function E527K variant in SRC was previously found to cause thrombocytopenia and bleeding in patients from two unrelated families.[1,2] These patients also presented syndromic features such as myelofibrosis, osteoporosis, premature edentulism, behaviour problems and mild facial dysmorphia. Cellular studies showed that SRC-E527K resulted in hyperactive SRC signaling with increased overall tyrosine phosphorylation patterns. Platelets from patients are larger with a paucity of alpha-granules. The expression of lentiviral SRC-E527K in healthy CD34+ hematopoietic stem cells (HSC) was shown to decrease proplatelet formation during megakaryopoeisis in contrast to SRC-WT.

Aims: Our aim is to study the effect of SRC-E527K during megakaryopoiesis as it remains unknown how this kinase influences megakaryocyte (MK) differentiation.

Methods: SRC-WT and SRC-E527K lentiviral transduced MK were subjected to RNAseq and proteomics.

Results: RNAseq of day 12 differentiated MK derived from healthy CD34+ HSC transduced with SRC-WT versus SRC-E527K showed 691 significant differentially expressed genes (44% upregulated and 56% downregulated). Proteomic analysis of these MK cultures shows 141 significant differentially expressed proteins (29,8% upregulated and 70,2% downregulated) and alterations in 45 tyrosine phosphorylation sites were detected in 37 proteins. A combined analysis identified 20 upregulated genes/proteins versus 24 downregulated genes/proteins downregulated in SRC-E527K MK. A pathway analysis of these hits revealed a major role of interferon-stimulated

proteins in hyperactive SRC signaling during megakaryopoiesis (p=2.35E-14).

Conclusions: The interferon pathway and other interesting candidate proteins will be further validated in MK generated from control, patient and patient-corrected inducible pluripotent stem cell models that have been generated.

Turro E et al. (2016) Science Translational Medicine.
[2]De Kock L et al. (2019) Platelets.

PB1617 | Role of Platelet Production/Destruction Imbalance on the Interindividual Variability in Aspirin Response in Diabetic and Non-diabetic Patients

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Background: An interindividual variability in response to aspirin (ASA) has been recognized. A faster recovery of platelet cyclooxygenase (COX)-1 activity during the 24-h dosing interval, possibly related to enhanced platelet turnover, may explain incomplete thromboxane (TX) inhibition in a fraction of ASA-treated subjects.

Aims: To characterize the determinants of less-than expected low-dose ASA-response in patients with or without type 2 diabetes mellitus (T2DM).

Methods: One-hundred patients with and 100 without T2DM in chronic treatment with low dose ASA (100 mg/day) for cardiovascular prevention were recruited. Blood sampling was performed at 10 and 24 hours after a witnessed ASA administration. Serum TXB_2 ($sTXB_2$) was measured at each time point, and the slope of $sTXB_2$ during the 10-24 h dosing interval was calculated to characterize the kinetics of platelet COX-1 recovery and to stratify patients in $sTXB_2$ tertiles. Plasma glycocalicin (GC) and glycocalicin index (GCI), indexes of platelet destruction, and plasma thrombopoietin (TPO), index of thrombopoiesis, were measured in patients in the first and third tertiles.

Results: Platelet count was significantly increased in the third *vs* first tertile only in T2DM patients (p=0.0310) (Figure 1). In the whole group and both in subjects with and without T2DM, GC was significantly lower (p< 0.0001, p< 0.0001 and p< 0.0004, respectively), and in all and T2DM TPO was significantly higher (p=0.004 and p=0.02 respectively) in the third *vs* first tertile (Figure 2).

GC correlated inversely with TPO (p=0.013) and $sTXB_2$ slope (p< 0.000) in all patients; GCI correlated inversely with TPO (p=0.003), $sTXB_2$ slope (p< 0.000), PLT count (p< 0.000), and PLT mass (p< 0.000).

Conclusions: We showed for the first time that a suboptimal response to ASA, as reflected by accelerated kinetics of COX-1 recovery, is associated with reduced platelet destruction and enhanced platelet production, the latter at least in T2DM patients.

PB1618 | Rap1a/b Isoforms Differentially Regulate Megakaryocyte Biology

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Background: Thrombocytopenia, or low circulating platelet count, is often caused by a defect in megakaryocyte (MK) development. MKs reside primarily in the bone marrow, where they develop from undifferentiated multipotent cells into highly specialized, polyploidy cells that undergo the process of proplatelet formation (PPF). It is currently not clear if and how Rap1, a member of the Ras small GTPase superfamily, contributes to platelet generation.

Aims: We hypothesized that the two Rap1 isoforms, Rap1a and Rap1b, are critical in MK development and/or PPF.

Methods: We studied MK development in primary murine MKs isolated from conditional knockout mice lacking Rap1a ($Rap1a^{mKO}$), Rap1b ($Rap1b^{mKO}$), or both isoforms ($Rap1a,b^{mKO}$) in MKs and platelets. Flow cytometry, immunohistochemistry and 2-photon/confocal/electron microscopy imaging were used to analyze MKs in vivo, ex vivo, in situ, and in vitro.

Results: $Rap1a^{mKO}$ mice exhibited a normal peripheral platelet count (PPC) but significantly increased MK numbers in the bone marrow and spleen. Compared to controls, MKs from $Rap1a^{mKO}$ mice were smaller and less differentiated (lower ploidy). $Rap1b^{mKO}$ mice exhibited a mild macrothrombocytopenia but normal MK numbers and ploidy. However, PPF in $Rap1b^{mKO}$ MKs was impaired, both in vivo and ex vivo (few but very large proplatelets). $Rap1a,b^{mKO}$ mice exhibited a marked macrothrombocytopenia, impaired MK differentiation and a dramatic defect in PPF. $Rap1b^{mKO}$ and $Rap1a,b^{mKO}$ MKs exhibited defects in demarcation membrane system (DMS) biogenesis and the association of the DMS with F-actin in proplatelet extensions. Consistent with the abnormal DMS, LIM kinase and cofilin signaling was altered in $Rap1a,b^{mKO}$ mice, and LIM kinase inhibitor treatment normalized the platelet count in the mutant mice.

Conclusions: These results provide the first definitive proof for a critical role of Rap1 GTPases in MK biology. Future studies will have to show whether some inherited or acquired thrombocytopenias are caused, at least in part, by altered Rap1 signaling in MKs.

PB1620 | Ikaros/AhR Interaction Favors Expansion of Megakaryocytic Precursors with a High Potential to Produce Proplatelets

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Background: The aryl hydrocarbon receptor (AhR), a transcription factor better known for detoxification processes via the regulation of P450 cytochromes such as CYP1B1 (canonical pathway), also participates in several unrelated biological pathways. Indeed, we recently showed that an antagonist of AhR, stemregenin-1 (SR1), promoted the generation of a thrombocytogenic $CD34^{+}CD41^{low}$ megakaryocytic precursor capable to mature into MKs displaying a high potential to generate platelets. Remarkably, this effect was mimicked by the coculture of CD34+ progenitors with mesenchymal stromal cells (MSCs).

Aims: This study aimed to decipher the AhR-dependent molecular mechanisms associated to the development of these highly competent MK precursors.

Methods: $CD34^{+}$ hematopoietic progenitors were differentiated into MKs in presence of SR1 or MSCs. Using pharmacological inhibitors and shRNA inhibition techniques, we evaluated the involvement of AhR pathway constituents on the appearance of our population of interest (POI) now phenotypically defined as $CD34^{+}CD9^{-}CD41^{+}$.

Results: The negative regulation of the canonical CYP1B1 pathway (shRNA) did not impact the emergence of the POI and of platelet production. This result downplayed a role of the AhR-canonical pathway in POI emergence and suggested the involvement of an AhR-alternative pathway. A targeted transcriptomic analysis of the POI coupled to literature survey oriented us towards the involvement of Ikaros. In line with this hypothesis, the downregulation/inhibition of Ikaros (shRNA or lenalidomid) significantly reduced the emergence of SR1-induced POI. Interestingly, using a proximity ligation assay, we could demonstrate a physical interaction between AhR and Ikaros. This interaction appeared to also control the development of the POI differentiated in the presence of MSCs.

Conclusions: While the CYP1B1 canonical pathway of AhR has a weak impact on MK differentiation, we revealed a previously unknown AhR/Ikaros-dependent pathway required to prompt the expansion of the thrombocytogenic $CD34^{+}CD9^{-}CD41^{+}$ precursors. These findings open new perspectives to platelet production engineering.

PLATELET FUNCTION AND INTERACTIONS

PB1621 | Association of Platelet Derived - Microparticles and Endothelia Dysfunction in NVAF Patients Undertaking PCI

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Background: This study attempted to reveal platelet derived- microparticles (MPs) interact with endothelial cells in the circulation after platelet activation in patients with atrial fibrillation.

Aims: We hypothesized that the elevated MPs was closely related to the prognosis of NVAF patients.

Methods: Aenous blood samples were drawn from 33 NVAF patients and 30 sinus rhythm controls from October 2018 to September 2019. Activation and mitochondrial $\Delta\psi m$ depolarization of platelets were measured. Plasma levels of platelet and endothelial- derived MPs, von Willebrand factor (vWF) and endothelin (ET) were observed. The relationship between PMPs and prognosis of NVAF patients was assessed.

Results: Activation and higher mitochondrial $\Delta\psi m$ depolarization of platelets were found in NVAF patients preoperatively. PMPs, EMPs, vWF and ET increased in NVAF group prior to PCI when compared to patients prooperatively or controls. Patients with elevated PMPs had higher rates of recurrences compared with those who had lower PMPs. Levels of PMPs but not EMPs were positively correlated with CHADS2 score.

Conclusions: NVAF accompanied with platelet activation and endothelia dysfunction. Increased PMPs released from activated platelets may predict the recurrence rates of atrial fibrillation.

PB1622 | Thrombotic Disease Caused by Hypothermia-Induced Activation of the Splenic Platelet Pool in a Mouse Model

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Background: Hypothermia, which can be induced either by therapeutic processes or accidentally (i.e. an involuntary decrease in core body temperature to < 35°C), results in hemostatic disorders. However, it remains unclear whether hypothermia enhances or inhibits coagulation, especially in cases of severe hypothermia.

Aims: The present study evaluated the thrombocytic and hemostatic changes in hypothermic mice.

Methods: C57Bl/6 mice were placed at an ambient temperature of -20°C under general anesthesia. When the rectal temperature decreased to 15°C, 10 mice were immediately euthanized, while other

10 mice were rewarmed, kept in normal conditions for 24 hours, and then euthanized. These treatments were also performed in 20 mice that had undergone splenectomy.

Results: The hypothermic mice had adhesion of CD62P-positive platelets with high expression of von Willebrand factor (vWF) in their spleens, while the status of the peripheral platelets was unchanged. Furthermore, the plasma levels of platelet factor 4 (PF4) and pro-platelet basic protein (PPBP), which are biomarkers for platelet degranulation, were significantly higher in hypothermic mice than in control mice, indicating that hypothermia activated the platelets in the splenic pool. Thus, we analyzed these biomarkers in asplenic mice. There was no increase in either PF4 or PPBP in splenectomized hypothermic mice. Additionally, the plasma D-dimer level and microthrombosis were elevated in rewarmed mice, but not in asplenic rewarmed mice.

Conclusions: Our results indicate that hypothermia leads to platelet activation in the spleen via the upregulation of vWF, and this activation causes hypercoagulability after rewarming.

PB1624 | Kupffer Cells Clear Aged Platelets through Macrophage Galactose Lectin

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Background: Every day, human megakaryocytes produce billions of platelets that patrol the vasculature for several days. At the end of their lifespan, platelets get cleared in the liver through a process that is incompletely understood. Loss of sialic acid residues is thought to feature in the aging and clearance of platelets. Recent studies suggest that desialylation causes platelet clearance via the Ashwell-Morell receptor (AMR) on hepatocytes, however, this process has never been observed *in vivo*.

Aims: Therefore, our aim was to study the process of platelet clearance *in vivo* using intravital microscopy of the mouse liver.

Methods: We performed spinning-disk intravital confocal microscopy of the liver of anesthetized naive mice to observe platelet-Kupffer cell interactions in the steady state. Mice were treated mice with sialidase to induce *in vivo* desialylation of platelets or transfused with *ex vivo* desialylated platelets. We also performed flow cytometric and platelet analyses.

Results: We found that under basal conditions, platelets continuously touch down and let go on Kupffer cells. A small percentage of platelets, however, remained attached to the Kupffer cells and were ingested. Similarly, when we desialylated platelets *in vivo* or *ex vivo* we observed rapid accumulation almost exclusively on Kupffer cells but not on hepatocytes or endothelium. Combining AMR deficiency with blocking the macrophage galactose lectin (MGL) resulted in almost completely abolished adhesion of desialylated platelets to Kupffer cells and their subsequent uptake. This process was also relevant for the clearance of cold-stored platelets. Interrupting platelet clearance by depleting Kupffer cells led to the accumulation of aged platelets and increased bleeding.

Conclusions: Our data provides evidence that the MGL of Kupffer cells plays a significant role in the removal of desialylated platelets through a collaboration with the AMR thereby maintaining a healthy and functional platelet population.

PB1625 | Cold over Room Temperature Storage of Apheresis Platelets Better Preserves Hemostatic Function out to 21 Days

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Background: Current regulatory guidelines dictate that platelet products (PLT) be stored at 22°C (RT) for five days. The burden

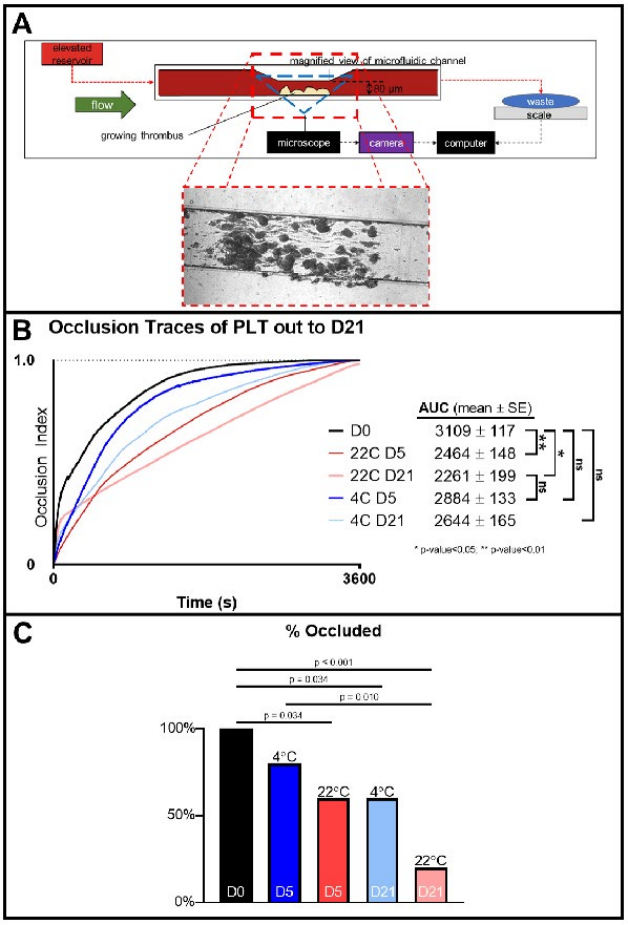

FIGURE 1 (A) Microfluidic assessment of PLT. (B)-(C) CS better preserved hemostatic function by rate and incidence of occlusion

imposed by this shelf-life results in roughly USD$90,000,000 in waste. Cold storage (CS) decreases the risk of bacterial contamination and reduces platelet metabolism, thus potentially prolonging shelf-life. However, the effect of CS duration on hemostatic function remains unclear.

Aims: The objective of this study was to determine the effect of CS out to 21 days on hemostatic function of PLT under flow.

Methods: Human apheresis PLTs (n=10) were collected in plasma at a licensed blood collection center. PLT were obtained post-collection and tested the same day (D0). PLT were split into equal volumes and stored agitated at RT or in the cold (4°C). PLT were also tested on days 5 and 21 (D5, D21) of storage.

PLT were perfused through a collagen-coated stenotic microfluidic chamber (mean shear rate 2100 1/s; Fig 1A) with a constant pressure head. Perfusion continued until thrombus filled the stenosis and complete cessation of flow was achieved. Phase-contrast images were collected in real time.

Results: By rate of occlusion, D0 PLT occluded the quickest (leftmost curve, Fig 1B). Analysis of variance (ANOVA) of the area under curve (AUC) was significant for temperature (p=0.025) but neither time nor donor. The rate of CS-PLT (D5 and D21) occlusion was not different than D0, while D5 and D21 RT-PLT were different (p=0.007 and p=0.011, respectively). By incidence of occlusion, D5 and D21 RT-PLT were again significantly different from D0 (p=0.034 and p=0< 0.001), while D5 CS-PLT were not (Fig 1C). By both rate of occlusion and incidence, D21 CS-PLTs were not different than D5 RT-PLT, the current regulatory standard.

Conclusions: CS of PLT may allow for extension of the shelf-life out to 21 days based on hemostatic function in a microfluidic flow chamber.

PB1626 | Video Rate, High Contrast Morphological Imaging of Blood Thrombus using Label - Free Coherent Optical Scattering and Interferometry (COSI) Microscopy

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Background: High resolution morphological imaging *ex-vivo* using electron and super-resolution microscopy demonstrated that surface- and shear-activated platelet aggregates display a distinctive morphology, adjacent to prothrombotic sites as compared to weakly activated platelets within a developing thrombus, but are devoid of any real time spatial temporal information.

Aims: Here we propose an integrated label-free microscopy platform "COSI", a rapid spatio-temporal and high contrast label-free imaging technique that provides full morphological characterisation of a developing thrombus under flow.

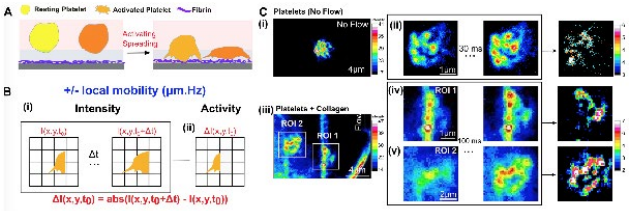

FIGURE 1 Backscattering signal from single platelet and platelet activity quantification

Methods: Thrombi were firstly formed by drawing PRP into a collagen-coated microfluidics channels (width*height*length: 400*100*1200 µm) with a syringe pump (7500s^{-1} shear rate for 5 minutes). Saline buffer is then used to mimic embolisation events (7500s^{-1} and 12500s^{-1} for 5 and 2 minutes).

The embolisation event is captured using COSI, which collects the backscattering signal and transmitted light (using interferometry) that raised from refractive index difference to achieve volumetric imaging and determination of activity level without fluorescence labelling.

Results: We demonstrate backscattering signal of single platelet by loading washed platelets onto fibrin coated coverslip (Fig 1A) and the subtraction method we used to define platelet activity (Fig 1B). Examples of high contrast imaging of platelet are shown (Fig 1C).

To quantify embolisation event, we use the volumetric difference from interferometry imaging (Fig 2B) and platelets activity at basal surface from back scattering signal (Fig 2C) to calculate volume map (Fig 2D) and averaged platelet activity map (Fig 2E). We also spatially map the volume and activity map in Fig 2F. The effect of proteolytic receptor shedding in thrombus stability and embolisation is investigated, by testing the roles of metalloproteinases and using a broad metalloproteinases inhibitor GM6001 (250µM) to pre-treated the PRP.

Conclusions: With COSI, we can achieve label-free imaging of volumetric information of the thrombus and platelet activity at basal surface with 150Hz acquisition rate, which provide a new perspective for interpreting prothrombotic and embolisation events.

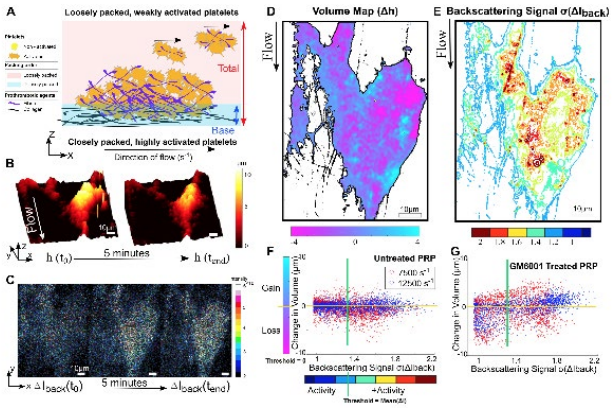

FIGURE 2 Volumetric imaging and platelet activity quantification of the thrombus during embolisation

PB1627 | Exercise Training Enhances Mitochondrial Bioenergetics of Platelets in Patients with Peripheral Arterial Disease

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Background: Exercise training (ET) may influence aerobic fitness and vascular thrombotic risk in patients with peripheral arterial disease (PAD). Mitochondrial functionalities in platelets involve the cellular bioenergetics and thrombogenesis.
Aims: This study aimed to elucidate the effect of ET on systemic aerobic capacity and platelet mitochondrial bioenergetics in patients with PAD.
Methods: Forty randomly selected patients with PAD engaged in general rehabilitation (GR) with ET (i.e., work-rate at ventilation threshold for 30 min/day, 3 days/week for 12 weeks) (GR+ET, n=20) or to a control group that only received GR course (n=20). Systemic aerobic capacity and platelet mitochondrial bioenergetics [oxidative phosphorylation (OXPHOS) and electron transport system (ETS)] were measured through automatic gas analysis and high-resolution respirometry, respectively.
Results: The GR+ET for 12 weeks significantly (i) increased VO_{2peak} and lowered V_E-VCO_2 slope, (ii) enhanced capacities of mitochondrial OXPHOS and ETS in platelets by activating FADH2 (Complex II)-dependent pathway, and (iii) heightened the Short Form-36 physical/mental component scores. However, no significant changes in exercise performance, platelet mitochondrial bioenergetics, inflammatory status, and health-related quality of life occurred following GR alone. Moreover, changes of VO_{2peak} level was positively correlated with changes of maximal OXPHOS and ETS capacities in platelets.
Conclusions: The GR with ET effectively increases the capacities of platelet mitochondrial bioenergetics by enhancing Complex II activity in the PAD patients. Moreover, the exercise regimen also enhanced aerobic fitness and depresses circulatory inflammatory status, consequently improving health-related quality of life in patients with PAD.

PB1628 | Evaluation of Platelet Count and Platelet Indices of Male *Cannabis Sativa* (Marijuana) Consumers in Calabar South, Cross River State, Nigeria

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Background: *Cannabis sativa* is one of man's oldest and most commonly used illegal drug and countries with the highest use among adults as of 2018 are Zambia, United States, Canada and Nigeria. It is widely consumed as marijuana (the dried leaves extract) inhaled and smoked.
Aims: This work brings cannabis to light, studying its reaction on "platelet indices" in the Calabar man.
Methods: 100 subjects made up of 50 voluntary marijuana male consumer and 50 non-consumers (control group), same age range group recruited from Calabar South L.G.A, Cross River State, for this study which lasted for a period of four months. Informed consent and a filled questionnaire were gotten from test and control subjects, along with ethical approval from CRS MOH. Blood sample in the concentration 1.5-2mg/ml of blood K2EDTA was used for complete blood count using an automated method (HB 7501 Haematology analyzer) for platelet count and their indices. SPSS version 20.0 was used in the analysis of the data and an error probability of ≤ 0.05 was considered significant.
Results: Mean age (23.00±0.45) shows to have no effect on the study. Moreover, there was a significant (P< 0.001) decrease in platelet count (PLT), plateletcrit (PCT) and platelet distribution width (PDW) (186.42X109/L, 0.172% and 15.9fL), as mean platelet volume (MPV) (8.9fL) showed slight increase (P< 0.05) in test group when compared to control group. There was a mild positive and negative significant relationship between duration of smoking versus PLT, PCT, MPV and PDW (r=0.111, r=0.112, r=0.224 and r=-0.172) respectively. The various marijuana types (weed, skunk, weed+skunk) smoked revealed an overall non-significant difference among groups.
Conclusions: Cannabis smoking alters platelet characteristics in blood and may consequently lead to certain disease conditions such as the dreadful myocardial infarction, thromboembolism, atherosclerosis and immune thrombocytopenic purpura among others.

TABLE 1 Comparison of mean age, platelet count and platelet indices of Marijuana smokers and control groups

Parameters	Marijuana Smokers n = 50	Non-Smokers (Control) n = 50	P value
Age (years)	23.66±3.305	23.00±3.130	0.308
PLT (X109/L)	186.42±48.843	252.60±33.111	0.000
PCT (%)	0.172±0.075	0.218±0.028	0.000
MPV (fL)	8.89±0.692	8.55±0.668	0.014
PDW (fL)	15.98±0.782	16.27±0.532	0.039

TABLE 2 Comparison of platelet count and platelet indices among the various types of Marijuana (weed, skunk and weed+skunk) consumed

Parameters	Weed (n =15)	Skunk (n = 9)	Weed+Skunk (n = 26)	P value
PLT (X109/L)	177.33±13.82	178.11±12.67	194.54±9.34	0.790
PCT (%)	0.159±0.017	0.163±0.012	0.183±0.018	0.727
MPV (fL)	8.667±0.154	9.167±0.135	8.938±0.155	0.187
PDW (fL)	16.14±0.18	15.98±0.23	15.90±0.17	0.598

PB1629 | Epinephrine Enhances Platelet Aggregation at the Expense of Procoagulant Activity

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Background: Platelet activation is characterized by shape change, granule secretion, activation of fibrinogen receptor (glycoprotein [GP] IIb/IIIa) sustaining platelet aggregation, and externalization of negatively charged aminophospholipids contributing to platelet procoagulant activity. Epinephrine alone is a weak platelet activator. However, it is able to potentiate platelet activation initiated by other agonists.

Aims: We were interested to investigate the role of epinephrine co-stimulation in the generation of subpopulations of activated platelets, especially its role for the formation of aggregating versus procoagulant platelets.

Methods: Human platelets were activated with convulxin (CVX, a specific agonist of the collagen receptor GPVI), thrombin (THR), epinephrine (EPI), and combination thereof. Platelet aggregation was assessed by light transmission aggregometry (LTA) and with PAC-1 binding by flow cytometry, recognizing activated GPIIb/IIIa. Procoagulant COAT platelets, induced by combined activation with CVX and THR, were visualized by flow cytometry as Annexin-V positive and PAC-1 negative platelets. Cytosolic calcium fluxes following platelet activation were monitored by flow cytometry using Fluo-3 indicator.

Results: EPI increased platelet aggregation induced by low doses of CVX alone, THR alone, and combined CVX+THR. On the other hand, EPI dose-dependently reduced the formation of procoagulant COAT platelets generated by combined CVX+THR activation. We observed a decrease (relative -10%) of Annexin-V positivity and an increase (relative +10%) binding of PAC-1 with the triple activation (CVX+THR+EPI) compared to the standard activation (CVX+THR). Interestingly, we observed a decreased calcium mobilization with the triple activation compared to CVX+THR alone, in line with the decreased procoagulant phenotype.

Conclusions: While CVX+THR induced the formation of procoagulant COAT platelets (known to mobilize high levels of cytoplasmic calcium, express negatively charged phospholipids at their surface, and lose aggregatory properties), the addition of EPI (triple stimulation) modulated platelet activation reducing cytosolic calcium mobilization, decreasing the procoagulant response, and enhancing platelet aggregation.

PB1630 | MiR-204-5p Regulates Platelet Adhesion to Fibrinogen

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Background: Circulating miRNA profile is a biomarker of platelet reactivity and cardiovascular outcome. Recent studies functionally validated miRNAs as regulators of platelet function using mice or human-derived cells, but the exact mechanism is largely unknown.

Aims: To investigate the impact of miR-204-5p on platelets, and to address the mechanistic issues mediating its functional effect using a model of human hematopoietic stem cells.

Methods: Human hematopoietic stem cells were differentiated into megakaryocytes, allowing transfection of miR-204-5p and recovery of subsequent platelet like structures (PLS). Surface receptors of PLS were characterized by flow cytometry. The impact of miR-204-5p overexpression on fibrinogen binding was assessed by measuring inactive and active form of GPIIbIIIa, PLS spreading on immobilized fibrinogen and PLS binding of soluble labeled fibrinogen.

Quantitative PCR and western blot were used to assess the impact of miR-204-5p on Cdc42 a target of miR-204-5p. The impact of the Cdc42 modulation was investigated using silencing strategy.

Results: Flow cytometry revealed that miR-204-5p was associated with an overexpression of the two subunits of the GPIIbIIIa at the surface of PLS. Moreover, miR-204-5p increased the expression of the activated form of GPIIbIIIa and binding of PLS to both immobilized and soluble fibrinogen.

MiR-204-5p transfection decreased both Cdc42 mRNA and protein level in human-derived cells. Silencing of Cdc42 induced similar results than miR-204-5p transfection of PLS.

Conclusions: We functionally validated miR-204-5p as a regulator of platelet reactivity that occurs through the regulation of the fibrinogen receptor expression and activation. These data support a potential role of miR-204-5p as a biomarker of platelet reactivity and cardiovascular outcome.

PB1633 | Platelet Aggregation is Suppressed in the Quinonoid Dihydropteridine Reductase Knockout Mice

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Background: *Quinonoid* dihydropteridine reductase (QDPR) functions in recycling pathway of tetrahydrobiopterin (BH4), an essential cofactor of catecholamines and serotonin
(5-hydroxytryptamine, 5-HT) synthesis. 5-HT is secreted during platelet activation and contributes to haemostasis.

Aims: The objective of this study is the evaluation of platelet function in QDPR gene knockout (*Qdpr*$^{-/-}$) mice.

Methods: Citrated blood was collected from wild type (Qdpr+/+) mice and Qdpr-/- mice: (OYC35, Lexicon Pharmaceuticals Inc.). For platelet rich plasma (PRP) preparation, the whole blood sample was centrifuged, and adjusted to 250,000/μL. Platelets aggregation was triggered with ADP (5~20 μM) or collagen (3~4 μg/mL), monitored for 10 minutes using light transmission method. Intraplatelet 5-HT level was determined by high-performance liquid chromatography (HPLC) with electrochemical detection.

Results: The area under the platelet aggregation curve (AUC) of the *Qdpr*$^{-/-}$ mice was significantly reduced when stimulated with ADP (*Qdpr*$^{+/+}$: 4,197±295, *Qdpr*$^{-/-}$: 2,452±266, $p < 0.05$ by Tukey-Kramer test) or with collagen (*Qdpr*$^{+/+}$: 6,061±600, *Qdpr*$^{-/-}$: 3,424±514, $p < 0.05$). The intraplatelet 5-HT content was significantly decreased in the *Qdpr*$^{-/-}$ mice (*Qdpr*$^{+/+}$: 48.1±4.0 pmol/10^6 platelets, *Qdpr*$^{-/-}$: 22.1±2.8 pmol/10^6 platelets, $p < 0.01$). There were no significant differences in the blood cell counts between *Qdpr*$^{+/+}$ mice.

Conclusions: These results suggest that the secondary platelet aggregation via 5-HT is suppressed in *Qdpr*$^{-/-}$ mice.

PB1634 | Platelets form Fibrillar Adhesions: Implications for Force-Regulated Thrombus Remodeling

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Background: Platelets are crucial for thrombus formation and clot contraction upon vascular injury, but do they directly participate in thrombus remodeling? During early stages of wound healing, invading fibroblasts drive the formation of a provisional fibronectin matrix. Whether platelets might assemble fibronectin fibrils already during thrombus formation is unclear.

Aims: To characterize the mechanism underlying fibronectin fibrillogenesis by platelets

Methods: Blood was obtained from healthy consenting volunteers in accordance with relevant ethics and data protection guidelines. Washed platelets were seeded on protein-coated micropost arrays to measure single cell traction forces. Platelets were seeded on protein-coated coverslips in the presence of fluorescently labeled fibronectin and the formation of fibrils was assessed by 3D super-resolution microscopy. Different adhesion protein coatings and specific integrin or myosin IIa inhibitors were used to disentangle the contributions by different adhesion receptors or by contractility.

Results: Platelets assembled fibronectin fibrils using αIIbβ3 rather than α5β1 integrins, in contrast to fibroblasts. Fibrillar adhesion formation depended distinctly on matrix identity and platelet contractility. Platelets in contact with thrombus proteins (fibronectin, fibrin) were highly contractile and pulled fibronectin fibrils along their apical membrane, whereas platelets on basement membrane proteins (collagen IV, laminin) were less contractile and formed less stretched planar meshworks beneath themselves (Figure 1). As probed by vinculin-decorated talin unfolding, platelets on fibronectin generated similar forces in apical fibrillar adhesions as fibroblasts.

Conclusions: Our results reveal major novel mechanobiological mechanisms by which platelets develop different levels of contractility and orchestrate the formation of anisotropic 3D versus 2D fibronectin network architectures. We suggest that these processes contribute to thrombus heterogeneity and potentially pave the way for cell infiltration.

PB1635 | An Elevated Platelet Count Increases the Rate of Prothrombin Activation, but Not the Total Amount of Thrombin Formed

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Background: Platelets play an important role in coagulation and are known to be crucial in the development of arterial thrombosis. An algorithm based on thrombin generation (TG) was created to study thrombin dynamics more in detail. This computational approach takes into account both pro- and anticoagulant mechanisms during TG, as well as plasma levels of fibrinogen, α2Macroglobulin (α2M) and antithrombin. By quantifying prothrombin conversion and thrombin inactivation, more information is gained about the pro- and anticoagulant processes that underlie TG in platelet rich plasma (PRP). Especially for patients with a platelet disorder or for patients taking drugs that affect platelet function, it is interesting to investigate the overall thrombin potential kinetics in PRP.
Aims: To determine the effect of platelet count on TG and thrombin dynamics in PRP.
Methods: PRP was collected from 117 healthy individuals. PRP TG was measured undiluted and diluted to a platelet concentration of $50*10^9$/L and $150*10^9$/L. Pearson correlation test was used to determine correlations between parameters.
Results: Thrombin dynamics was established in PRP of 117 healthy individuals. Peak, velocity index and the maximum rate of prothrombin conversion increased linearly with platelet count, but endogenous thrombin potential reached a maximum at $150*10^9$/L. Correlation analysis indicated that the platelet count mainly affected the rate of prothrombin conversion. Inhibition of thrombin by antithrombin and α2M increased with increasing TG, but the ratio of inhibition by antithrombin or α2M remained the same independently of the total thrombin formed.
Conclusions: TG and thrombin dynamics were assessed in PRP of healthy donors and demonstrated that increasing the platelet count significantly affected the rate of prothrombin conversion and TG, rather than the total amount of thrombin formed. As venous thromboembolism (VT) can be caused by insufficient attenuation of coagulation by the anticoagulants, patients with high platelet counts could have an increased risk of developing VT.

PB1636 | Effect of Three Types of Mixed Anesthesia (MMB) for Platelet Function

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Background: Three types of mixed anesthesia (MMB), consists of medetomidine, midazolam, and butorphanol has been widely used instead of pentobarbital as anesthesia for laboratory animals.
Aims: We evaluated the effect of MMB and pentobarbital on platelet aggregation and considered the validity as anesthesia in hemostatic thrombus formation studies.
Methods: ICR mice, 6-month-old males, were used. For MMB, 4 mg/kg of midazolam, 5 mg/kg of butorphanol, and two different concentrations of medetomidine 0.3 mg/kg or 0.75 mg/kg (0.3MMB and 0.75MMB), and 80 mg/kg of pentobarbital (PENT) were adopted. Platelet-rich plasma (PRP) was collected from each group and was adjusted to 250,000 cells / μL. The aggregation rate induced by collagen or ADP was measured by a light transmission method. We have also examined the pretreatment effect of medetomidine (+MED) or butorphanol (+BUT) on platelets before PENT administration. Furthermore, the α_2 receptor blocker atipamezole was administered in vitro, and the aggregation rate was measured.
Results: The maximum platelet aggregation stimulated with collagen 0.8 μg/mL was 37.2 ± 7.7% in the PENT group and was enhanced to 59.2 ± 5.0 and 86.6 ± 2.9% in the 0.3MMB and 0.75MMB groups, respectively. Aggregation was similarly enhanced in ADP-induced platelet aggregation. In the +MED group, the platelet aggregation was significantly enhanced in comparison with single PENT anesthesia, while not in the +BUT group. Furthermore, the enhancement of platelet aggregation in MMB was suppressed by the addition of atipamezole.
Conclusions: Since the adrenergic α_2 receptor on the platelet membrane is known to send an aggregation-enhancing signal upon stimulation, medetomidine in MMB seems to enhance platelet aggregation via adrenergic α_2 receptor. It would be necessary to pay attention that the aggregation rate is modified by MMB anesthesia, compared with classical pentobarbital.

PB1637 | Antibodies Protect Platelets against Streptococcus Pneumoniae Toxin Pneumolysin

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Background: Community-acquired pneumonia is one of the most common acute infections and a leading cause of morbidity and mortality worldwide and mostly caused by *Streptococcus pneumoniae*.

Its toxin pneumolysin renders platelets non-functional, preventing them from covering endothelial cell lesions leading to extravasation of fluid and respiratory impairment.

Aims: To investigate whether antibodies protect platelets from damage by pneumococcal pneumolysin.

Methods: Platelets were isolated from healthy donors (n≥3) and incubated with 300ng/mL pneumolysin in the absence or presence of a polyclonal rabbit-anti-pneumolysin-antibody, a monoclonal mouse-anti-pneumolysin-antibody (abcam) and human derived pharmaceutical i.v.IgG. We assessed the impact of pneumolysin in the presence or absence of antibodies on platelet viability using RealTime-Glo cell viability kit and on platelet activation by measuring cytosolic Ca^{2+} and CD62P expression. Subsequent TRAP6-stimulation was performed to assess remaining platelet functionality. Adhesion and pore-sealing capacity was tested in a Boyden-chamber-assay and thrombus formation on collagen-surfaces was measured in a flow chamber.

Results: Both antibodies and i.v.IgG protect platelets from pneumolysin-induced cell death (Fig. 1A). They also prevented increase of free cytosolic Ca^{2+} and CD62P-expression induced by pneumolysin (Fig. 1B). In presence of pneumolysin and antibodies, platelets remained sensitive to subsequent TRAP-6 stimulation (Fig. 1B). Platelets can adhere to Boyden-chamber membranes and seal pores, which is severely impaired in the presence of pneumolysin. Addition of i.v.IgG preserved the pore-sealing capacity of platelets (Fig. 2A) and thrombus formation in whole blood (Fig. 2B).

Conclusions: Specific antibodies as well as i.v.IgG can protect platelets against pneumolysin-induced perforation and preserve platelet functionality. This indicates that antibody treatment might be able to prevent acute respiratory distress syndrome in pneumococcal infections by preserving platelet function and prevention of capillary leakage.

This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - Project number 374031971 - TRR 240.

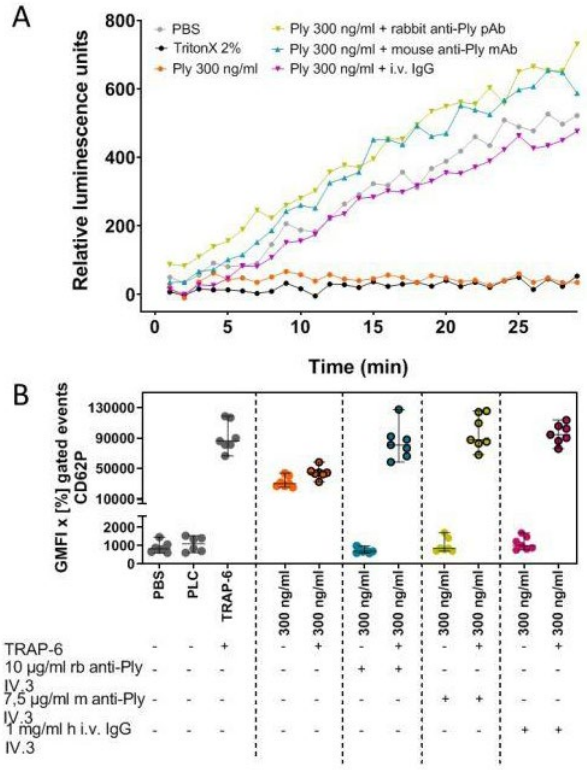

Figure 1:
(A) Platelet viability as measured by luminescence and (B) CD62P-expression of platelets treated with pneumolysin without and with subsequent TRAP-6 stimulation in absence and presence of polyclonal rabbit-anti-pneumolysin-antibody, monoclonal mouse-anti-Pneumolysin-antibody or i.v.IgG.

FIGURE 1

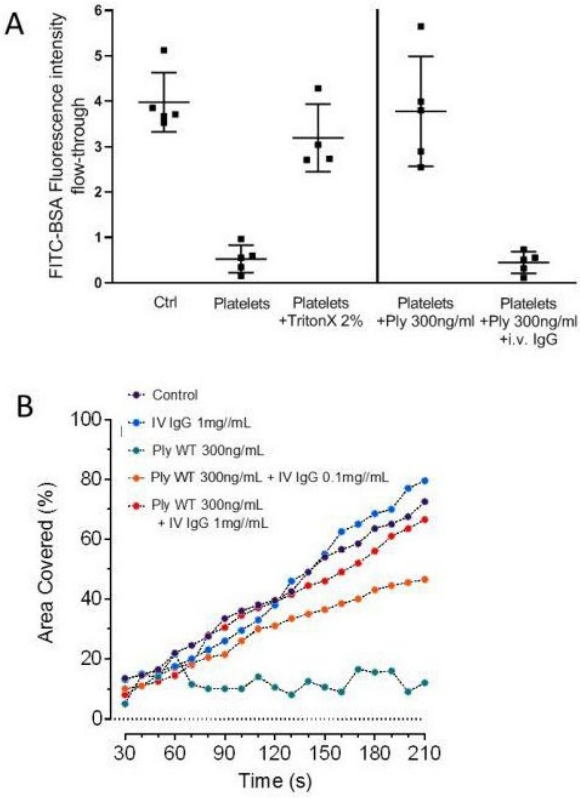

Figure 2:
(A) FITC-BSA flow-through Boyden-chamber membranes covered with platelets or pneumolysin-treated platelets in absence and presence of i.v.IgG. (B) Collagen surface area coverage under flow with and without pneumolysin in presence and absence of i.v.IgG.

FIGURE 2

PB1638 | Modified Plasma Recalcification Assay for Evaluation of Platelet Procoagulant Activity

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Background: Platelet procoagulant activity is usually assessed by expression of phosphatidylserine (PS) which appeared on the surface of activated platelets and served as a substrate for assembling of coagulation complexes. However, direct effects of activated platelets on fibrin formation are not thoroughly studied so far.
Aims: To develop the method for evaluation of the effects of activated platelets on fibrin formation in blood plasma.
Methods: Washed human platelets were activated by thrombin receptor activating peptide (TRAP), collagen and ionophore A23197. Platelets were spun down on the bottom of multiplate wells (0.5 x 10^7 platelets per well). After supernatant removal, 100 µl of plasma diluted at 1/1 ratio with Owren-Koller buffer supplemented with 150 µg/ml of corn trypsin inhibitor was added into the wells. Clotting was initiated by addition of 50 µl 25 mM CaCl2 and followed in a multiplate reader at 25°C by increase of adsorption at 450 nm. PS expression on activated platelets was measured by annexin V binding using flow cytofluorimetry.
Results: Platelets localized on the bottom of multiplate wells accelerated recalcified plasma clotting. The shortest clotting lag phases were detected in the presence of platelets activated by ionophore A23187. Significant shortage of the lag phases in comparison with not activated platelets was also observed when platelets were activated by collagen and to a less extent when they were activated by TRAP. Not activated platelets also accelerated clotting in comparison with plasma alone, presumably due to their activation by endogenous thrombin in recalcified plasma. Procoagulant effects of activated platelets correlated with the expression of PS detected by flow cytofluorimetry in parallel samples.
Conclusions: A new method based on modification of plasma recalcification assay was developed for evaluation of platelet procoagulant activity.
This work was supported by Russian Foundation for Basic Research (grant #20-015-00106).

PB1639 | Importance of Platelet Contractility at Different Stages of Thrombus Formation

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Background: Re-organization of the actin cytoskeleton accompanies platelet activation and is a pre-requisite for the development of platelet contractility, which is important in thrombus compaction and wound contraction. Defective platelet contractility can give rise to bleeding diathesis and increases the likelihood of thrombus embolization. However, how platelet contractility affects other aspects of platelet function is poorly understood. Moreover, reports suggest that current platelet function tests may be unaffected by changes in contractility.
Aims: To determine the consequences of compromised contractility on platelet adhesion under flow or static conditions, on platelet secretion, and on aggregation measurements in solution.
Methods: Blood was obtained from healthy consenting volunteers as approved by the RCSI Research Ethics Committee (REC1405). Different aspects of platelet function were assessed:

i translocation dynamics of single platelets on von Willebrand Factor (vWF) at arterial shear;
ii actin cytoskeletal morphology in spread platelets on fibrinogen;
iii P-selectin exposure in response to collagen-reactive peptide, and
iv aggregation in response to arachidonic acid or ADP.

In all cases, myosin IIa activity was dose-dependently inhibited by pre-incubation with para-amino blebbistatin (paBBT). The effect of paBBT on platelet contractility was directly measured by single platelet traction forces on micropost arrays.
Results: Platelet traction forces were significantly inhibited by doses as low as 1 µM paBBT. Myosin inhibition caused longer and faster platelet rolling on vWF. Actin filament bundling in spread platelets was reduced sensitively by paBBT whereas cytoskeletal rearrangements were inhibited only above 16 µM. Platelet alpha granule secretion in solution and aggregometry were unaffected by paBBT up to 40 µM.
Conclusions: Our preliminary results suggest that contractility critically affects several functions in adherent platelets and thus plays an important role beyond mere clot retraction. Current clinical tests insufficiently reflect this important aspect.

PB1641 | The Effect of IL-6-Primed Platelets on ADAMTS13-Mediated Clearance of Platelet-Bearing ULVWF and its Mechanism

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Background: Inflammation plays an important role in the development of thrombosis. Therefore, studying effects of inflammatory cytokines on platelet-bearing von Willebrand factor (VWF) clearance by a disintegrin-like and metalloproteinase with thrombospondin

type I repeats 13 (ADAMTS13) as the first step in thrombus formation is pathophysiologically significant.

Aims: This study investigated the mechanism by which interleukin-6 (IL-6)-induced platelet characteristic changes are involved in the regulation of ultra-large VWF (ULVWF)-platelet strings by ADAMTS13.

Methods: For this, we used platelets washed after incubation with IL-6 and compared their capability to form platelet-bearing ULVWF strings with that of mock-treated platelets following perfusion through the microfluidic channel with a basal surface lined with stimulated endothelial cells.

Results: The perfusion of IL-6-treated platelets significantly increased the formation of ULVWF-platelet strings and decreased their cleavage by ADAMTS13 in Figure 1. We found that platelets treated with IL-6 increased the phosphorylation of the serine/threonine kinase Akt and extracellular signal-regulated protein kinases 1/2. To examine whether the activation of the IL-6-induced signaling pathway in platelets regulates the formation and cleavage of ULVWF-platelet strings by ADAMTS13, IL-6-treated and untreated platelets were incubated with inhibitors of the IL-6 signaling pathway before perfusion through the microfluidic channel. Treatment of IL-6-treated platelets with either the phosphoinositol-3 kinase inhibitor LY294002 or the mitogen-activated protein kinase kinase inhibitor U0126 reduced the formation of platelet strings and restored the ability of ADAMTS-13 to cleave IL-6-treated platelet strings compared to the group treated with IL-6 alone. Furthermore, IL-6 increased the phosphorylation of the intracellular adaptor molecule 14-3-3 zeta, which is involved in regulation of VWF binding to the glycoprotein Ib-IX receptor on platelets. The 14-3-3 antagonist R18 significantly enhanced ADAMTS-13 cleavage of IL-6-treated platelet strings in Figure 2.

Conclusions: These results indicate that IL-6 increased the formation of ULVWF-platelet strings and reduced their cleavage by ADAMTS13.

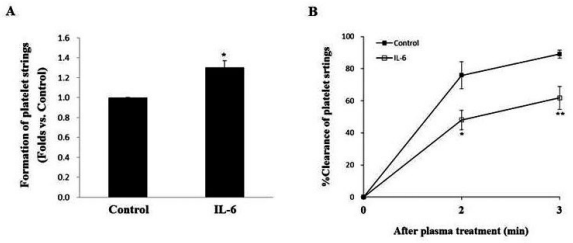

Figure 1. Formation of IL-6-treated platelet-ULVWF strings and clearance by ADAMTS13. (A) Effect of IL-6-induced platelet characteristic changes on the formation of ULVWF-platelet strings under flow conditions. Mean±standard error of the mean (SEM), n=5, $^{*}P < 0.01$ compared to control. (B) Effect of IL-6-treated platelets on the cleavage of ULVWF-platelet strings by ADAMTS13. Mean±SEM, n=5, $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the untreated group (control).

FIGURE 1 Formation of IL-6-treated platelet-ULVWF strings and clearance by ADAMTS13

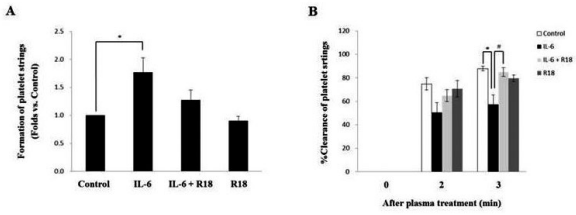

Figure 2. Involvement of 14-3-3 in the cleavage of ULVWF-platelet strings by ADAMTS13 under flow conditions. (A) Effect of a 14-3-3 inhibitor on platelet string formation. Mean±SEM, n=4, $^{*}P < 0.05$ compared to control. (B) Involvement of 14-3-3 in the cleavage of ULVWF-platelet strings by ADAMTS13. Mean±SEM, n=4, $^{*}P < 0.05$ compared to the control group, $^{\#}P < 0.05$ compared to IL-6 alone-treated group.

FIGURE 2 Involvement of 14-3-3 in the cleavage of ULVWF-platelet strings by ADAMTS13 under flow conditions

PB1642 | Reduced GPVI Signalling in Genetic and Disease Models of Thrombocytopenia

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Background: It is the current consensus that younger platelets are more functional than their older counterparts, however, recent work suggests otherwise. In *Bcl-x*$^{PLT20/PLT20}$ mice, which have thrombocytopenia and an increased proportion of immature platelets, we detected a prolonged bleeding time following 3-mm tail amputation. This phenotype was not solely attributable to the moderate thrombocytopenia present in these mice, therefore, we hypothesised these mice also possess a platelet function defect.

Aims: We evaluated platelets from mice with thrombocytopenia and a high proportion of reticulated platelets to determine if function was affected.

Methods: The *Bcl-x*$^{PLT20/PLT20}$ mouse model was used as a genetic model of thrombocytopenia. Additionally, two disease models exhibiting moderate to severe thrombocytopenia at high disease burden following intravenous transplant of a small-cell lung cancer (SCLC) cell line or an Eμ-Myc lymphoma cell line, were used. Platelet function was evaluated by light-transmission aggregometry and platelet integrin activation and degranulation by flow cytometry, in response to thrombin and convulxin.

Results: In all models, a high percentage of thiazole orange positive platelets was present, indicating an increased proportion of immature platelets. FACS analysis of platelet activation revealed a significantly diminished response to the GPVI agonist convulxin, but not thrombin. Likewise, platelet aggregation and Syk phosphorylation downstream of GPVI in response to convulxin was significantly reduced in all models. Surface expression of GPVI as assessed by FACS was unchanged in platelets from the *Bcl-x*$^{PLT20/PLT20}$ and SCLC transplanted mice, compared to WT or non-transplanted mice, but was slightly reduced in platelets from Eμ-Myc transplanted mice.

Assessing GPVI cleavage by western blot revealed no cleaved GPVI in any model.

Conclusions: Here, we report a functional defect in GPVI signalling present in three models of thrombocytopenia, possibly attributable to an increased proportion of young platelets. These results challenge the view that young platelets are more functional than older platelets.

PB1643 | Microparticles Released from Stored Platelet Increase Coagulation Ability

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Background: Quality management of blood platelet products is important for successful platelet transfusion therapy.

Although function of stored platelets is usually assessed by platelet aggregation ability, morphological change and P-selectin expression, senescence markers such as exposure of phosphatidylserine (PS) and microparticle (MP) have not been well evaluated in stored platelets.

In the previous study, we demonstrated that the number of PS-exposed platelets and MP were time-dependently increased during storage of platelets. Furthermore, activated partial thromboplastin time (APTT) was accelerated by the addition of PS-exposed platelets. However, the function of MP released from stored platelets is still unknown.

Aims: The aim of this study was to elucidate the role of MP released from stored platelets.

Methods: Expression of P-selectin, PS exposure and MP release were assayed by using flow cytometry after platelet rich plasma (PRP) were stored at 22°C for 0-14 days with continuous gentle agitation.

Results: Initial pH in stored platelets was pH 7.51±0.38 and sustained from day0 to day14. The population of P-selectin-expressing platelets significantly increased in a time-dependent manner during incubation (day 0, 8.8%; day10, 37.8%). Similar results were obtained for PS-exposed platelets. MPs were not detected at day 0, but they appeared at day 3 and gradually increased thereafter. Interestingly, PS-exposure was detected on MPs like platelets after incubation, suggesting a functional role of PS-exposed MP in coagulation as observed for PS-exposed platelets in my previous study. Actually, coagulation time was significantly shortened by the addition of PS-exposed MP to the APTT assay.

Conclusions: PS-exposed MPs are released from stored platelets and accelerate coagulation activity.

PB1644 | Characterization of the Hypercoagulable State and NETosis in Systemic Lupus Erythematosus

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Background: Many mechanisms are involved in thrombotic processes that contribute to morbidity in patients with systemic lupus erythematosus (SLE). Global coagulation tests may be valuable to evaluate SLE hypercoagulable state.

Aims: To characterize the prothrombotic state by Rotational Thromboelastometry (ROTEM®) and Calibrated Automated Thrombogram (CAT®) in SLE not mediated by antiphospholipid antibodies (aPL).

Methods: 32 patients with SLE without aFL and 88 healthy controls were recruited after signing the informed consent. Experimental protocol was approved by La Paz University Hospital Ethics Committee. Kinetic clot formation was determined by ROTEM®. Platelet activation markers, exposure of phosphatidylserine (PS) and caspase activities were determined by flow cytometry. To generate neutrophil extracellular traps (NETs) neutrophils from controls or SLE patients were stimulated with 100 nM PMA and cocultivated with PRP from healthy controls adjusted to 10^5 platelets/µL with/without corn trypsin inhibitor (CTI) to block contact phase. Thrombin generation associated to NETs and to microparticle's (MPs) content of tissue factor (TF) was measured by CAT. Plasma levels of cfDNA and of PAI-1 were also determined. Statistical analysis was performed by Prism-Graphpad.

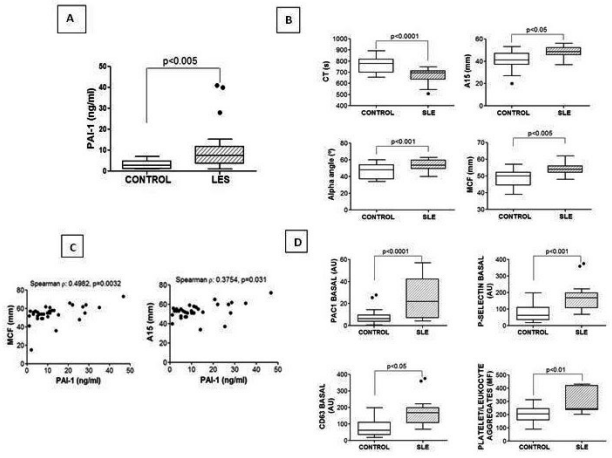

Fig. 1. Procoagulant profile in patients with SLE. A) PAI-1 plasma level was increased in SLE patients. **B)** ROTEM was performed in whole blood. SLE, systemic lupus erythematosus; CT, clotting time; A15, amplitude at 15 min; MCF, maximum clot firmness. **C)** Correlation between PAI-1 plasma levels with ROTEM parameters. **D)** Basal activity of fibrinogen receptor, surface exposition of P-selectin, CD63 and PS (determined by annexin V binding). Data are expressed as arbitrary units (mean fluorescence X % of positive cells) or only as % of positive cells. p≤0.05 was stablished as statistical significance.

FIGURE 1

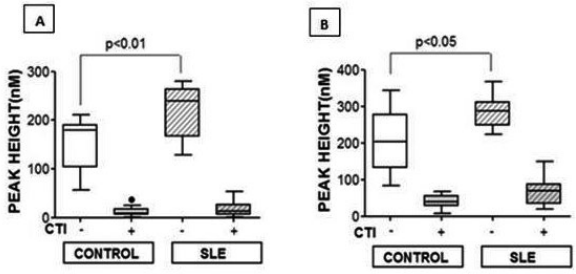

Fig. 2. Thrombin generation associated to NETs. A) SLE patients showed a higher peak of thrombin without and **B)** with 100 nM PMA stimulus and without CTI (an inhibitor of FXIIa). Presence of CTI decreased thrombin generation in control and SLE patients. p≤0.05 was stablished as statistical significance.

This work was supported by grants from the FIS-FONDOS FEDER (PI19/00772, NB). EMM holds a predoctoral fellowship from Federación Española de Trombosis y Hemostasia (FETH-SETH).

FIGURE 2

Results: ROTEM® showed a procoagulant profile in SLE patients (Fig 1 A). Plasma PAI-1 was increased in SLE patients (Fig. 1B) and its levels correlated with several ROTEM parameters (Fig. 1C). SLE patients showed a basal platelet activation and a higher PS exposure (Fig. 1D) that was not due to an enhanced apoptosis and an increased thrombin generation associated to TF content of MPs, to cfDNA and to NETs (Fig. 2). cfDNA- and NETs- associated thrombin generation were prevented by CTI.

Conclusions: ROTEM® detected a hypercoagulable state in SLE patients that might be linked to increased plasma PAI-1, basal platelet activation with a consequent enhanced exposure of PS, augmented TF-containing MPs and cfDNA. Moreover, neutrophils from SLE patients seemed more prone to form NETs than those from healthy controls.

PB1645 | The Active Recruitment of Red Blood Cells to Activated Platelets via CD36 and FasR Is Crucial for Arterial Thrombus Formation

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Background: Recently, a direct interaction of platelets and red blood cells (RBCs) via FasL-FasR interaction was found to be important for the externalization of phosphatidylserine (PS) at the RBC membrane that attributes a direct role for RBCs to thrombin generation and thrombus formation and stabilization upon hemostasis and thrombosis.

Aims: –.

Methods: Analysis of the recruitment of RBCs to activated platelets under flow conditions using blood samples from healthy volunteers.

Results: The inhibition of integrin aIIbb3 with Abciximab resulted in reduced platelet adhesion on recombinant FasR (CD95) and decreased PS exposure on the RBC membrane indicating an interaction between FasR on RBCs and integrin aIIbb3 at the platelet surface. This observation was further supported by increased externalization of CD61 at the platelet membrane in the presence of RBCs. Increased externalization of CD61 was blocked by specific anti-CD36 and anti-FasR antibodies as well as by FasR knock-out RBCs. The interaction of RBCs and platelets via aIIbb3 and FasR was not important for the recruitment of RBCs to activated platelets. In contrast, first results of dynamic adhesion and thrombus formation using a blocking CD36 antibody imply a contribution of CD36 of RBCs to be involved in the active recruitment of RBCs into the growing thrombus. Furthermore, flow chamber experiments with collagen-adherent platelets provide first evidence for a role of FasR and PS exposure of RBCs and the release of thrombospondin from platelets to be involved in the active recruitment of RBCs to activated platelets.

Conclusions: Taken together, the recruitment of RBCs to collagen-adherent platelets is an active process that is controlled by several surface proteins on the platelet and the RBC membrane including CD36 and FasR on RBCs and the release of thrombospondin from platelets. This initial mechanism might play a role in hemostasis and thrombosis.

PB1646 | Interaction between Platelets and *Streptococcus pneumoniae*

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Background: *Streptococcus pneumoniae*is a human pathogen causing serious health issues including community-acquired pneumonia and pneumonia induced acute respiratory distress syndrome (ARDS). Recent studies have shown that platelets are important players in infection processes and influence the growth of pathogenic bacteria.

Aims: Investigation of the interplay of platelets and different *S. pneumoniae* serotypes.

Methods: Platelets were isolated from blood of healthy donors (n≥3). Pneumococcal serotypes 2 (D39), 4 (TIGR4), 6B and 19F and *pneumolysin*deficient mutants (D39D*ply*; TIGR4D*ply*) were grown in complex THY-Medium to mid-exponential phase. Platelets, releasates of activated platelets (stimulated with 5µg/ml collagen, 20µM TRAP-6)and platelet lysates were incubated at a ratio of 5 platelets per *S. pneumoniae*for 2, 3 or 4 hours. Colony forming units (CFU) were determined. Platelet activation was analyzed by CD62P expression using flow cytometry.

Results: All four wildtype strains induced CD62P expression on platelets (positive cells ≥29%). Pneumolysin deficient mutants

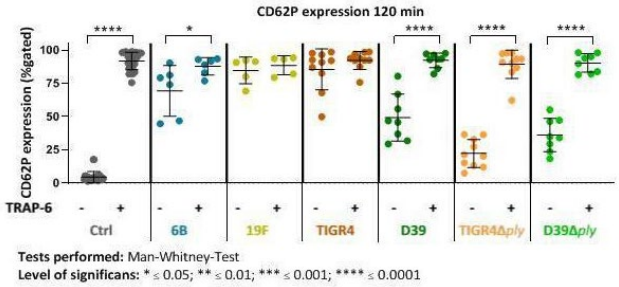

FIGURE 1 CD62P expression on platelets after incubation with different S. pneumonia strains

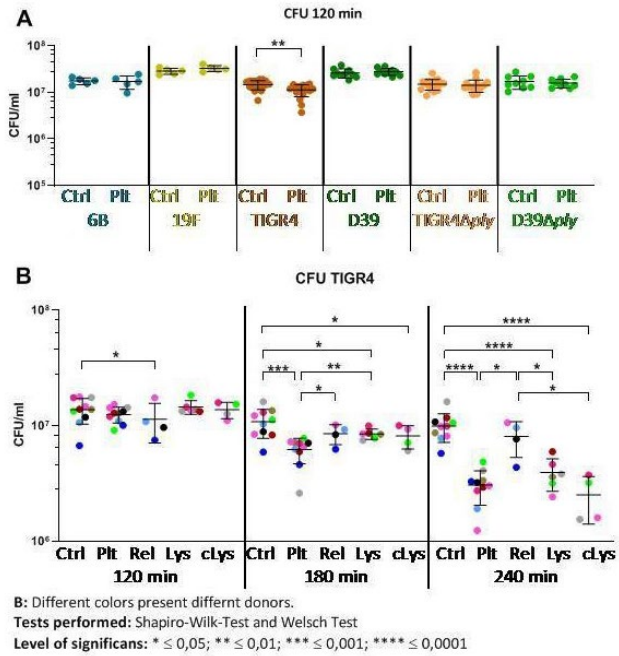

FIGURE 2 Influence of platelets and platelet components on the growth of different S. pneumonia strains

caused only moderate CD62P expression (positive cells ≥7%) and subsequent TRAP-6 stimulation further increased activation at all conditions tested (Fig. 1).Platelets did notinfluence growth of strains D39 (wildtype and *ply*mutant), 6B and 19F while growth of TIGR4 was significantly affected. (Fig. 2a: p=0.002 (2h)). Living platelets (p=0.0001 (4h)) and platelet lysate (p=0.0001 (4h)) inhibited bacterial growth, while platelet releasate had no effect (Fig. 2b).

Conclusions: The interaction between platelets and *S. pneumoniae*seems to be strain specific with a dominant role of pneumolysin. Interestingly, growth of *S. pneumoniae*can be inhibited by whole platelets and platelet lysates but not by supernatant of activated platelets (releasate) indicating that the inhibition of *S. pneumoniae* growth is not mediated by components stored in the platelet granules but rather in the cytoplasm.

PB1647 | *Streptococcus pneumoniae* Toxin Pneumolysin Renders Platelets Non-Functional

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Background: *Streptococcus pneumoniae* (pneumococcus) is a commensal of the upper respiratory tract but also causative agent of severe diseases like community acquired pneumonia associated acute respiratory distress syndrome (ARDS), meningitis and sepsis. During dissemination via the blood stream, pneumococci associate with platelets. Platelets are an important diagnostic marker in pneumococcus infections, because low platelet counts correlate with high mortality rates. A major virulence factor of pneumococci is the pore-forming toxin pneumolysin (Ply). This cholesterol-dependent cytolysin has earlier been reported to activate platelets as indicated by high CD62P surface expression.

Aims: To investigate the impact of pneumococcal Ply on platelet activation and function.

Methods: Platelets were isolated from healthy volunteers. The impact of Ply on activation and function of platelets was assessed by measuring CD62P and PAC-1 binding, platelet aggregation, release of intracellular Ca^{2+}, and platelet viability. The effect of Ply on platelet adhesion to collagen and thrombus formation was measured in a flow chamber.

Results: Ply mediated pore formation in platelets (Fig. 1A) occurred in a concentration dependent manner and was associated with Ca^{2+}-release (Fig. 1B) and loss of platelet viability. Ply did not activate platelets as indicated by unaltered PAC-1 binding. Platelets aggregated in response to agonists only at sublytic Ply concentrations. Increased levels of CD62P was due to pore formation and intracellular CD62P staining. Consistent with Ply induced platelet lysis, thrombus formation in whole blood was reduced by Ply treatment in a concentration dependent manner (Fig. 1C).

Conclusions: Pneumococcal Ply perforates platelet membranes, which leads to loss of platelet viability and function. The severe impact of Ply on platelet function and viability suggests that Ply contributes to capillary leakage during severe cases of pneumococcal pneumonia associated ARDS.

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PB1648 | Septins Contribute to Platelet Shape and Functionality

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Background: Platelets are blood cells that play an important role in hemostasis and thrombosis. Activated platelets change shape due to reorganization of cytoskeleton. Septins, a family of GTP-binding cytoskeletal proteins, are implicated in cytokinesis and mitosis of nucleated cells, but their role in platelets is largely unknown.
Aims: To study the septins in resting and activated platelets and their contribution to platelet morphology and functions.
Methods: Confocal microscopy, flow cytometry, biomechanical and biochemical assays were used to examine structural and functional changes in human platelets. Septins 2 and 9 were immunostained in resting and activated platelets, either spread on a fibrinogen-coated surface or contracting within a 3D plasma clot. Clot contraction was assessed by tracking the clot size.
Results: In resting platelets, septin 2 concentrated at the cell periphery, colocalizing with the microtubule marginal ring, while septin 9 was distributed as small patches over the cell volume, often with a peripheral localization. In thrombin-activated platelets, septins were also observed in filopodia. Activation with thrombin resulted in a 2-fold increase of septin intensity. Inhibition of septin dynamics with forchlorfenuron (FCF) caused dose-dependent perturbations, including a substantial decrease of platelet roundness and surface curvature. In FCF-pretreated platelets activated with TRAP, expression of activated integrin αIIbβ3 was significantly suppressed. FCF impeded clot contraction with a 6-fold increase of the lag-time and up to a 3-fold decrease of the extent of contraction. Alterations in septin structure caused by FCF abrogated platelet spreading by 50% and accelerated thrombin-induced platelet fragmentation.
Conclusions: Septins are important for stabilizing platelet shape and supporting platelet integrity; septins are involved in platelet biomechanical functions, such as contractility and adhesion.
Funding: Work supported by the Program of Competitive Growth at KFU and grants 18-415-160004, 19-015-00075, 20-015-00257 from the Russian Foundation for Basic Research, and 17SDG33680177 from AHA.

PB1649 | LV-AO Angle Is Associated with Platelet Reactivity in Patients with Aortic Stenosis

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Background: The pathogenesis of aortic stenosis (AS) is not fully understood. However, local inflammation of the valve appears to be a main player. Except haemostasis, platelets contribute to inflammatory processes in various ways. Furthermore, platelet function is altered in patients with AS. Moreover, a steep angle between the left ventricle and the aorta (LV-AO-angle) leads to turbulent blood flow.
Aims: However, it is not known if platelet reactivity is associated with steep LV_AO angle in patients with AS.
Methods: We included 289 patients with severe AS and performed cardiac computertomography to assess the LV-AO-angle. Platelet function was evaluated by light transmission aggregometry by using collagen and adenosine-diphosphate to induce platelet activation.
Results: ADP- and collagen induced aggregation showed a significant negative correlation with LV-AO-angle (ADP: r=-0.19, p=0.0009, R^2=0.022; collagen: r=-0.21, p=0.0004, R^2=0.027). ADP-induced MoA was significant higher in patients with a LV-AO-angle < 160° in comparison to patients with an angle ≥160° (< 160°: 66.99% ± 20.72% vs. ≥160°: 60.66% ± 19.85%, p=0.009). Collagen-induced platelet reactivity was significant higher in patients with a LV-AO-angle < 160° in comparison to patients with an angle ≥160° (< 160°: 78.67% ± 13.19% vs. ≥160°: 73.85% ± 14.44%, p=0.003). Multivariate cox-regression revealed that LV-AO angle < 160 was a robust predictor of ADP- and collagen-induced platelet aggregation.
Conclusions: A steep LV-AO-angle is associated with enhanced platelet reactivity in patients with AS. Platelet activation is known to lead to local inflammation. Therefore, enhanced platelet reactivity could play crucial in the progression of AS. The clinical significance of a steep LV-AO-angle needs be evaluated in further trials.

PB1650 | Collagen-Induced Thrombocyte Aggregation in Essential Arterial Hypertension with Subclinical Organ Damage

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Background: Many trials proved that arterial hypertension (AH) influenced on the reliable increase of the atherotrombotic damage of vasculature. The study of the peculiarities of the collagen-induced thrombocyte aggregation at the patients with arterial high blood pressure can testify the degree of increased clotting formation depending on heart and vessel remodeling.
Aims: To set the character of collagen-induced thrombocyte aggregation changes depending on different sites of vasculature remodeling in AH of subclinical vessel damage.
Methods: We have examined 188 patients (102 women and 82 men, aged 31 to 72 years) with moderate and low-risk AH and 30 healthy as control group. Ultrasound investigation was used to set the remodeling of heart and vessels. Gromerular filtration rate (GFR) was served as indicator of small vessel injury. Three indexes (the level, time and rate) of collagen-induced platelet aggregation were measured.

Results: We have examined 188 patients (102 women and 82 men, aged 31 to 72 years) with moderate and low-risk AH and 30 healthy as control group. Ultrasound investigation was used to set the remodeling of heart and vessels. Gromerular filtration rate (GFR) was served as indicator of small vessel injury. Three indexes (the level, time and rate) of collagen-induced platelet aggregation were measured.

Conclusions: The peculiarities of collagen-induced aggregation depend on the local cardiovascular alterations which forward to turbulent blood flow and can increase risk of thrombotic complications.

PB1651 | The Effects of E.coli Lipopolysaccharides on Platelet Function Are Limited to Re-Sensitization

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Background: Lipopolysaccharides (LPSs), are well-known mediators of the inflammatory response. A plethora of groups have reported that LPSs potentiate platelet responses after weak stimulation. However, it has also been reported multiple times that LPSs do not affect platelet functioning.

Aims: Investigation of platelet functioning in the presence of LPSs and determination of the cause of the discrepancy in the previously published data.

Methods: Whole anticoagulated blood of healthy volunteers (12 persons, 18-25 years of age) within 3 hours of collection was used for all experiments. Platelet responses, activation of polymorphonuclear neutrophils (PMN) and platelet-PMN heteroaggregate formation were analysed by flow cytometry, light transmission aggregometry and western blotting. Thrombus growth and PMN activity were assessed by means of fluorescent microscopy in parallel-plate flow chambers with fibrillar collagen type I. LPSs from *E.coli* O111:B4 and O127:B8 at concentration 10 μg/ml (for experiments with PMNs) or 100 μg/ml (for whole blood experiments) were utilized in experiments.

Results: The activity of all utilized LPSs was confirmed by their ability to activate PMNs. 30-min incubation of LPSs with PMNs with PRP or washed platelets enhanced platelet-PMNs heteroaggregate formation without activation of platelets. LPSs also increased the amount of highly activated PMNs during thrombus formation and slightly increased PMNs crawling velocity. LPSs did not substantially affect either thrombus growth or platelet aggregation. 0.5-2 hours incubation of platelets or whole blood with LPSs did not affect platelet functional responses (integrin activation, granule release, calcium signalling, phosphatidylserine exposure) to activation with ADP, collagen-related peptide of TRAP. LPSs were capable of restoring platelet responses reduced by sub-threshold doses of ADP upon P2 receptor desensitisation.

Conclusions: LPSs effects on platelet activation are limited to their effects on PMNs and their capability to compensate ADP-induced desensitisation of platelets. The study was supported by the Russian Science Foundation grant 17-74-20045.

PB1652 | Emerging Roles of CLEC-2 during Development of Monocrotaline-Induced Liver Injury in Mice

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Background: Hepatic sinusoid obstruction syndrome (SOS) is characterized by a drug and/or radiation-induced liver injury. SOS may negatively influence the patients' outcome after chemotherapy or hematopoietic stem cell transplantation, and is diagnosed by the clinical symptom of hepatomegaly, fluid accumulation, weight gain, and bilirubinemia. We identified C-type lectin-like receptor 2 (CLEC-2) on the surface of platelets as a receptor for platelet-activating snake venom, rhodocytin. Although liver sinusoidal endothelial cell is also reported to express CLEC-2, it is unknown whether CLEC-2 has some roles in the promotion of SOS.

Aims: The aim of this study is to uncover a previously unknown role of CLEC-2 in the pathogenesis of SOS.

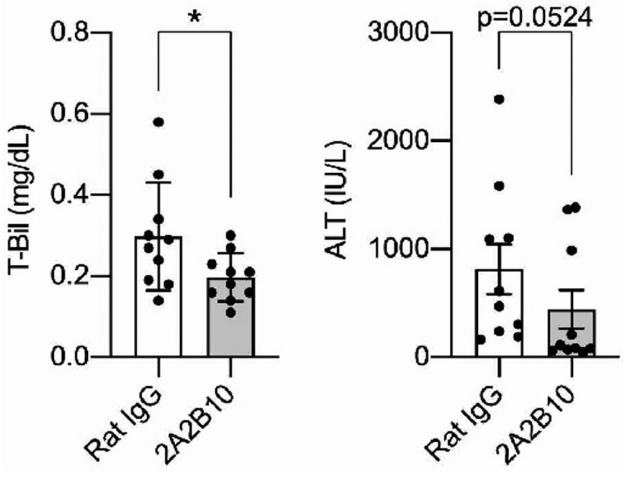

FIGURE 1 Biochemical examination of the serum obtained from control and CLEC-2-depleted mice

Methods: We used monocrotaline (MCT)-induced mouse model of SOS. Control rat IgG or 2A2B10 (CLEC-2 depleting antibody) were intravenously injected for two consecutive weeks (n = 10, each group). After confirming the absence of CLEC-2 on the surface of platelets, MCT was intraperitoneally injected. Forty-eight hours after MCT administration, mice were sacrificed, and complete blood count, biochemical examination and liver-tissue section were analyzed.

Results: We checked macroscopic findings and analyzed hepatic injury markers in control and CLEC-2 depleted mice after MCT injection. Interestingly, 5 mice out of 10 in CLEC-2 depleted group showed normal macroscopic appearance, although all mice in the control group showed reddish appearance to some extent, indicating that liver injury was attenuated in CLEC-2 deplete mice. Moreover, the elevation of total bilirubin, the most important criterion for the diagnosis of SOS, was significantly decreased in CLEC-2-depleted mice (Figure). The elevation of ALT also tended to be attenuated in CLEC-2-depleted mice. In the liver-tissue section, injury score, evaluated by the morphological changes, was significantly lowered in CLEC-2-deplete mice.

Conclusions: Absence of CLEC-2 may attenuates SOS by protecting the progression of thrombosis in the liver sinusoids.

PB1653 | Assessment of Thrombus Formation under Flow-Conditions in Essential Thrombocythemia/ Polycythemia Vera

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Background: Essential thrombocythemia (ET) and polycythemia vera (PV) have an increased risk of both thrombosis and bleeding. Recent clinical studies have shown that patients with JAK2 gene mutation have a higher risk of thrombosis than those without mutation. However, the effect of JAK2 mutation on dynamics of thrombus formation in ET/PV is not well characterized.

Aims: To assess dynamics of thrombus formation under flow conditions in ET/PV.

Methods: We examined platelet thrombus formation on a collagen-coated microchip under flow conditions using total thrombus-formation analysis system (T-TAS). Whole blood from 48 patients including 36 ET, 12 PV patients and 20 healthy controls were subjected to T-TAS. T10 (time at the onset of thrombus formation) and T60-10 (time from T10 to occlusive thrombus formation) were evaluated. JAK2 mutation was determined by allele-specific PCR.

Results: The T10 in ET/PV patients was longer than that in control (184±195 sec vs 83±33 sec, p< 0.01), indicating delay in the onset of thrombus in ET/PV. In the ET/PV patients, T10 was negatively correlated with hematocrit, but not platelet count while T60-10 was negatively correlated with platelet count, but not hematocrit. T10 in patients with JAK2 mutation was shorter than T10 in patients without JAK2 mutation (139±153 sec vs 318±246 sec, p=0.03), suggesting faster start of thrombus formation in patients with JAK2 mutation. Von Willebrand factor activity was not associated with T10 or T60-10.

Conclusions: These results suggest that increased hematocrit and JAK2 mutation accelerate start of thrombus formation (T10) and increased platelet count promotes thrombus growth under flow conditions. These dynamics of thrombus formation may be involved in pathogenesis of hemostatic manifestations in ET/PV.

PB1654 | Platelets May Be Involved in the Regulation of ITP Autoimmunity

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Background: Primary immune thrombocytopenia is an autoimmune disease mediated by antiplatelet autoantibodies that cause platelet destruction and suppression of platelet production.Platelets are non-nucleated cells that play central roles in the processes of hemostasis, innate immunity, and inflammation. Platelets express numerous receptors and contain hundreds of secretory products. These receptors and secretory products are instrumental to the platelet functional responses. Much evidence suggests that platelets themselves play a key role in regulating inflammation and immune processes.However, whether and how platelets are involved in the autoimmune response of ITP patients is unknown.

Aims: To explore the differences between CD9, CD31, GPVI, CD62P, CD63, CD31, TSP, PD-1, PDL-1,CD36,CD226,CD47,and CD59 on platelets of ITP patients and normal controls, and the relationship with clinical parameters. To study the changes of platelet surface molecules after treatment with different drugs.

Methods: 1.Platelet-rich-plasma (PRP) was separated by centrifugation of the blood for 10 minutes at 800rpm.The PRP was then

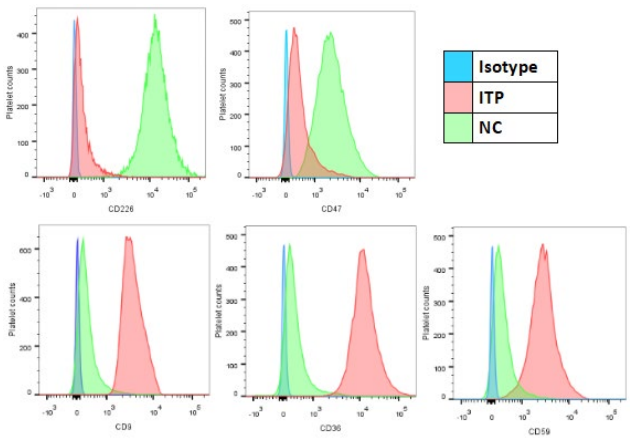

FIGURE 1 Flow chart of platelet surface molecules in normal controls and ITP patients

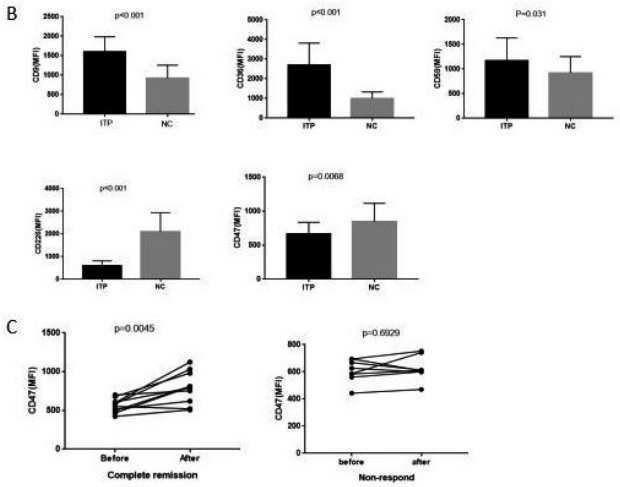

FIGURE 2 B and C

centrifuged again at 1700 g for 10 min at 20 °C to separate plasma from the platelets.

2.Flow cytometry was used for detecting the level of those molecules from platelets.

Results: 1. There are significant differences in platelet surface molecules between ITP patients and normal controls.

2. In ITP patients, the mean fluorescence intensity of CD9, CD36 and CD59 on platelet surface was significantly higher than that of normal control, while the mean fluorescence intensity of CD226 and CD47 was significantly lower than that of normal control.(FigureA,B)

3. The expression of CD47 on the platelet surface of the complete remission group increased significantly after treatment, but there was no significant change in the ineffective group.(FigureC)

Conclusions: The results of our current research indicate that some molecules have abnormal expression on the surface of platelets in ITP patients, so we speculate that platelets may play an important regulatory role in ITP autoimmunity.

PB1655 | Healthy Pediatric Platelets are Moderately Hyporeactive But Hyper-Coagulant within a Wide Age Range

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Background: Studies on platelet function in children older than neonatal period are few and their results are controversial. There are reports of both hypo- and hyper-reactivity in pediatric platelets compared to adults.

Aims: The aim of our study was to compare platelet function in children aged from 1 year to 18 years old with adults.

Methods: Venous blood was collected into vacuum plastic tubes with sodium citrate, final concentration 3.8%. 20 μL of blood was diluted 1:20 in HEPES-buffered Tyrode buffer. For platelet activation, a mixture of collagen-related peptide (1.25 μg/mL), PAR-1 activating peptide SFLLRN (12.5 μM) and 2.5 mM CaCl2 was used. We determined forward scatter (FSC), side scatter (SSC), CD42b-PE, CD61-PE, CD62P-Alexa Fluor 647, PAC-1-FITC, annexin V-Alexa Fluor 647 binding and mepacrine release levels.

Results: Children were divided into 3 age groups: 1 to 5 years old (n=16), 6 to 10 years old (n=23) and 11 to 18 years old (n=32); the control was healthy adults aged 19 to 40 years old (n=49). Procoagulant platelets level in all children groups was 1.5-fold higher than in adults, both in resting and activated states. Resting platelets of children aged 1 to 5 years old had 1.3-fold fewer dense granules than adults' platelets and lower relative degranulation. Pediatric platelets from all groups had 1.2-fold lower CD62p after activation than adults. CD42b and CD61 on stimulated platelets of children were higher than in adults. No difference between children and adults was found for PAC-1 in resting and activated states, CD42b, CD61 and CD62p in resting state.

Conclusions: Pediatric platelets are characterized by lower granule release and higher procoagulant response than adult platelets, with the same integrin level.

PB1656 | Bcl-Xl Deamidation State Tells Platelets Age

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Background: Deamidation is commonly viewed as a post-translational modification that sets an expiration date on proteins. This spontaneous reaction accounts for the erosion, with time, of Asn residues within proteins, that are converted to Asp/IsoAsp residues. Such changes entail structural and functional alterations. Among apoptosis regulators of the Bcl-2 family, Bcl-xL shows the unique characteristic to be either singly or doubly deamidated. We developed tools to highlight the sequential deamidation reactions targeting Asn52 first, and later Asn66 in the protein. While monodeamidation provides Bcl-xL with a gain of autophagic activity, double deamidation impairs Bcl-xL anti-apoptotic functions. One of the unique traits of platelets is to rely on the balance between anti-apoptotic Bcl-xL and pro-apoptotic Bak for their survival in circulation at steady state. How Bcl-xL is regulated to terminate platelet life is currently unknown.

Aims: As Bcl-xL regulates the life span of platelets and proplatelet-forming megakaryocytes, we aimed to investigate the status of Bcl-xL deamidation among young and old platelets.

Methods: We therefore analyzed Bcl-xL deamidation state in platelets from mice models where platelets lifespan was manipulated. In parallel, we compared human platelets obtained at steady state from healthy controls, to platelets newly synthesized after recovery from acute thrombocytopenia

Results: We found that while expression levels of Asn52 monodeamidated Bcl-xL remains unchanged, Asn52Asn66 doubly-deamidated Bcl-xL is virtually absent in young platelets and accumulates in old platelets.

Conclusions: Bcl-xL deamidation status therefore fulfills all the criteria of a bona fide aging marker of platelets. Ongoing work addresses whether Bcl-xL deamidation is responsible for platelets death, and whether it can be instrumental in the detection of hematologic malignancies.

PB1657 | Platelet Functions Defects in Calreticulin and JAK2V617F MPN Patients

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Background: The two most frequent molecular causes of Phi negative myeloproliferative neoplasms (MPN) are JAK2V617F and mutations of Calreticulin. They are responsible for increased activation of the JAK/STAT pathway and subsequent increased production of platelets. The main risk in these diseases is thrombosis, but patients with Calreticulin mutations are at a lesser risk of thrombosis than JAK2V617F patients. The calreticulin protein, through its C domain, regulates calcium homeostasis. The mutations that are found in MPN impair this domain. Given the role of calcium in platelet activation, we hypothesized that calreticulin mutated platelets would have platelet function defects, that may explain why these patients are at lesser risk of thrombosis.

Aims: Compare platelet function depending on the molecular marker of MPN.

Methods: We analysed platelet function in 9 MPN patients with calreticulin mutations, 6 with JAK2V617F and 20 healthy controls. To rule out a possible effect of treatment on platelet function, none of the patients were taking antiplatelet therapy nor cytoreductive drugs.

We performed platelet agregometry, secretion assays, stained for glycoprotein receptor expression, analysed the content of alpha and dense granules, looked for phospholipid expression using annexin, and studied fibrinogen binding.

Results: We observed significant defects of platelet aggregation, secretion, CD41 expression, fibrinogen binding and dense granule content in calreticulin platelets compared with normal ones. But we also observed the same results with JAK2V617F platelets compared with normal ones. There was no significant difference in platelet function tests between calreticulin and JAK2 mutated platelets. We did not observe any significant difference in annexin binding among different subtypes of MPN platelets.

Conclusions: Using various platelet function tests, in patients without any antiplatelet nor cytoreductive treatment, we did observe platelet function defect in calreticulin mutated platelets but these defects were not specific for calreticulin mutations as we observed comparable defects in JAK2V617F platelets.

PB1659 | Characterizing Platelet Granules of von Willebrand Disease Patients Using Super-Resolution Structured Illumination Microscopy

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Background: Platelets play an essential role in thrombus formation by secreting a variety of prothrombotic proteins from their alpha and dense granules upon activation. The mechanisms that control granule release and organization are still incompletely understood, in part because we lack the ability to obtain quantitative morphometric data from large numbers of platelets imaged at sufficient resolution to discern their individual granules.

Aims: Our aim is to develop an automated method for quantitative analysis of platelet granules using super-resolution microscopy that can be applied to patients with bleeding abnormalities.

Methods: Platelets were isolated from healthy donors and patients with Von Willebrand Disease (VWD). Degranulation was induced

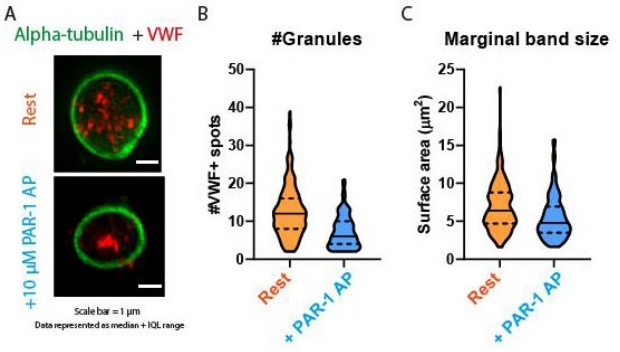

FIGURE 1 Representative image (A) and quantification (B,C) of resting vs. PAR-1 AP activated platelets

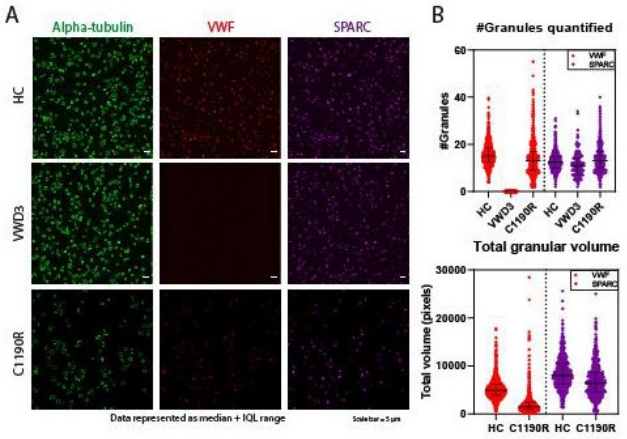

FIGURE 2 Representative images (A) and quantification (B) of healthy donor and VWD patient VWF+ and SPARC+ granules

by activating platelets with 10 μM PAR-1-AP. Fixed platelets were immunostained for alpha-tubulin (marginal band), VWF and SPARC (both alpha-granules). Samples were imaged employing Structured Illumination Microscopy. After reconstruction, images were analyzed by custom-developed ImageJ macros to quantify platelet (granule) morphometric parameters.

Results: Activation of platelets led to clear morphological and granular changes, such as loss of VWF+ granules (mean granule number 12.6±0.50 vs. PAR-1-AP 7.2±0.35, p< 0.0001) and compression of the marginal band (7.0±0.24 μm^2 vs. 5.5±0.26 μm^2, p< 0.0001) (Figure 1). Analysis of platelets from a type 3 VWD patient revealed similar numbers and morphology of SPARC+ granules but no detectable VWF+ granules (Figure 2). Subtle granular changes were observed in a type 2A VWD patient with a C1190R mutation, which leads to defective VWF multimerization. The number of SPARC+ and VWF+ granules was similar to controls, but VWF+ granules were smaller (granule volume = 145.8±3.3 vs. 284.7±4.6). Taken together, this suggests that the C1190R mutation in VWF leads to defective packaging in platelet granules.

Conclusions: We have generated a platform for automated assessment of platelet granules in VWD patients. Our data highlights the potential of such analyses for the diagnosis of hemostatic disorders.

PB1660 | Effects of GPVI on Clot Structure: Reduction in Procoagulant Platelets in GPVI-Deficient Clots

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Background: Immunoglobulin receptor GPVI is involved in platelet activation and has recently been implicated in thrombus growth and stability.

Aims: To determine the effects of GPVI on clot structure and identify how this contributes to the GPVI-deficient phenotype.

Methods: Clots from purified fibrinogen were analyzed by turbidity with 0-10 μM GPVI-monomer/dimer. Clot pore size was determined by permeation ± 2.5 μM of GPVI-monomer/dimer, and the mechanical properties of the clot ± 5 μM GPVI were investigated using magnetic tweezers. Clotting/lysis was analyzed by ROTEM using whole blood from WT and $GPVI^{-/-}$ mice. Platelet-rich plasma (PRP) clots were used to determine clot pore size by permeation, and fiber density/size by confocal and scanning electron microscopy (SEM).

Results: Turbidity analysis showed an increase in clotting lag phase (18-23% GPVI-monomer; 11-38% GPVI-dimer, p< 0.05), suggesting impaired protofibril formation, and increased MaxOD: (4-12% GPVI-monomer; 16-17% GPVI-dimer, p< 0.05), suggesting thicker fibres, in the presence of GPVI. Fibrin-clots containing GPVI showed a reduced elastic modulus (G') (2.1-fold GPVI-monomer, p< 0.001; 0.8-fold GPVI-dimer, ns) and larger pore size (1.95-fold GPVI-monomer; 1.91-fold GPVI-dimer, p< 0.05). There were no differences in clotting/lysis or fiber density between WT and $GPVI^{-/-}$ mice clots, although a small reduction in pore size was observed in clots from $GPVI^{-/-}$ mice. SEM showed no changes in fiber size/number or platelet number between both groups, however, a clear reduction in the number of procoagulant platelets was observed in $GPVI^{-/-}$ mice compared to WT (1:2 procoagulant:non-procoagulant ratio for $GPVI^{-/-}$ vs 1:1 for WT, p≤0.05).

Conclusions: Our data indicate that clots are more porous, less stiff, and composed of thicker fibers in the presence of GPVI. Less procoagulant platelets were observed in PRP clots from $GPVI^{-/-}$ mice. These findings support GPVI as a possible therapeutic target to reduce thrombosis.

PB1661 | Targeting the Chemokine Receptor CXCR7 Favours an Anti-thrombotic Platelet Lipidome and Regulates Thromboinflammatory Functions

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Background: Elevated platelet surface expression of the chemokine receptors CXCR4 and CXCR7 in coronary artery disease-(CAD) patients influences prognosis following myocardial infarction-(MI). Although CXCR4 supports a pro-thrombotic profile, CXCR7 mediates an anti-thrombotic response following ligation with its physiological ligand.

Aims: To validate the significance of CXCR7-axis in regulating thromboinflammatory response following myocardial infarction and in the context of heparin induced thrombocytopenia-(HIT).

Methods: MI/RI model in mice, HIPA, aggregometry, flowcytometry, *ex vivo* thrombus formation, lipidomic (UHPLC-ESI-QTOF-MS/MS) analysis, were performed. Sera and corresponding IgG fractions were isolated from patients with clinically and serologically confirmed HIT (4Ts score 6-8, ELISA and HIPA positive) and non-HIT subjects.

Results: CAD patients having platelet CXCR7 surface expression ≥median showed significantly reduced aggregation. Administration of a pharmacological CXCR7 agonist improved functional recovery, regulated platelet activation and thrombotic response 24hrs-post-MI/RI, without affecting bleeding time or coagulation profile. It also reduced HIT^+ sera/IgG-induced platelet aggregates in HIPA, and thrombus formation *ex vivo*. CXCR7 agonist checked the formation of platelet-leukocyte aggregates in peripheral circulation post-MI/RI *in vivo*; similarly, counteracted HIT^+ sera/IgG induced platelet-neutrophil aggregate formation *ex vivo*. Release of pro-inflammatory mediators from HIT^+IgG activated platelets and levels of pro-inflammatory chemokines-cytokines in murine plasma post-MI/RI were reduced in presence of CXCR7 agonist. Mechanistic basis of this anti-thrombotic action revealed that CXCR7 agonist significantly altered the platelet lipidome favouring the generation of several anti-thrombotic lipid mediators including 12-HETrE, which acted through prostacyclin receptor, triggering adenylyl cyclase activity, and elevation of platelet inhibitory cAMP levels. On the contrary, CXCR7 agonist counteracted generation and release of pro-thrombotic lipid mediators (lysophosphatidylinositol, lysophosphatidylcholine, arachidonic acid, thromboxane, 12-HETE) from stimulated platelets, which affected intraplatelet activatory signalling cascade and therefore functional response.

Conclusions: CXCR7 ligation alters the platelet lipidome to regulate thrombotic and thromboinflammatory response, which might be utilized as a potential anti-thrombotic strategy following MI and in HIT without enhancing bleeding risk.

PB1662 | Platelets and Aortic Valve Calcification: Insights from a Rabbit Model and from Patients with Severe Aortic Stenosis

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Background: Calcific aortic stenosis (CAS) is the most common heart valve disease and a major cause of cardiac impairment. Recent preclinical studies indicate that platelets would play an active role in valve calcification through the release of transforming growth factor-β (TGF-β) or via the autotaxin-lysophasphatidic acid (LPA) axis. Platelets may therefore represent new biomarkers of CAS progression and outcome.

Aims: To study the correlation between platelet markers and aortic valve calcium score in patients with severe aortic stenosis (AS) and in a new rabbit model of aortic valve calcification.

Methods: Platelet count, number of activated platelets ($CD62P^+$), plasma levels of TGF-β, LPA, autotaxin and CT aortic valve calcium score (CT-AVC) were studied in 35 symptomatic AS patients.

New Zealand White rabbits were fed for 16 weeks with palm oil-enriched diet (5% palm oil) supplemented with vitamin D2 (25.000U/day/2.5kg for 15 days). Macrocalcification appearance was analyzed by computed tomography (CT) performed at regular intervals. Blood samples were collected at the same intervals to study the evolution of platelet count. After 16 weeks, the hearts were collected for hematoxylin-eosin and alizarin red stainings.

Results: In AS patients, CT-AVC was inversely correlated with platelet count and with the number of circulating $CD62P^+$ platelets. Plasma TGF-β levels showed positive correlation with the number of $CD62P^+$ platelets.

The rabbits exhibited massive aortic wall calcification, which was detectable by CT in 5 rabbits out of 6. Calcification nodules were detected in the aortic valve of 3 rabbits out of 6. Platelet count decreased after 8 weeks of diet, when calcification started to be visible by CT.

Conclusions: Platelet consumption is associated with CAS in AS patients and in our rabbit model. This supports a role for platelets in this process, possibly via TGF-β. Platelet parameters might help assessing AS severity and provide prognostic information.

PB1664 | Dietary Intake of Zinc Modulates Platelet Intracellular Levels and Haemostatic Parameters

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Background: Platelets are the main circulating pool of zinc, which is implicated as a cofactor in haemostasis. Zinc is chiefly stored in a-granules and is subsequently released following stimulation. Zinc deficiency is estimated to affect one-third of the world's population, but despite this the number of human studies on the impact of ion shortage are limited.

Aims: To investigate the direct impact of dietary restriction of zinc on haemostasis.

Methods: Thirty-six healthy volunteers were recruited after taking written consent. Dietary intake of zinc was restricted for 2 weeks (1 mg/day) followed by a two-week repletion (≥4.5 mg/day). Blood was collected at baseline and after depletion and repletion phases from which platelet rich plasma (PRP) was isolated.

Results: Inductively Coupled Mass Spectrometry data showed a reduction of zinc in platelets during dietary restriction compared to

habitual phase (6.78 ± 0.52 vs. 9.63 ± 1.19 μM). Collagen-induced platelet aggregation was significantly decreased during zinc depletion (45.06 ± 6.9%) compared to habitual phase (90.80 ± 6.62%). The lagtime to thrombin generation was increased during depletion compared to habitual period (16.39 ± 4.05 vs. 13.07 ± 1.07 min). Thrombodynamics data indicated that zinc depletion slowed the rate of clot growth (37.52 ± 3.02 μm/min) compared to habitual period (51.55 ± 2.35 μm/min). The rate of tissue plasminogen activator-induced lysis of PRP clots was delayed by depletion of zinc compared to the habitual phase (99.46 ± 5.19 vs. 78.30 ± 3.85 min). All parameters were found to return to baseline upon dietary repletion of zinc.
Conclusions: Our data indicate that dietary restriction of zinc adversely affects clot formation by attenuating platelet aggregation and thrombin generation. We provide the first direct evidence that the platelet pool of zinc in humans is directly related to dietary intake and demonstrate the key role of this ion as a cofactor in haemostasis.

PB1665 | Platelet Ageing Is Associated with Cytoskeletal Degradation and a Reduction in Cytoskeletal Dynamics

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Background: Platelet lifespan within the circulation of healthy humans is about 10 days but is shortened in a number of disease states. Despite reports indicating associations between increased percentages of newly formed platelets and risk of thrombosis, evidence of age-related differences in platelet composition is lacking.
Aims: To determine changes in composition of platelets across their lifespan using proteomic analysis coupled with immunofluorescence.
Methods: Platelets were stained with thiazole orange (TO) and separated into two subpopulations by cell sorting based on TO staining intensity (Aria IIIu). The top 10% of TO-positive platelets were categorised as 'young' and the bottom 30% as 'old'. The platelet proteome profile was determined by label-free quantitative mass spectrometry (nanoHPLC-Q Exactive MS/MS) and analysed by MaxQuant and Perseus software. Confocal microscopy was used to assess changes in the cytoskeleton of resting and adherent platelet subpopulations.
Results: Proteomics revealed a significant alteration in 78 proteins between young and old platelets. Young platelets had a relatively higher content of a number of cytoskeletal-associated proteins including emerin, gelsolin and twinfilin-2. Microscopy confirmed that young platelets had a more abundant and complex cytoskeleton compared to old platelets, with a significant reduction in the fluorescence intensity of α-tubulin (2435±354AU vs. 1034±79AU) and f-actin (2166±110AU vs. 1364±139AU; $p< 0.05$, n=4), but without any differences in platelet cross-sectional area. When spread on fibrinogen, young platelets readily formed lamellipodia with a larger spread area than old platelets (24.2±2.8μm^2 vs. 7.9±0.5μm^2; $p< 0.05$, n=4), with a higher abundance of actin nodules (63±1% vs. 24±1% of spread platelets; $p< 0.05$, n=4).
Conclusions: Platelet ageing is associated with a decline in cytoskeletal content and a subsequent reduction in cytoskeletal reorganisation. These reductions may contribute to a decrease in haemostatic function and an increase in clearance of platelets as they age.

PB1666 | Direct Temporal Labelling of Platelets *in vivo* Allows Monitoring and Examination of Newly Formed and Aged Platelets to Reveal Changes in Receptor Expression Levels and Loss of Functional Ability

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Background: Newly formed platelets, also known as reticulated or immature platelets, have been clinically correlated with adverse cardiovascular outcomes. However, much of what is thought about young platelets is largely based upon indirect measures or follows a pathological insult.
Aims: To develop direct temporal labelling of platelets in mice *in vivo* to allow tracking and molecular phenotyping without the need to induce experimental thrombocytopenia.
Methods: Fluorescently conjugated antibodies (Emfret, Germany) were administered (*i.v.*) to C57BL/6 mice at specific timepoints, to label platelets. Fluorescence activated cell sorting was used to isolate platelets in blood samples and mRNA levels quantified by qPCR. Alternatively flow cytometry was used to track or assess platelets in blood samples. Whole blood *ex vivo* aggregation and *in vivo* arterial injury model were used to examine platelet function.
Results: Newly formed platelets (< 24 hours old) contained the greatest levels of mRNA and contributed disproportionately to thrombi forming *ex vivo* and *in vivo*. Consecutive sampling demonstrated that new platelets are heterogenous in size, with a similar size distribution to older populations, and that their average size does not change as they age. Finally, paired flow cytometric analysis revealed that expression of MHC1, PECAM-1 (CD31), GPV (CD42d), integrin α2 (CD49b) and GPVI significantly decreased as platelets aged. In contrast CD9 expression significantly increased.
Conclusions: Unlike approaches that induce pathophysiological insult such as thrombocytopaenia, our approach allows the study of platelets throughout a normal life span. Our observations confirm (mRNA content and function) and dispel (platelet size) certain commonly held beliefs. Our studies also indicate that loss of key receptors including PECAM1 and GPVI, with concurrent increase in CD9 may underpin changes in hemostatic function associated with the ageing platelet. Finally, this approach will provide further functional and molecular insights into how different platelet-age profiles contribute to health and disease.

PB1667 | Omega-3 Fatty Acids Significantly Improve the Antiplatelet Profile of Triflusal

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Background: Triflusal irreversibly inhibits cycloxygenase-1, thus inhibiting platelet activation induced by Arachidonic Acid (AA). Meanwhile, the omega-3 fatty acids, EPA and DHA inhibit AA- and collagen-induced platelet activation, whereas they do not affect thrombin- and ADP-induced platelet aggregation.
Aims: We investigated the antiplatelet effect of Triflusal, EPA, DHA and their combination on platelet activation induced by various agonists.
Methods: Platelet Rich Plasma (PRP), isolated from blood of healthy volunteers was pre-incubated with Triflusal, EPA, DHA or their combination at various concentrations for 10min at 37°C, prior to activation by AA, TRAP-6, and ADP (10μM, 0.3mM and 5μM, respectively). Platelet aggregation was determined by Light Transmittance Aggregometry.
Results: The minimum inhibitory effect of Triflusal, DHA or EPA towards AA-induced platelet aggregation (13.00±5.80%, 4.00±0.57% and 14.00±2.82%, respectively) was observed at final concentrations of 400μM, 150μM and 50μM, respectively. Importantly, Triflusal combined with DHA (400μM and 50μM, respectively), inhibited AA-induced platelet aggregation by 85.00±12.12% (n=4). The inhibitory effect of Triflusal+EPA was only 18.00±2.08% (n=3) whereas its combination with DHA and EPA inhibited AA-induced platelet aggregation by 92.00±6.92% (n=3). The minimum inhibitory effect of Triflusal, EPA or DHA towards TRAP-6-induced platelet aggregation (11.50±5.64%, 32.00±7.52% and 18.00±11.01%, respectively) was observed at final concentrations of 500μM, 50μM and 100μM, respectively. Importantly, Triflusal combined with EPA (500μM and 50μM, respectively) inhibited TRAP-6-induced platelet aggregation by 75.00±2.82%. Similar inhibitory potency exhibited the combination of Triflusal+EPA+DHA (71.00±10.40%), whereas the inhibitory effect of Triflusal+DHA was only 37.00±0.70%. ADP-induced platelet aggregation wasn't significantly inhibited by Triflusal, DHA, EPA or their combination.
Conclusions: DHA significantly improves Triflusal's inhibitory effect towards AA-induced platelet aggregation, whereas in the presence of EPA, Triflusal expresses a potent inhibitory effect towards TRAP-6-induced platelet activation. The underlying mechanisms and the importance of these findings at the clinical level, in patients treated with Triflusal for the secondary prevention of ischemic events, need further investigation.

PB1668 | Platelet-Derived Extracellular Vesicles Actively Process Proteins and Present Antigen via MHC Class I

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Background: In addition to their role in hemostasis, platelets are also recognized for their contribution in immunity. Like megakaryocytes, platelets contain proteasome and present antigen-MHC class I molecules. Once activated, platelets release extracellular vesicles (PEV) formed by the budding of cytoplasmic membranes. PEVs are heterogeneous in terms of content (e.g. nucleic acid, enzymes, mitochondria) and surface molecules, suggesting that they may play diverse functions. However, whether the proteasome is also transferred and can support protein processing has never been examined.
Aims: We hypothesized that a functional antigen processing and presenting machinery is transferred to PEV by activated platelets.
Methods: Immunoblotting, flow cytometry and an enzymatic activity assay were used to reveal if PEV from activated platelets contains active 20S proteasome and presentation molecules. Ovalbumin antigenic peptide and whole protein were used to determined that PEV could process it through their active proteasome and load antigenic peptide in MHC-I molecule.
Results: These proteasome-containing PEV were found to express MHC-I and lymphocyte co-activation proteins such as CD40L and OX40L. Proteasome-containing PEV exposing MHC-I molecules were identified in blood in healthy volunteers, were increased in inflammatory conditions, and were found in the lymphatic system. Intravenous injection of PEV in mice revealed that they could reach lymphoid organs where they could interact with lymphocytes. In OT-1 mice, all T lymphocyte TCR recognize processed ovalbumin peptide loaded in MHC-I molecule, thereby stimulating these cells to secrete cytokines and to proliferate. Importantly, the co-incubation of PEV and lymphocytes from OT-1 mice led to the production of cytokines and lymphocyte proliferation, a process that was blunted by PEV 20S proteasome inhibition.
Conclusions: Contrarily to platelets, which cannot circulate in lymph, PEV have privileged access to immune cells through the lymphatic system. With their machinery capable of protein processing and antigen presentation, this study reveals a novel role for PEV in immunity.

PB1669 | A Novel Reporter Mouse Line Enables *in vivo* Imaging of Intracellular Calcium Fluxes in Platelets

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Background: Platelets are increasingly recognized as important players of inflammation, interacting with immune and endothelial cells. Ca^{2+} is an essential second messenger in virtually all cells, regulating a wide range of fundamental cellular processes. In platelets, the elevation in Ca^{2+} contributes to various steps of cellular activation, such as reorganization of the actin cytoskeleton necessary for shape change, degranulation or platelet aggregation. So far, measuring Ca^{2+} influx in platelets is mainly performed using fluorescent Ca^{2+} indicators like Fura-2. However, this approach is limited to *in vitro* studies.
Aims: We aim to overcome this limitation by generating a new mouse model that allows monitoring rises in intracellular Ca^{2+} levels in platelets *in vivo*.
Methods: We generated mice that express the fluorescent calcium indicator protein GCaMP5G, along with tdTomato (marks reporter-positive cells) exclusively in megakaryocytes and platelets using the *Pf4*-Cre system. The expression of the calcium sensor was investigated by fluorescence microscopy. Platelet function was assessed by *in vitro* assays, such as flow cytometry, aggregometry, spreading and flow adhesion assays. Intravital imaging was performed to assess the feasibility of the sensor in living animals.
Results: Expression of the sensor in platelets was confirmed and neither Ca^{2+} influx, nor basic platelet functions like platelet activation and aggregation were altered. Using a flow adhesion system to assess thrombus formation on a collagen-coated surface, we were able to visualize Ca^{2+} fluxes in platelets in a growing thrombus *ex vivo*. More importantly, calcium signals in platelets could be visualized *in vivo*, using different models of arterial thrombosis and thrombo-inflammation.
Conclusions: The presented mouse model allows imaging of Ca^{2+} rises in platelets *in vivo*. This provides a valuable tool not only to study platelet signaling in the context of thrombus formation but provides the possibility to visualize platelet responses upon interaction with other cells in the context of (patho-)physiological processes.

PB1670 | Hyperglycemia Primes Procoagulant Platelet Formation and Exacerbates Ischemic Stroke Independent of Platelet Glucose Uptake

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Background: Hyperglycemia is a common comorbidity for ischemic stroke and is associated with worsened outcomes. Platelets are critical mediators of stroke and hyperglycemia mediates platelet hyperactivity and thrombosis through platelet glucose metabolism.
Aims: In this study, we investigated the role of platelet glucose metabolism in hyperglycemia induced exacerbation of ischemic stroke.
Methods: Mice with platelet-specific double-knockout (DKO) of Glut1 and Glut3 are unable to metabolize glucose in platelets. DKO mice and their littermate controls (WT) were subjected to one-hour transient middle cerebral artery occlusion under normoglycemic and streptozotocin-induced hyperglycemic conditions. Brain infarct size, platelet activation and brain platelet-neutrophil complexes were analyzed 24 hours after stroke.
Results: Under normoglycemic conditions, DKO mice were protected from ischemic stroke as apparent by reduced infarct volumes (62.8±19.5 vs. 34.4±20.7 mm^3, p=0.01). Hyperglycemia significantly increased infarct size in WT mice (94.4±17.8 vs. 62.8±19.5 mm^3, p=0.0036). Remarkably, hyperglycemia also increased infarct volume in DKO mice (88.6±8.1 vs. 34.4±20.7 mm^3, p< 0.0001), completely abolishing the protective effect observed under normoglycemia. Hyperglycemia increased platelet P-selectin and platelet-neutrophil aggregate trafficking to the brain after stroke, independent of platelet glucose metabolism. Phosphatidylserine positive (PS^+) procoagulant platelets mediate injurious trafficking of neutrophils to the brain after stroke. Interestingly, under normoglycemic conditions, DKO mice are unable to form procoagulant platelets in response to dual agonist stimulation. Conversely, hyperglycemia potentiated procoagulant platelet formation in WT animals (16.4±3% vs. 11.3±3.1% PS^+ platelets, p=0.042) and restored procoagulant platelet formation in DKO animals (11.3±4.3% vs 2.2±0.5% PS^+ platelets, p=0.0003).
Conclusions: Hyperglycemia aggravates ischemic stroke outcomes independent of platelet glucose uptake. Furthermore, we demonstrated that hyperglycemia primes procoagulant platelet formation. We hypothesize that the increase in procoagulant platelets drives the increase in injurious platelet-neutrophil interactions in the hyperglycemic stroke brain. This underscores the therapeutic potential for strategies targeting procoagulant platelet formation for the treatment of ischemic stroke.

PB1671 | C-type Lectin-like Proteins D and E, but Not Botrocetin, Cause Thrombocytopenia during Bothrops jararaca Snakebite Envenomation

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Background: Thrombocytopenia is frequently encountered in patients bitten by *Bothrops jararaca* snakes. Although various proteins

that activate or inhibit platelets *in vitro* have been isolated and characterized from *B. jararaca* venom (BjV), it has not been determined which ones account for the development of thrombocytopenia *in vivo*. We showed previously that BjV incubated with catalytic inhibitors of metalloproteinases or serine proteinases, and/or anti-botrocetin (a VWF-dependent C-type lectin-like protein, CTLP) antibodies, and injected in rats and mice, still evoked platelet consumption.

Aims: To identify and characterize BjV toxins that cause thrombocytopenia *in vivo*.

Methods: Proteins were purified using ultrafiltration and liquid chromatography, and their primary structures were characterized by mass spectrometry. The biological activity of venom fractions was tested *in vivo* and *in vitro*. All procedures involving the use of animals or human donors were approved by IACUC from Instituto Butantan and Plataforma Brasil, respectively.

Results: By ultrafiltration, we observed that only venom components passing 50-kDa molecular weight cutoff membranes caused thrombocytopenia in mice, and this activity was completely independent of the hemorrhagic or coagulant activity of BjV. Ultrafiltered proteins were further separated by hydrophobic interaction, ion exchange and reversed-phase chromatography. Two 30-kDa proteins that induced thrombocytopenia in mice were isolated, and they showed primary structures similar to CTLPs, and were called CTLP-D and CTLP-E. Surprisingly, they did not induce platelet aggregation in platelet rich plasma or whole blood from humans or mice. Currently, experiments are being carried out to understand the mechanisms of action of CTLP-D and -E.

Conclusions: These findings reinforce previous results that thrombocytopenia occurs simultaneously to hemorrhage and coagulation following snakebites, but they are independent phenomena. Such results are important to understand the pathophysiology of *B. jararaca* envenomation, particularly of hemostatic disorders that may complicate the clinical picture of bitten patients. Funding: FAPESP, CNPq, ONICET, CAPES, Fundação Butantan.

PB1672 | Variability in Platelet Function among Patients with von Willebrand Disease Type 2B (VWD2B): Focus on Patients Historically Diagnosed with Montreal Platelet Syndrome (Historic-MPS)

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Background: (1) In VWD2B, spontaneous VWF binding via gain-of-function A1 domain variants to platelet GPIbα results in loss of high-molecular-weight VWF multimers, variable thrombocytopenia and a bleeding phenotype. Intrinsic platelet dysfunction has been reported in VWD2B; this can contribute to bleeding.

(2) The historic-MPS kindred was shown to have VWD2B with the V1316M variant (Jackson et al, Blood 2009).

Aims: To compare platelet function among VWD2B patients, with a focus on historic-MPS.

Methods: 9 patients, including 3 of the historic-MPS kindred, with genetically-confirmed VWD2B (6 V1316M; 2 R1341Q; 1 R1309C), with platelet counts ranging from 16-167x10^9/L and VWF:activity/VWF:antigen ratios, from 0.15-0.56, were studied. Informed consent was obtained from all subjects, and the study was approved by the institutional REBs. Platelets were studied by flow cytometry after activation in citrated-whole blood with: thrombin (T; 1U/mL); collagen (C; 20μg/mL); TRAP (50μM); or T+C or TRAP+C in the presence of GPRP and 2mM Ca^{2+}. Platelets were identified using anti-CD41-FITC or anti-CD42a-PE. α-granule release was determined by anti-CD62P-PE binding, and GPIIb-IIIa activation, by PAC1-FITC binding. Procoagulant, phosphatidylserine (PS)-exposing platelets were identified by annexinA5-Alexa647 binding, and platelet-derived extracellular vesicles (EVs), as CD41-FITC positive events < 0.75μm.

Results: Historic-MPS patients' platelets were larger than those of the other VWD2B patients, with platelet sizes (expressed as median forward scatter) of 30,900±5,200 (mean±SEM) vs 11,100±2,200 ($P< 0.005$). CD62P expression on activated historic-MPS platelets ranged from 40%-60% that on the other patients' platelets ($P< 0.004$-0.0001), while PS exposure was increased by 1.5-3-fold ($P< 0.026$-0.0001). Neither GPIIb-IIIa activation nor EV formation differed between the 2 groups.

Conclusions: This study uncovers an unexpected variability in platelet function among VWD2B patients: historic-MPS patients with the V1316M variant exhibit decreased α-granule secretion upon platelet activation and increased PS exposure compared with platelets from other patients with the same V1316M variant or the R1341Q or R1309C variants.

PB1673 | Mepacrine Uptake & Release Assay in Thrombocytopenic Patients

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Background: Thrombocytopenia is one of the most challenging limitations for lumi-aggregometry assays and even more if platelet size is increase. Interpretation in such situations requires deep knowledge and some cases need complementary assays to get a conclusion. Flow cytometry (FC) assays assess platelet behavior individually and in combination with lumi-aggregometry, provides a better approach about platelet function. Mepacrine uptake/release assay by FC (Mep u/r) is used to characterized storage pool disease (SPD) and evaluates granule content release.

TABLE 1 Comparation between means

Platelet count	Mepacrine	
	Uptake	Release (TRAP-6 60µM)
(.10^9/L)	log (mep/aut)/log FSCH	log (mep/mep-r)
	0.55±0.09 (mean ±2SD)	0.55±0.17 (mean± 2SD)
50 (n=5)	0.54±0.12	0.56±0.11
100 (n=5)	0.55±0.04	0.55±0.10
300 (n=5)	0.53±0.08	0.53±0.03
ANOVA	p< 0.05 (ns)	p< 0.05 (ns)

aut= platelets MFI without mepacrine; mep= resting platelets MFI with mepacrine;FSCH= mean of frontal scattering histogram; mep-r= platelet MFI after TRAP-6 stimulation

Aims: To evaluate some technical aspects of Mep u/r and the contribution along with lumi-aggregometry in functional characterization of patients with thrombocytopenia.

Methods: We evaluate a normal group (NG, n: 37), a SPD group (SPD, n: 4) as pathologic control, and 14 patients with thrombocytopenia (TP1-14; platelet count < 150.10^9/L). Mep u/r was carry out as Cai et al. 2016, using platelet rich plasma, Trap-6 60uM as agonist inducing maximum degranulation. Platelet count dependency was tested in 5 individuals from NG at 50-100-300.10^9platelets/L. ATP release by lumi-aggregometry (ATPr) were performed using ADP, ADR, arachidonic acid and collagen. TP group results was interpreted according to platelet count.

Results: Mep u/r was independent of the platelet count in the evaluated range (table 1). SPD group had normal platelet count, absent ATPr and low Mep u/r. ATPr in PT group was normal in PT1-11 and correlates well with Mep u/r except in 2 cases (PT10-11). Degranulation of PT10-11 was reevaluated with PMA on FC showing normal results. PT12-14 had decrease ATPr or its inconclusive due to big platelet size and were diagnosed as SPD through abnormal Mep u/r (table 2).

Conclusions: Mep u/r can be used on thrombocytopenia and correlates well with ATPr. PT10-11 showed abnormal Mep u/r with TRAP-6 exclusively, suggesting some alteration in its signaling pathway. In such situations it may be necessary use more than one agonist.

PB1674 | Liver Diseases Contribute to Functional Platelet Aggregation Defects

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Background: The role of platelets in hemostasis involves their adherence to sites of vessel injury, aggregation to form plugs. Agents that physiologically activate platelets in vivo include adenosine diphosphate (ADP), epinephrine, collagen, thromboxane A2 (TxA2) and serotonin. Vascular and platelet dysfunction represent complications of hepatic disease.

Aims: The purpose of this study is to investigate the effect of various agonists on platelet aggregation profile of patient with hepatic disease.

Methods: Icteric Samples from the clinical lab from Loyola University medical center were randomly selected for this study (n=25). Blood samples from the healthy donors were included to prepare platelet rich plasma (PRP) and platelet poor plasma (PPP). PRP and PPP from various donors were separated to make pools. Four different pools were made namely Normal PRP, Icteric PRP, Icteric PRP + Normal PPP 1:1, Normal PRP + Icteric PPP1:1. Agonists such as ADP, arachidonic acid (AA), epinephrine and collagen were used to activate the platelets. The final assay concentration for ADP is 20 uM, AA is 500 ug/ml, collagen is 190 ug/ml and epinephrine is 10 ug/ml.

Results: All results were compiled in terms of percent aggregation of platelets and represented as mean ± standard deviation. Normal PRP showed platelet aggregation response reaching a peak aggregation of 93.± 27.70 with ADP agonist. Icteric PRP, and two different

TABLE 2 Lumy-Aggregometry and FC results

	Platelet count (range= 150-450) (.10^9/L)	ATPr* (according to platelet count)	Mepacrine Uptake (range= 0.46-0.64)	Mepacrine Release (range= 0.38-0.72)
NG (n= 37)	150-490**	Normal	0.46-0.53**	0.46-0.70**
SPD (n=4)	150-338**	Absent with all agonist	0.19-0.33**	0.04-0.24**
PT1-9 (n=9)	58-146**	Normal	0.53-0.62**	0.47-0.79**
PT10	55	Normal	0.59	0.08
PT11	110	Normal	0.60	0.15
PT12	108	Absent with all agonits	0.28	0.12
PT13	82	Absent with all agonist	0.30	0.18
PT14	74	Inconclusive	0.34	0.29

* AA: 0,5-1 mM; ADP: 2,5-5 µM; ADR: 10-50 µM: COL1 : 1- 8µg/ml.
** Min-max range.

pools did not show any significant platelet aggregation with ADP. Varying degrees of the inhibition of platelet aggregation was noted with epinephrine, collagen and arachidonic acid in comparison to normal PRP.

Conclusions: On the basis of these results it is concluded that while the normal PRP showed platelet aggregation responses to various agonists' icteric plasma, when mixed with normal PRP produced inhibition of platelet aggregation. This data suggests that the icteric plasma contains an endogenous substance which produces the inhibitory effect on agonist induced platelet aggregation.

PB1675 | Large and Small Platelets Differently Contribute to Whole-Blood Thrombus Formation

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Background: Human blood platelets circulate as heterogeneous populations of different age, size, and composition. In response to agonists, platelets separate into subpopulations with differential expression of activation markers, including P-selectin and phosphatidylserine. So far, it has remained unclear, how platelet size and structure within a subject relate to activation properties.

Aims: To assess the activation potential of size-separated human platelet populations under stasis and shear flow.

Methods: Fractions of large and small platelets were separated from healthy blood donors (n=6) by differential centrifugation. Glycoprotein (GP) expression and activation markers were measured by multicolor flow cytometry; platelet aggregation by light transmission aggregometry. Platelet and leukocyte depleted whole blood was reconstituted with large, small (same count or same crit = platelet count x volume) or unsorted platelets and examined for kinetics of multiparameter thrombus formation on collagen.

Results: Separated platelet fractions markedly differed in mean volume: large (10.3-13.8 fL) versus small (6.8-9.5 fL, p< 0.0001). In response to collagen, large platelets aggregated faster than small platelets. Expression of GP as well as activation markers increased with platelet size. In the total platelet pool, activated platelets with high levels of GPVI or GPIba (upper quartiles) showed highest expression of secretion and other markers (P-selectin, CD63, TLT1, aIIbb3). In leucodepleted whole blood reconstituted with large, small (*N.B. crit-adjusted*) or unsorted platelets, significant differences in thrombus formation were observed. For the large platelets: (i) kinetics of adhesion under high-shear flow were faster; (ii) thrombus multilayer formation was increased; (iii) the activation marker P-selectin was increased. In contrast, for crit-adjusted small platelets, phosphatidylserine exposure was increased.

Conclusions: Within subjects, platelets with high expression levels of surface glycoproteins, such as present in the fraction of large platelets, show an increased activation potential, which however did not extend to procoagulant activity.

PB1677 | A Novel Methodology to Study αIIbβ3 Interactions with Polymerizing Cross-linked Fibrin

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Background: Vascular injury leads to platelet deposition, followed by the generation of cross-linked fibrin within seconds to minutes, leading to the formation of a stable platelet thrombus. Fibrin formation requires thrombin's action on fibrinogen, producing fibrin monomers, and catalysis by factor XIIIa of the reciprocal transamidation of the C-terminal γ-chain peptides from adjacent fibrinogen molecules. While the interaction of platelets with fibrinogen has been shown to be mediated by the interaction of fibrinogen γ12 peptide with the RGD pocket on integrin αIIbβ3, the interaction with cross-linked fibrin is poorly defined, despite its biological and clinical importance in thrombus stabilization and clot retraction. We recently studied the interaction of washed platelets with polymerizing fibrin in an aggregometer by preventing the interaction of γ12 with the RGD pocket with 150 μM RGDW, a concentration that completely eliminates aggregation to a thrombin receptor activating peptide (T6) (Figure 1A). When stimulated with 0.2 u/ml thrombin, platelets undergo a delayed wave (DW) of aggregation ~4-5 min after thrombin stimulation (Figure 1B). The DW correlates with the appearance of cross-linked γ-chains, (γ-γ dimers) (Figure 1C) and is inhibited by 5 mM Gly-Pro-Arg-Pro (Figure 1B), demonstrating a requirement for fibrin polymerization.

Aims: To expand our studies to HEK-293 cells expressing WT integrin αIIbβ3.

Methods: Platelet Aggregometry.

Results: HEK-αIIbβ3 cells undergo a marked DW in response to thrombin activation in the presence of fibrinogen (100 μg/ml), that parallels the platelet response. In comparison, HEK cells not expressing αIIbβ3 have no significant DW (Figure 2). HEK-αIIbβ3 cells expressing the MIDAS mutation D119A, which is known to disrupt

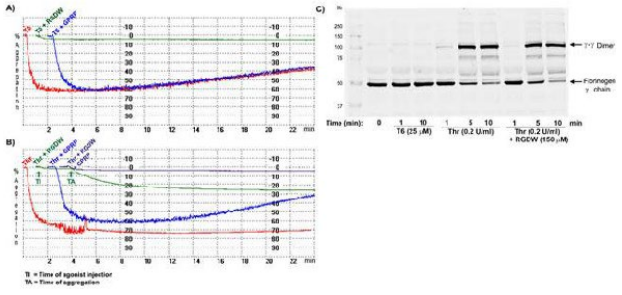

FIGURE 1

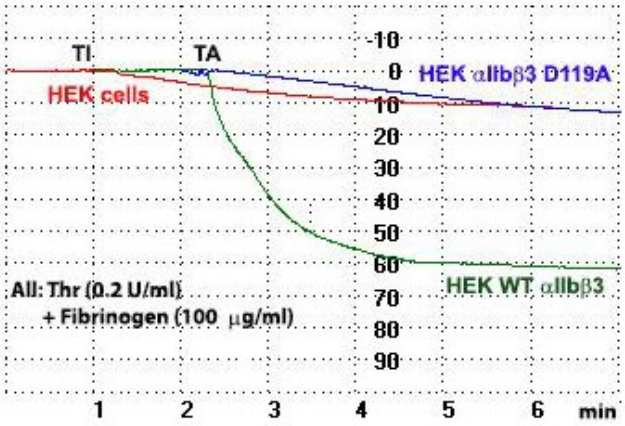

FIGURE 2

binding to fibrinogen, fragment D98, and D-Dimer do not undergo DW (Figure 2).

Conclusions: HEK-αIIbβ3 are able to interact with polymerizing fibrin providing an novel methodology to study the αIIbβ3-specific residues that mediate the interaction.

PB1678 | The Role of Coagulation-generated Components on GPVI-mediated Platelet Activation

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Background: Platelets and the coagulation system contribute to haemostasis and thrombosis in an interactive manner. Thrombin activates platelets through PAR1 and PAR4 receptors, whereas collagen-related peptide (CRP) stimulates through glycoprotein (GP)VI. Coagulation triggering is known to enhance the platelet-stimulating effect of CRP. It is so far unclear if this enhancement is solely thrombin dependent, or also mediated by other (anti)coagulation factors. Factors known to bind to platelets include amongst others FXa, FXIIIa and activated protein C (APC).

Aims: To investigate the modulating effects of FXa, FXIIIa and APC on GPVI-induced platelet activation processes.

Methods: Washed platelets were treated with FXa, FXIIIa or APC, combined with a suboptimal concentration of CRP. Markers of platelet activation, active integrin $\alpha_{IIb}\beta_3$ and P-selectin expression, were evaluated by flow cytometry. Changes in cytosolic $[Ca^{2+}]_i$ of Fura-2 loaded platelets were assessed in a well-plate based assay.

Results: Platelet treatment with the (anti)coagulation factors FXa, FXIIIa or APC significantly enhanced all CRP-induced activation markers (integrin activation, P-selectin expression). Of these, FXIIIa or APC were ineffective by themselves, whereas FXa alone had a mild stimulating effect. All three (anti)coagulation factors further enhanced the CRP-induced rises in $[Ca^{2+}]_i$ in a dose-dependent way. Again, FXIIIa and APC were not stimulating by themselves. Markedly, FXa alone caused a measurable calcium flux, which was abolished by the addition of thrombin inhibitors, suggesting that those effects are thrombin-mediated and not due to the direct action of FXa.

Conclusions: Coagulation FXIIIa and anticoagulation factor APC have a positive priming effect on GPVI-mediated platelet responses. The platelet-activating and -enhancing effects of FXa rely on the in-situ formation of traces of thrombin.

PB1679 | Evaluation of a New Agonist (ADP-HS®) for Platelet Function Testing with Light Transmittance Aggregometry: A Bi-Center Study

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Background: ADP is a platelet agonist used in aggregation assays when screening for platelet disorders or platelet response to P2Y12 blockers. The addition of divalent cations in the ADP reagent could potentially minimize P2Y receptor desensitization due to blood sampling-associated platelet activation and/or hemolysis (Baurand A *et al*. Mol Pharmacol. 2005;67(3):721-33).

Aims: To compare the results obtained between a new ADP High Sensitivity reagent (ADP-HS®, Agro-Bio) using a formulation including divalent cations and the in-house ADP reagent, after assessing platelet aggregation with light transmittance aggregometry (LTA) in a bi-center evaluation.

Methods: During 5 months, we enrolled patients assessed with LTA for platelet disorder or suspicion of abnormal antiplatelet response and healthy donors as controls. Platelet functions were tested in parallel using ADP-HS® reagent and the in-house reagent in each center at two concentrations: 2 and 10 μM (ADP-Chrono-Log® on TA-8V® SD-Medical aggregometer or ADP-Helena® on APACT-4004® aggregometer, respectively).

Results: We enrolled 128 patients (39 with aspirin, 54 with aspirin+P2Y12 blockers, 16 with P2Y12 blockers, 19 with platelet disorder suspicion) and 70 controls. In the control group, platelet aggregation maximum amplitudes were similar between ADP-HS® and in-house ADP reagent in both centers: 71.6 ± 18.7 vs 68.9 ± 16.5%

and 89.6 ± 3.2 vs 89.3 ± 2.6%, respectively (10 μM). The values did not significantly differ (p > 0.05 Wilcoxon) in patients with P2Y12 blockers monotherapy (56.8 ± 26.4 vs 57.6 ± 27.9% and 64.6 ± 9.4 vs 58.6 ± 10.8% at 10 μM, respectively), with aspirin monotherapy (76.5 ± 11.2 vs 78.1 ± 11.6% and 83.2 ± 6.7 vs 80.7 ± 7.7% at 10 μM, respectively), with aspirin+P2Y12 blocker (50 ± 18.6 vs 47.1 ± 19.9% and 66.9 ± 10.4 vs 64.6 ± 12.4% at 10 μM, respectively), or with platelet disorder suspicion (77.8 ± 23.9 vs 79.9 ± 24.4% and 78.5 ± 9.7 vs 76.8 ± 16.7% at 10 μM, respectively).

Conclusions: ADP-HS® reagent displayed similar performances than current ADP reagents used in LTA platelet aggregation assays with two different centers.

PB1681 | Glycans on the Surface of Platelet Microparticles Mediate Binding by Hepatocytes

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Background: Platelets shed platelet microparticles (PMP) when activated or stored. Procoagulant PMP may enhance efficacy of components once transfused, and it is important they are cleared efficiently to prevent vaso-occlusion. The majority of PMP are phosphatidylserine (PS)-negative, suggesting other clearance mechanisms. As desialylation is important for platelet clearance, we hypothesised similar mechanisms for PMP.

Aims: To investigate the role of glycans in PMP binding.

Methods: Apheresis platelets were collected, stored in 70% SSP + 30% plasma at 22°C with agitation, and tested at day 1, 5 and 7 post-collection. Platelet and PMP were characterised using flow cytometry and annexin V (AnV, PS-exposure), CD41 antibody and/or glycan-binding lectins *Ricinus communis* Agglutinin-1 (RCA-1; galactose), succinylated Wheat Germ Agglutinin (sWGA, βGlcNAc), *Sambucus nigra* (SNA, α2-6-linked) and *Maackia amurensis* (MAA, α2-3-linked sialic acid).

PMP were stained with Deep Red (5 μM) and isolated by centrifugation and binding of PMP was assessed using HepG2 cells. PMP were treated with 20 U/mL galactosidase and neuraminidase to cleave galactose or sialic acid respectively. PS was inhibited with lactadherin (8 μg/mL). p < 0.05 was considered significant using 1-way ANOVA or unpaired t-tests.

Results: Following platelet storage, there was a 2-fold increase in RCA^+/AnV^+ PMP (p = 0.0323), indicating desialylation, accompanied by an increase in RCA^+/AnV^+ platelets (p = 0.0584, n = 6). RCA^+/AnV^- PMP numbers were not changed at day 7. $MAA^+/CD41^+$ PMP numbers (n = 4) decreased by a 3-fold, whereas $SNA^+/CD41^+$PMP did not change, indicating loss of α2-3-linked sialic acid. $sWGA^+/AnV^+$ PMP numbers remained unchanged, however, there was a 2-fold increase in $sWGA^+/AnV^+$ platelets (n = 5, p = 0.0115), indicating degalactosylation of procoagulant platelets.

At day 1, in contrast to neuraminidase, galactosidase induced a 30% increase (p = 0.0654), while lactadherin reduced HEPG2-binding (p = 0.0137), indicating PS was not the main mechanism.

Conclusions: PMP and platelets expose glycans, which are differentially modulated by storage. PMP bind to HepG2 cells in a glycan-mediated mechanism.

PB1683 | The Role of Platelets in the Adhesion of Normal Freshly Isolated Lymphocytes to Fibrin

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Background: The processes of primary hemostasis, which are caused by adhesion, aggregation and subsequent retraction of the platelet plug, cannot be considered in isolation from the processes of coagulation hemostasis. The final manifestation of both coagulation pathways is the appearance of fibrin filaments and the organization of a dense fibrin clot due to a reduction in its platelet component.

Aims: Investigate the role of fibrin in the interaction of lymphocytes and platelets isolated from blood.

Methods: The blood of practically healthy donors, with an adhesive surface in static conditions, we used fibrin-coated millipore filters. Served as control filters, impregnated with Na CL 0.9% (control 1) or autologous platelet-poor plasma (control 2). Pre-prepared filters were placed on the bottom of the wells of a plastic plate and pre-prepared lymphocyte-platelet suspension isolated from the blood of practically healthy individuals was placed. After incubation under static conditions and subsequent washing, the cell pool was resuspended and the number of lymphocyte-platelet coaggregates remaining in suspension was counted in a light microscope.

Results: It was established that after incubation of platelets and lymphocytes on the surface of fibrin filters, their amount in suspension was reduced almost 2.5 times compared to filters impregnated with NaCL 0.9% or platelet-poor plasma. At the same time, both the average number of platelets per lymphocyte and the total number of lymphocytes decreased (p < 0.05).

Conclusions: The decrease in their number in the suspension indicates the adhesion of lymphocytes in the composition of coaggregates with platelets to the fibrin coating.

Thus, lymphocytes are able to contact with fibrin, being in the composition of lymphocyte-platelet coaggregates and the presence of fibrin itself, and not of plasma, is of primary importance.

PB1684 | Platelet Activation and Aggregation Induced by NS1 Dengue Protein

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Background: Platelets role as immune mediators in infection has recently been recognised. Dengue virus (DENV) infection is an increasing public health problem worldwide. NS1 is a highly conserved DENV glycoprotein associated to the infective capacity of the virus and the disease clinical outcome; recent data suggest a critical role of NS1-DENV in the thrombocytopenia present in severe dengue infection.
Aims: To study platelet response to DENV NS1 protein.
Methods: NS1-DENV was cloned into pHLSec plasmid, competent E. coli cells were transformed and plasmid DNA was extracted. HEK293 cells were transfected with plasmid DNA, liquid chromatography was used to purify the protein in its full form and in its individual domains. Platelet-rich plasma (PRP) was obtained from healthy donors and dengue patients by centrifugation of citrated blood and incubated with DENV NS1 proteins. After protein stimulation, platelets were stained with specific antibodies and cells fixed with 1% paraformaldehyde solution. Platelet activation was assessed by flow cytometry using an anti-CD41-PC7 antibody. Expression of platelet activation markers P-selectin and GpIIb-IIIa was assessed using anti-CD62P-PE and PAC1-FITC antibodies, respectively. The activation patterns obtained were compared with those obtained with classical platelet agonists ADP, collagen and epinephrine. Platelet aggregation was also assessed using NS1 DENV full protein and its individual domains as agonists.
Results: Flow cytometry analysis showed that the NS1 full protein as well as NS1 wing domain are able to induce the expression of P-selectin receptor and GpIIb-IIIa complex on platelet surface. DENV NS1 Full but not its domains induce platelet aggregation. Dengue patients show increased platelet activation and aggregation patterns.
Conclusions: DENV Protein NS1 induces platelet activation and aggregation. These results may partially explain the cause of thrombocytopenia in DENV virus infection and emphasize both the critical role of DV NS1 in dengue pathogenesis and the important role of platelets in host defense against viruses.

PB1685 | Platelet PN-1 Regulates Clot Structure and Retraction

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Background: Protease Nexin-1 (PN-1), is a strong inhibitor of serine proteases. We recently established platelet PN-1 as a negative regulator of thrombin generation and thrombosis. Altered clot architecture and retraction are known to be associated with abnormal thrombin generation.
Aims: To evaluate the role of platelet PN-1 on clot structure and retraction.
Methods: Clot retraction was assessed after recalcification in glass tubes of human platelet-rich plasma (PRP) incubated or not with a PN-1-blocking IgG, or of PRP obtained from wild-type (WT) or PN-1 knock-out (PN-1$^{-/-}$) mice. Clot architecture was evaluated with confocal microscopy of human or mice platelet-rich clots (PRC). Platelet adhesion and spreading were assessed on immobilized fibrinogen at 37°C. Clot viscoelasticity properties were analysed with ROTEM and the phosphorylation state of platelet tyrosine proteins and myosin light chain (MLC) by western blot of solubilized clots.
Results: Surprisingly, clot weight was increased by 66% in human PRC incubated with a blocking anti-PN-1 IgG and by 40% in PN-1$^{-/-}$ PRC compared to their controls. Confocal microscopy images showed a fibrin network structure more porous in PRC from PN-1$^{-/-}$ mice or in human PN-1 inhibited PRC. TEMograms showed that the blocking anti-PN-1 IgG induced a 26% decrease of the maximum clot elasticity. Adhesion to fibrinogen was markedly decreased ($P < 0{,}001$) in TRAP-4 or convulxin- activated platelets from PN-1$^{-/-}$ mice compared to WT. Platelets PN-1$^{-/-}$ display a more pronounced actin polymerization in response to TRAP-4 activation. Interestingly, platelets from PN-1$^{-/-}$ mice exhibit a persistence of MLC phosphorylation during clot formation.
Conclusions: Despite its anti-thrombin effect, platelet PN-1 accounts for the formation of a tight structure of PRC. We thus identify a critical positive role of PN-1 on platelet-driven clot retraction. Platelet PN-1 has an inhibitory effect on F actin polymerization and MLC phosphorylation.

PB1686 | Tyrosine Kinase Inhibitor Sunitinib Delays Platelet-induced Coagulation: Additive Effects of Aspirin

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[2]*Maastricht University Medical Center (MUMC+), Medical Oncology, Maastricht, the Netherlands*

Background: Sunitinib is a multi-target tyrosine kinase inhibitor (TKI) used for treatment of patients with solid tumors, and is associated with an increased bleeding risk. We previously showed that platelets sequester sunitinib, which affects activation responses. Cardiovascular disease is a common co-morbidity in cancer patients, resulting in additional anti-platelet treatment. The additive effect of anti-platelet drugs on top of sunitinib treatment is unclear.
Aims: Investigating consequences of the antiplatelet drug aspirin in combination with sunitinib treatment on platelet activation properties and secondary coagulation.
Methods: Whole blood or isolated platelets from healthy volunteers were incubated with sunitinib and/or aspirin or vehicle. In addition,

blood was used from aspirin-treated healthy volunteers. Platelet activation by flow cytometry, changes in $[Ca^{2+}]_i$, light transmission aggregometry and whole blood perfusion over collagen alone or co-coated with tissue factor (TF) were measured.

Results: Sunitinib dose-dependently suppressed glycoprotein (GP) VI-induced platelet procoagulant activity and rises in $[Ca^{2+}]_i$ in washed platelets. Reduced exposure of phosphatidylserine (PS) was also observed in thrombi after perfusion of sunitinib-treated whole blood over collagen at high shear rate; this was paralleled by decreased thrombus formation. In the presence of TF triggered coagulation through PS exposure, we found that sunitinib delayed and decreased fibrin formation.

Next, we tested the effects platelet inhibition by aspirin in combination with sunitinib. This resulted in a further reduction (±75%) of GPVI-dependent platelet aggregation, when compared to sunitinib alone. Moreover, addition of sunitinib to blood from aspirin-treated healthy volunteers further reduced thrombus formation and PS exposure (by 30% and 50% respectively) on collagen under flow both in the presence and absence of coagulation.

Conclusions: Sunitinib treatment suppresses GPVI-induced platelet procoagulant activity and ensuing fibrin formation. This effect is enhanced by additional treatment with aspirin. These results ask for awareness among clinicians for the combined antiplatelet effects of TKIs together with aspirin.

PB1687 | Implication of Platelet Adhesiveness in the Risk of Venous Thromboembolic Disease

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Background: Previous studies show association between global platelet function, measured by the PFA-100 test, and risk of venous thromboembolic disease (VTE). This relation does not involve increased platelet aggregation, but changes in platelet adhesiveness have not been explored.

Aims: Evaluate the platelet adhesive function in patients with VTE.

Methods: Differences in platelet adhesion characteristics between VTE patients (n = 54) and matched healthy individuals (n = 51) were evaluated by using whole blood samples in: i) PFA-100, to measure global platelet function as closure times with epinephrine and ADP as inductors, ii) the IMPACT test, to evaluate platelet adhesiveness on a plastic surface (750 rpm), iii) microfluidic devices, to explore platelet adhesion on collagen type I (800 s-1) with 2D and 3D evaluation by confocal microscopy, and iv) membrane glycoprotein evaluation by flow cytometry. Plasma levels of VWF were measured.

Results: PFA-100 closure times were significantly shorter ($p < 0.01$) in patients than in controls for both, ADP and Epinephrine (70.5 ± 11.2s vs 93.5 ± 25.2s, and 78.4 ± 13.5s vs 102.3 ± 24.7s, respectively). The IMPACT test showed a significant increase in platelet aggregates size when using blood from VTE patients vs. controls (118.9 ± 5.4 μm^2 vs 100.2 ± 5.7 μm^2, $p < 0.05$, respectively) with a reduction in the interactions number (760.2 ± 44.9 vs. 846.9 ± 46). Platelet adhesion on collagen was enhanced area for VTE patients (16.8 ± 0.9% vs. 13.3 ± 0.8%, $p < 0.05$), with an increased aggregate volume (80.9 ± 14.9 μm^3 vs 69.7 ± 45.9, $p < 0.05$, n = 5). Platelet glycoprotein expression was not altered in VTE patients. Plasma VWF levels were significant higher in VTE patients than in controls (238.3 ± 13.8 vs 153.9 ± 13.6, $p < 0.01$).

Conclusions: Platelets from VTE patients exhibit a proadhesive phenotype under flow conditions that could be related to the shortened occlusion times observed at the PFA-100. This enhanced adhesiveness may be explained by increased levels of VWF. Therefore, primary hemostasis plays a consistent role in venous patophysiology of VTE.

PB1688 | Assessing Platelet Function in Large Cohorts, a Means to Donor Stratification

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Background: Changes in platelet function underpin both hemostasis and thrombotic disease but there is wide variation in how platelets from different donors respond. Anti-platelet drugs are used to supress platelet function but fail to work in some individuals. Better methods to assess platelet function within and across cohorts of donors would enhance ability to identify factors that alter platelet function and determine a donor's susceptibility to anti-platelet medication.

Aims: To develop a reproducible consistent high-throughput method to analyse platelet function in large cohorts of blood donors that can assist in donor stratification.

Methods: Platelet rich plasma from two independent cohorts of 50 donors and 16 donors recalled 18 months after original assessment were processed using a high-throughput high-dimensional 96 well plate flow cytometry assay. Bespoke software was developed to automatically characterise changes in platelet function and facilitate machine learning approaches to stratify results.

Results: The assay enabled a high number of platelet function metrics to be generated quickly and consistently. Machine learning approaches divided participants into distinct sub-populations displaying combinations of high to low capacity of response and sensitivity to agonists, metrics that cannot be identified in traditional single concentration assessments of platelet function. Data from both cohorts was easily compared and combined, being found to display the same platelet function phenotypes which are stable on recall.

Conclusions: We have developed a reproducible high-dimensional assay to measure platelet function in large groups of donors. This is aided by bespoke software that facilitates easy, quick and standardised results allowing the combination and comparison of data from independent laboratories. The analysis reveals that healthy cohorts can be stratified in distinct platelet function phenotypes, these exist in two independent populations and are stable on recall.

PB1690 | PCSK9 in Human Platelets: Characterization and Mechanism of Platelet Activation

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Background: We have previously shown that circulating PCSK9 significantly enhanced agonist-induced platelet aggregation and that formation and stability of arterial thrombus is reduced in PCSK9 knock-out mice. Liver is not the only source of PCSK9. Other cells, including platelets, can express the protein.

Aims: To:

1) deepen PCSK9 presence in human platelets;
2) analyze protein content regulation upon platelet activation;.
3) identify mechanisms beyond the potentiating effect of extracellular PCSK9 on platelet activation.

Methods: Platelets were from healthy subjects. Human megakaryoblastic Meg-01 cell line was cultured and differentiated in vitro (MK) in the absence of exogenous PCSK9. MK-PCSK9 expression was analyzed by flow cytometry and microscopy. Regulation of platelet-PCSK9 content was analyzed upon cell stimulation (epinephrine 0.6 μM; ADP 10 μM). Involvement of LDL receptor in the enhancement of epinephrine-induced aggregation by exogenous PCSK9 was evaluated by means of LDL-neutralizing antibody or EGF-blocking peptide.

Results: Only a subset (22.5%±5.9) of the human platelet express PCSK9. This was also observed in Meg-platelets as the result of the transfer of the protein from a subset of PCSK9$^+$ MK (18.4 ± 2.1%). In *ex vivo* experiments, epinephrine-stimulated platelets secreted PCSK9. The protein was then re-uptaken over 30 minutes. No re-internalization of the protein was observed after stimulation with stronger agonists. Extracellular PCSK9 enhanced epinephrine-induced platelet aggregation, an effect that was almost completely reduced when interaction with LDL-receptor was hampered. Further studies however need to be performed in order to highlight the role of intraplatelet PCSK9.

Conclusions: Data show that one mechanism responsible for the presence of PCSK9 in human platelets is through the direct transfer of the protein from PCSK9$^+$-MK. Circulating PCSK9 could also be taken-up by platelets since they are able to reuptake the endogenous PCSK9 secreted upon stimulation. The potentiating effect of PCSK9 on platelet activation is through a LDL receptor-mediated signaling.

PB1691 | The Effects of Platelet Agonists and Priming on Platelet Populations

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Background: Platelets from healthy donors display heterogeneity in responses to activating stimuli, resulting in different platelet populations with aggregating, secreting and/or procoagulant properties. The activation thresholds of platelets depend on a balance between multiple bioactive molecules, so called priming substances, which either increase or decrease the thresholds. The priming substances adenosine and succinate, elevated in conditions of ischemia or inflammation, affect these thresholds in a cAMP-dependent way. Hypercoagulation also influences the platelet activation state.

Aims: We investigated how negative or positive priming affects the formation of aggregatory and secretory platelet populations induced by physiologically relevant agonists.

Methods: Washed platelets were primed before stimulation with suboptimal doses of CRP-XL, stable ADP or TRAP6. Platelet populations were evaluated using flow cytometry after labelling with PAC1 (active integrin αIIbβ3; aggregating platelets) and anti-Pselectin (Psel; secretory platelets).

Results: With intermediate doses of the agonists, fractions of PAC1 + /Psel- (20-40%) and PAC1 + /Psel+ (30-40%) were distinguished, while only a minor fraction was PAC1-/Psel+ (5%). Regardless of the agonist dose, a considerable fraction (10-30%) failed to express either activation marker. Stimulation with Me-S-ADP typically produced the largest fraction of PAC1 + /Psel-, when compared to other agonists. Negative priming of platelets with adenosine dose-dependently decreased the PAC1 + /Psel+ fraction with all agonists, although did not change the PAC1 + /Psel- and PAC1-/Psel+ fractions. On the contrary, positive priming with increasing succinate doses enhanced the fraction of PAC1 + /Psel+, but only with CRP-XL or TRAP6, again without affecting the single positive fractions. Interestingly, platelet priming with serum particularly increased the PAC1 + /Psel+ population after stimulation with the agonists. Exposing platelets to plasma of prothrombotic patients or to a condition of low shear also resulted in positive priming.

Conclusions: The agonist dose and type change the distribution profile of platelet populations. However, platelet priming alters the overall extent of highly activated platelets without changing the other populations.

Acknowledgement:
LSBR 1711

PB1693 | Platelet Interactions with von Willebrand Factor under Dynamic Shear Stress

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Background: Conformational changes of von Willebrand factor (VWF) molecules play a key role in initiating shear-induced platelet activation (SIPAct). The globule-stretch transition occurs under high shear stress and facilitates the interaction of VWF A1 domains with GPIb receptors on a platelet surface. Multivalent binding leads to the priming of platelets and their consequent activation. The described mechanism was investigated under steady shear stress conditions. However, a shear stress level under a broad range of pathophysiological conditions highly varies. Therefore, for the estimation of platelet activation, it is necessary to take into account not only the shear stress level but also the time of its action. The concept of cumulative shear stress as a measure of the action of shear stress on the platelets along the trajectory in blood flow was suggested.

Aims: The aim of the work was to develop an approach for estimation of platelet activation due to unfolding of VWF on its surface under dynamic shear stress.

Methods: The mathematical model was proposed for the describing of VWF conformational changes on a platelet surface. The dynamics of VWF conformational changes under shear stress influence was investigated analytically and numerically. The methods of nonlinear dynamics were used for analyzing the conditions of VWF unfolding.

Results: The explicit dependence of the critical cumulative shear stress on VWF multimer length was derived. The minimal level of dynamic shear stress below which SIPAct never occurs regardless of exposure time was established.

Conclusions: The obtained results open new possibilities for rational regulation of SIPAct not only by controlling of biomechanical factors, but also pharmacologically by influencing on VWF size distribution.

The work was supported by grant No 19-11-00260 of Russian Science Foundation.

PB1694 | Novel Role for Intracellular Lipases in Platelets

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Background: In platelets, the presence of cytosolic lipid droplets and the role of their lipolytic machinery to degrade triglycerides are still elusive. According to genome-wide RNA-sequencing data from human platelets, adipose triglyceride lipase (ATGL) and monoglyceride lipase (MGL) are expressed in platelets, but their role in platelet function is unknown.

Aims: Our aim is to examine the potential role of ATGL and MGL in platelet physiology.

Methods: We used whole-body and platelet-specific Atgl- and Mgl-knock-out (-/-) mice to study the role of ATGL and MGL in platelets. We visualized platelet spreading and morphology by electron microscopy. We determined platelet activation by measuring the expression of P-selectin and activated glycoprotein IIb/IIIa by flow cytometry and investigated mitochondrial respiration using a Seahorse XF analyzer. We performed bleeding assays to study hemostatic function and determined platelet aggregation using a Multiplate analyzer.

Results: Atgl-/- platelets do not accumulate lipid droplets as observed in other cells and tissues and have unchanged platelet activation. We found reduced mitochondrial respiration in platelets of whole-body Atgl-/- mice, however, oxygen consumption rate of platelets isolated from platelet-specific Atgl-/- mice was unchanged. Of note, the global loss of MGL in mice was associated with a decreased hemostatic function, as evident by an increased bleeding time. Moreover, platelet activation in Mgl-/- blood was significantly decreased in response to collagen-related peptide and convulxin, which was unaltered in platelet-specific Mgl-/- mice. Platelet aggregation in response to collagen was unchanged in all genotypes.

Conclusions: We conclude that the global, but not the platelet-specific loss of either ATGL or MGL affects platelet function in mice, which might be attributable to factors in the circulation.

PB1695 | Platelet Procoagulant Potential Is Reduced in Platelet Concentrates ex vivo But Appears to Be Restored in vivo Following Transfusion

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Background: The procoagulant profile of platelet concentrates (PCs) following transfusion has been difficult to evaluate, lacking specific markers.

Aims: This study aimed to characterise procoagulant platelets in PCs and following transfusion, including cold-storage (2-6°C) and cryo-preservation (-80°C with DMSO), using the novel, specific marker, GSAO.

Methods: Procoagulant platelets were assessed by flow cytometry (GSAO+/P-selectin+) at rest and after ex vivo thrombin 2U/mL ± collagen 10 μg/mL stimulation in fresh whole blood collected pre- and one-hour post prophylactic platelet transfusion (n = 6). Comparison was made with PCs: pooled buffy coat-derived PCs (12 donors each, n = 6), apheresis PCs (n = 3) and healthy control fresh whole blood (n = 25). At production, each pooled PC was split for conventional storage, cold-storage and cryopreservation. Procoagulant platelets and thrombin generation were measured on days 1, 2, 5, 9 and 14.

Results: Buffy-coat and apheresis-derived PCs were equivalent, with both having significantly fewer procoagulant platelets before and after agonist stimulation than healthy control whole blood (mean±SD stimulated: 4.2 ± 1.3%, 3.5 ± 0.8% and 11.1 ± 2.8%, respectively, p < 0.001). However, in post-transfusion samples, where most platelets were PC-derived, procoagulant platelet response was significantly increased (mean±SD: combined day 5 buffy coat and apheresis PCs 4.0 ± 1.6%, transfused platelets 15.8 ± 5.9%, p < 0.0001, figure 1). P-selectin expression following agonist stimulation was also blunted (mean±SD: combined day 5 PCs 77.0 ± 7.8%; healthy control 99.3 ± 0.4%; p < 0.0001), however, was not restored upon transfusion (79.4 ± 13.9%, p < 0.0001). Cryopreserved PCs had marked increase in procoagulant platelets (unstimulated: day 1 conventional PC 0.3 ± 0.2%; immediately post-thaw cryopreserved 25.6 ± 1.8%; p < 0.0001) and thrombin generation (peak thrombin: day 1 conventional 67.1 ± 6.5; cryopreserved 134.4 ± 32.7; p = 0.003). There was strong correlation between proportion of procoagulant platelets and thrombin generation: peak thrombin (r^2=0.62, p < 0.0001), time to peak (r^2=0.79, p < 0.0001, figure 2) and lagtime (r^2=0.81, p < 0.0001).

Conclusions: Utilising the novel marker, GSAO, PCs have reduced procoagulant potential, but this significantly increases following transfusion.

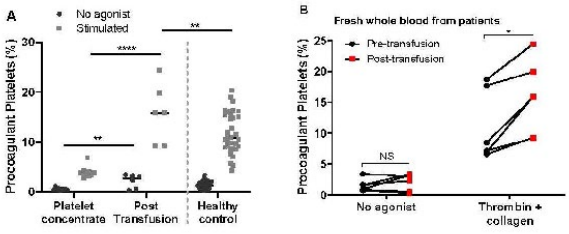

Figure 1. Procoagulant platelet proportions by flow cytometry in platelet concentrates on day 5 of storage, fresh whole blood post-transfusion and fresh whole blood from healthy controls, with and without thrombin 2U/mL and collagen 10μg/mL agonist stimulation (A). Change in procoagulant platelet proportions with and without agonist stimulation in fresh whole blood from patients before and after transfusion. n=9 for platelet concentrates, n=5 post-transfusion samples, n=25 fresh whole blood healthy controls.

FIGURE 1

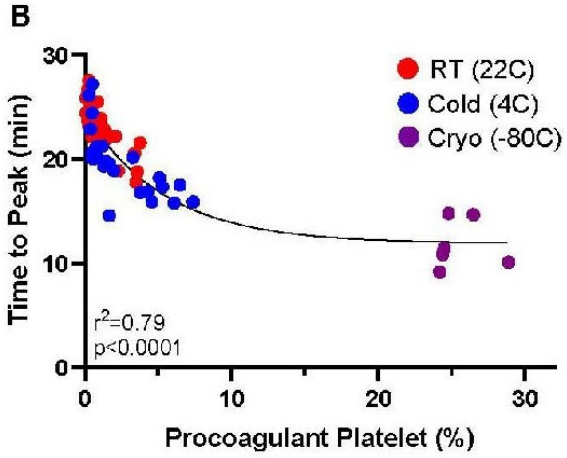

Figure 2. Correlation of time to peak thrombin generation and procoagulant platelet proportions of unstimulated conventional and cold stored platelet concentrates (PCs) over 14 days of storage and immediately post-thaw cryopreserved PCs. RT: room temperature conventionally stored PCs (n=30), Cold: cold stored PCs (n=24), Cryo: cryopreserved (-80°C) PCs immediately post-thaw (n=6).

FIGURE 2

PB1697 | Mean Platelet Volume, and Not Platelet Aggregation, Is Associated with Diabetes: Population Studies from 3,000 to 463,000 Individuals

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Background: Platelets play a critical role in the development of cardiovascular disease (CVD), and type 2 diabetes is a major risk factor for CVD. Despite this, evidence in large populations on the role of platelet biomarkers in diabetes remains limited.

Aims: We investigated the association of diabetes with platelet aggregation in response to multiple agonists or mean platelet volume in cross-sectional and prospective studies.

Methods: Multivariable linear mixed effect models were used to determine associations in the Framingham Heart Study (n = 3,244 individuals, n = 244 diabetics at baseline) between metabolic syndrome and/or diabetes and platelet aggregation, and the association of mean platelet volume (MPV) with diabetes was assessed by multivariable linear analysis in the UK BioBank cohort (n = 463,703, n = 36,297 diabetics).

Results: Contrary to the hypothesis, lower platelet aggregation was associated with prevalent diabetes in most agonists with the exception of a low concentration of epinephrine (0.1 μM) and only after data were stratified by male sex and/or metabolic syndrome. After a median of 18.1 years follow-up, no platelet aggregation trait was associated with increased risk of diabetes (n = 344). However, increased MPV was significantly associated with diabetes (β=0.0976; P = 8.62×10^{-33}). Interestingly, sex-stratified analyses indicated the association of MPV with diabetes is markedly stronger in males (β=0.1232; P = 1.00×10^{-31}) than females (β=0.0514; P = 7.37×10^{-5}). Increased MPV was associated with insulin (β=0.1341; P = 1.38×10^{-11}) but decreased MPV was associated with both metformin (β=-0.0763; P = 1.99×10^{-6}) and the sulphonylureas (β=-0.0559; P = 0.0034). These diabetic medications showed the same direction of effect in both sexes, however the association strength with MPV nearly doubled in women compared to men.

Conclusions: Platelet function as measured by aggregation to ADP, collagen or epinephrine is not consistently associated with diabetes, however, MPV is robustly associated. Future work may focus on how MPV segments pre-diabetics, diabetics, and diabetes drugs for risk prediction.

PB1698 | Physiological extracellular calcium levels impairs platelet aggregation as a result of iPLA2 inhibition due to lack of calcium stores' depletion

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Background: Classic platelet aggregation in citrated plasma is typically irreversible. However, in the presence of physiological extracellular calcium concentration, platelet aggregates are often unstable. The mechanisms of this are not clear.

Aims: We hypothesized that low extracellular calcium induces depletion of intracellular calcium stores (ICS) known to activate calcium-independent phospholipase A2 (iPLA2) producing additional TxA2.

Methods: Fresh whole blood of healthy volunteers was used for all experiments. Platelet aggregation was performed using turbidimetry for platelet-rich plasma (citrated or hirudinated) or washed platelets in Tyrode's buffer with or without 2 mM $CaCl_2$. ICS calcium level was measured using Fluo-5N. Structure of aggregates was analyzed by confocal fluorescent microscopy and flow cytometry.

Results: Reversible platelet aggregation was observed in the presence calcium in hirudinated platelet-rich plasma or washed platelet suspension in response to ADP or a combination of adrenalin and serotonin. At low extracellular calcium, the aggregation was irreversible and became reversible upon inhibition of TxA2 synthesis by aspirin or iPLA2 inhibitor bromoenol lactone. Analysis of small aggregates and single platelets by flow cytometry and of large aggregates by confocal microscopy demonstrated rapid depletion of ICS in response to activation only without extracellular calcium. Without stirring, no aggregates formed in response to activation and the ICS depletion did not occur. Depletion of ICS by thapsigargin (an inhibitor of ICS repletion) caused irreversible platelet aggregation even without ADP. Additional destabilization of aggregates in presence of extracellular calcium might occur due to the presence of non-adhesive procoagulant platelets. Approximately 1% of platelets within aggregates became procoagulant when stimulated by ADP in the presence of calcium and stirring; none were observed without stirring.

Conclusions: Without extracellular calcium platelet aggregates formed in response to ADP under stirring are stabilized by TxA2 synthesized by iPLA2 activated by the ICS depletion. The study was supported by the Russian Science Foundation grant 17-74-20045.

PB1699 | Characterization of Thrombus Heterogeneity and Dynamics of Formation on Collagen Spots in a Parallel-plate Flow Chamber

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Background: Although parallel-plate flow chamber is a widely used tool for whole blood arterial thrombus formation studies and has potential for diagnostic use, the details of thrombus dynamics and structure, spatial distribution in the chamber, pre-analytical and analytical conditions, and proper quantification methods are not clear.

Aims: To investigate parameters of thrombus formation induced by a collagen spot in a parallel-plate flow chamber.

Methods: Hirudinated whole blood was pumped at a constant velocity in a 50 um height parallel plate flow chamber to produce shear

rate of 1000 s^{-1}. DiOC6 and anti-P-selectin antibodies were used for labeling. Activation was by 1x1 mm collagen-coated spot. Spinning-disc confocal and epifluorescent videomicroscopy were used for imaging.

Results: Thrombi volume increased linearly for 10 minutes as observed by fluorescent intensity of DiOC6, followed by disruption and embolization. Local maximum of DiOC6 fluorescence was proportional to thrombi height, with the maximal height reached of about 40% of the channel height. Surface coverage increased as a sigmoid function of time and eventually reached the plateau value of 60% on average. P-selectin staining was present in the thrombi base but did not reach the tops of the thrombi. Although surface coverage was uniform throughout the collagen-covered spot, thrombi volume and height decreased downstream, with the largest thrombi located at the front border of the collagen-coated spot. Comparison of automatic image analysis algorithms has shown that fixed global thresholding applied to fluorescent images gives inaccurate results in determining surface coverage that can be improved using a density-based algorithm.

Conclusions: Thrombus formation in this flow chambers is characterized by heterogeneity and embolization.

PB1700 | An Activation-dependent Pathway Modulated by Dicer1 Neosynthesis and Down-regulating Platelet Reactivity Is Impaired in Diabetes

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Background: Platelets contain mRNAs and synthesize proteins upon activation but have also miRNAs and the cytoplasmic enzymes of the miRNAs machinery, including Dicer1, the function of which is poorly understood. We previously showed that thrombin induces Dicer1 neosynthesis in normal platelets leading to miRNA223 maturation. Previous results showed that Dicer1 and miRNA223 levels are reduced in resting platelets from diabetic patients (T2DM), however the role of Dicer1 in miRNA processing and in the regulation of platelet reactivity after thrombin stimulation in T2DM is unknown.

Aims: To assess whether Dicer1 neosynthesis occurs in T2DM platelets upon activation and if this has an impact on miRNA, mRNA and target protein expression thus regulating platelet function.

Methods: Platelets from T2DM patients and healthy volunteers were ultrapurified by immunomagnetic beads and stimulated with thrombin for different intervals. Dicer1 and $P2Y_{12}$ protein expression were analyzed by western blotting. Pre- and mature-miRNA223 and its target $P2Y_{12}$ mRNA were quantified by RT-PCR.

Results: Dicer1 and miR223 expression increase both in control and T2DM platelets early after thrombin stimulation, but in T2DM platelets they decrease after 60 min while remain elevated for upto 240 min in controls. Platelet $P2Y_{12}$ mRNA and protein expression decrease more rapidly in T2DM platelets (15 min) than in controls (60 min) but return to normal 4 hours after stimulation differently from controls where they remain suppressed.

Conclusions: Normal platelets regulate their own reactivity through the neosynthesis of Dicer1 and the downregulation of $P2Y_{12}$, a self-regulatory mechanism reducing the reactivity of platelets previously exposed to traces of thrombin finalized to prevent unwarranted thrombus formation. In T2DM platelets this occurs too, but then Dicer1 and miR223 expression return rapidly to normal allowing the recovery of $P2Y_{12}$ expression and thus of platelet reactivity. The derangement of this self-regulatory mechanism in T2DM may contribute to the platelet hyperreactivity of T2DM platelets.

PB1701 | Acquired GPVI Deficiency Is a Biomarker for Early Diagnosis and Prognostic Assessment of Patients with Sepsis

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Background: Sepsis is the leading cause of death at intensive care units (ICU) worldwide. During sepsis, patients frequently suffer from disseminated bleeding and thrombosis events as a consequence of hemorrhagic diathesis and edema formation due to loss of vascular integrity. Hemostasis and the maintenance of vascular integrity mainly depend on the function of platelet immunoreceptors including the immunoreceptor tyrosine activation motif (ITAM) receptor glycoprotein GPVI rather than on the mere platelet count. Thrombocytopenia during sepsis has been closely investigated and is a diagnostic criterium. Platelet function, in contrast, remains ill-defined due to few studies with overall contradictory results.

Aims: We assessed platelet function and immunoreceptor signaling longitudinally over the course of sepsis.

Methods: Blood withdrawal was scheduled for three time points (t_1) ICU admission day; (t_2) day five to seven; (t_3) ICU discharge. Platelet activation was determined in 15 patients with sepsis by flow cytometry indicated by P-selectin exposure and integrin GPIIb/IIIa activation. Light transmission aggregometry (LTA) was performed with washed platelets (500/nl). ITAM signaling cascades were assessed by immunoblot and ELISA.

Results: We found unaltered platelet receptor expression, but markedly reduced reactivity to various agonists (for all patients), assessed by flow cytometry and LTA. This defect was most prominent after GPVI stimulation with collagen-related peptide (Mean Geo-MFI ± SD; Control: 9356 ± 3460; Sepsis: 1438 ± 2090; $p < 0.0001$). In

14/15 patients GPVI-dysfunction was already present at ICU admission, considerably before the critical drop in platelet counts. Sepsis platelets failed to transduce the GPVI-mediated signal to induce tyrosine phosphorylation of Syk kinase or LAT. Platelet aggregation upon GPVI stimulation increased only in those patients whose condition ameliorated. We found a strong correlation ($r = 0.63$; $p < 0.01$) of restored platelet aggregation with patient survival.

Conclusions: GPVI dysfunction is a bona fide indicator for early sepsis diagnosis while restored GPVI function clearly indicates patient recovery and survival.

PB1702 | Towards the Correlation of Age with Size in Human Platelets under Steady State

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Background: Circulating platelets in humans are heterogeneous with regard to size, function, and age. Young platelets contain increased amounts of RNA ("reticulated platelets") and express more HLA-I on their surfaces. Furthermore, the loss of sialyl groups ("desialylation") is described as a feature of older platelets leading to platelet clearance via the Ashwell-Morell receptor. It is still debated, whether platelet size is associated with platelet age.

Aims: To assess the correlation of RNA content, HLA-I expression, and desialylation with platelet size in human large and small platelet subpopulations.

Methods: Large and small platelets were separated by differential centrifugation under steady state conditions. Using Cy5-labeled oligo-dT and oligo-dA the relative proportion of RNA-positive platelets was determined. HLA-I expression was quantified using

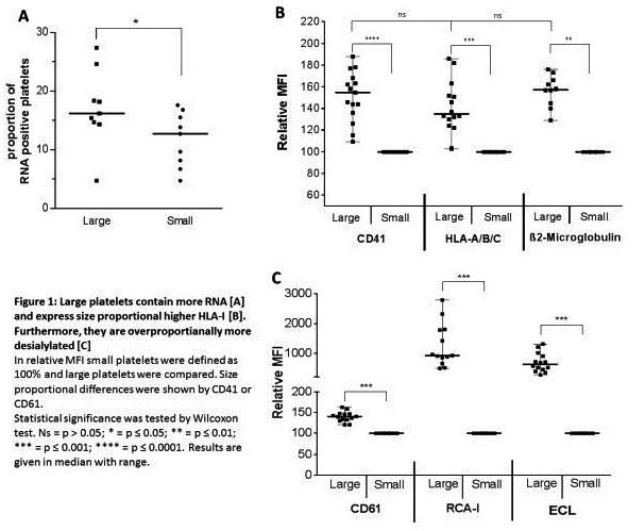

Figure 1: Large platelets contain more RNA [A] and express size proportional higher HLA-I [B]. Furthermore, they are overproportianally more desialylated [C]
In relative MFI small platelets were defined as 100% and large platelets were compared. Size proportional differences were shown by CD41 or CD61.
Statistical significance was tested by Wilcoxon test. Ns = $p > 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** = $p \leq 0.0001$. Results are given in median with range.

FIGURE 1 Large platelets contain more RNA [A] & express size proportional higher HLA-I [B]. Furthermore, they are overproportianally more desialylated[C]

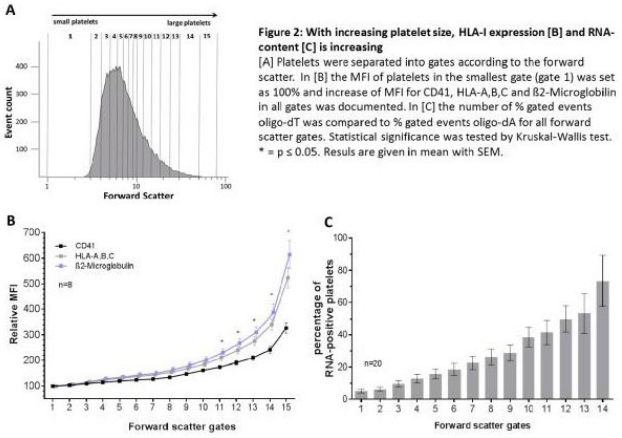

Figure 2: With increasing platelet size, HLA-I expression [B] and RNA-content [C] is increasing
[A] Platelets were separated into gates according to the forward scatter. In [B] the MFI of platelets in the smallest gate (gate 1) was set as 100% and increase of MFI for CD41, HLA-A,B,C and ß2-Microglobilin in all gates was documented. In [C] the number of % gated events oligo-dT was compared to % gated events oligo-dA for all forward scatter gates. Statistical significance was tested by Kruskal-Wallis test. * = $p \leq 0.05$. Resuls are given in mean with SEM.

FIGURE 2 With increasing platelet size, HLA-I expression [B] and RNA-content [C] is increasing

a-human HLA-A, B, C and a-human β2-Microglobulin antibodies. Lectins RCA-I and ECL were used to assess platelet desialylation status. All measurements were performed by flow cytometry. To adjust for platelet size, we introduced a relative MFI in which small platelets were defined as 100% and used CD41 or CD61 expression signals to show proportional size differences. Furthermore, RNA-content and HLA-I expression were assessed in unseparated platelets using defined forward scatter gates.

Results: In preseparated platelets the proportion of RNA positive large platelets was higher compared to small platelets ($p = 0.0273$; Fig. 1A); HLA-I expression was also higher in large platelets but proportional to the size difference (Fig. 1B). In unseparated platelets RNA-content and HLA-I expression were overproportionally higher in very large compared to midsize and small platelets (Fig. 2). However, large platelets showed overproportionally more desialylation compared to small platelets (Fig. 1C).

Conclusions: Large platelets concomitantly exhibit features of younger (higher RNA-content HLA-I expression) and older platelets (more desialylation). These features are overproportionally exhibited by very large platelets. This suggests that very large platelets are a distinctive subpopulation with specific function and reduced survival.

PB1703 | The Iron Uptake Receptor Transferrin Receptor 1 (TfR1) Is Required for Proper Platelet and Megakaryocyte Function in Mice

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Background: Dysregulated iron uptake is a risk factor for the development of thrombotic disorders in humans. Abnormal platelet

production is frequently detected in patients with iron deficiency anemia (IDA) which can be normalized with iron supplementation. Although a mouse model of IDA showed partially defective megakaryopoiesis, the underlying molecular mechanisms of cellular iron uptake in megakaryocytes have not been established. Transferrin receptor 1 (TfR1) has been proposed to regulate cellular iron uptake in mammalian cells. Recently, a missense mutation in TfR1 was identified in patients presenting combined immunodeficiency and thrombocytopenia, indicating an essential role of TfR1 in the hematopoietic system.

Aims: To assess the role of TfR1 in platelet and megakaryocyte function.

Methods: We utilized megakaryocyte- and platelet-specific TfR1 knockout mice (*Tfrc*$^{fl/fl\text{-}Pf4\text{-}Cre}$) and investigated the role of TfR1 in various *in vitro* experiments of platelet function. Ploidy levels of *in vitro* differentiated MKs were measured by flow cytometry

Results: Similar to human patients, *Tfrc*$^{fl/fl\text{-}Pf4\text{-}Cre}$ mice developed a thrombocytopenia, while the mean platelet volume was unaltered. This might be a consequence of impaired platelet production, since MKs that were differentiated from *Tfrc*$^{fl/fl\text{-}Pf4\text{-}Cre}$ bone marrow showed fewer cells of high ploidy as compared to their wild-type counterparts. Upon activation, platelets from TfR1-deficient mice showed defective activation in response to GPCR and (hem) ITAM-coupled receptor agonists. Notably, agonist-induced Ca^{2+} influx, as well as thapsigargin-triggered store-operated Ca^{2+} entry was strongly impaired in platelets lacking TfR1, directly linking iron and Ca^{2+} homeostasis.

Conclusions: These results establish a regulatory role for iron homeostasis in general and for TfR1 in particular in platelet function. This may have implications for patients with iron disorders.

PB1704 | Profiling of COX-dependent Eicosanoids Produced by Blood Cells and the Vasculature

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[1]*Queen Mary University London, Blizard Institute, London, United Kingdom;* [2]*Imperial College London, London, United Kingdom;* [3]*National Institute of Environmental Health Sciences, Durham, United States;* [4]*UCLA, Los Angeles, United Kingdom;* [5]*Queen Mary University London, William Harvey Institute, London, United Kingdom;* [6]*University of Reading, Reading, United Kingdom*

Background: Cyclooxygenase (COX) metabolism of arachidonic acid (AA) leads to the production of eicosanoids such as thromboxane (Tx)A_2, prostaglandin (PG)I_2 and PGE_2, important for thrombosis, inflammation and cancer. However, platelets, endothelial cells and leukocytes synthesize other metabolites not comprehensively profiled.

Aims: Here, we used platelet-COX-1 knockout (KO), global-COX-1 KO and aspirin-treated control mice to determine by mass spectrometry the eicosanoid profile associated with the activity of COX-1 in platelets and of COX-1 and COX-2 in other blood cells and vasculature.

Methods: *In vitro* stimulation of whole blood with A23187 (50 μM) or *in vivo* injection of AA (2.8 mg/Kg) were used to elicit eicosanoid release. The levels of metabolites were determined by mass-spectrometry.

Results: Hierarchical analysis identified 3 different clusters of AA-derived eicosanoids produced *in vitro*: 1) TxA_2, $PGF_{2\alpha}$, 11- hydroxyeicosatraenoic acid (HETE) and 15-HETE, that were absent in platelet-COX-1 and global-COX-1 KO mice; 2) PGD_2 and PGE_2, that were absent in global-COX-1 KO mice and partially reduced in platelet-COX-1 KO mice; 3) 5-HETE, 12-HETE and leukotriene B_4 that were unchanged. Eicosanoids produced *in vivo* clustered differently in platelet-COX-1 ko mice than in global-COX-1 KO and aspirin-treated control mice. All demonstrated absence of TxA_2 but, unlike global-COX-1 KO and aspirin (10 mg/Kg)-treated control mice, platelet COX-1 KO mice still produced PGI_2, PGE_2, $PGF_{2\alpha}$, 11-HETE and 15-HETE. From these data, Ingenuity Pathway Analysis (IPA) predicted enhanced cardiovascular protection for platelet-COX-1 KO mice, but increased thrombosis in global COX-1 KO and aspirin-treated mice. Lessened inflammation was predicted in global COX-1 KO and aspirin-treated mice, while cancer was predicted as decreased in platelet COX-1 KO mice, global COX-1 KO and aspirin-treated mice.

Conclusions: The activity of COX-1 in platelets and of COX-1 and COX-2 in other blood cells and the vasculature associate to distinct eicosanoid profiles underpinning important and different roles in cardiovascular, inflammation and cancer protection.

PB1705 | Bile Acids Maintain Platelet Homeostasis via p38 and Akt Pathway

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Background: Aberrant platelet activation causes thrombotic diseases. In physiological state, platelet homeostasis is maintained by inhibitory factors, including prostaglandin (PG) I_2, NO, and CD39. Antiplatelet drugs are needed for patients at risk of thrombosis, such as patients after percutaneous coronary intervention. However, existing antiplatelet drugs increase the risk of bleeding. Therefore, it is particularly important to further study physiological inhibition factors of platelet homeostasis. Recently, we found that bile acids inhibit aggregation of platelets.

Aims: Explore functions of bile acids in platelet activation and clarify mechanisms of bile acids in platelet homeostasis.

Methods: Platelet aggregation and flow cytometric analysis were used to explore function of bile acids in platelet activation. Phosphoproteomic profiling and Western blots were used to explore mechanisms of lithocholic acid (LCA) in platelet aggregation. Ferric chloride-induced carotid injury model was used to establish the influence of LCA on thrombus formation in Bile acids receptor (G Protein-Coupled Bile Acid Receptor 1, GPBAR1) knockout mice.

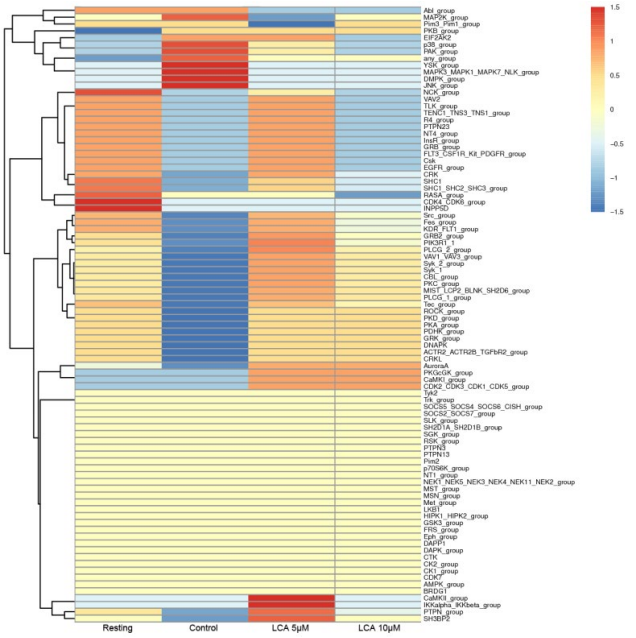

FIGURE 1 phosphorylation kinase analysis of LCA inhibitory effect on the collagen induced platelet aggregation

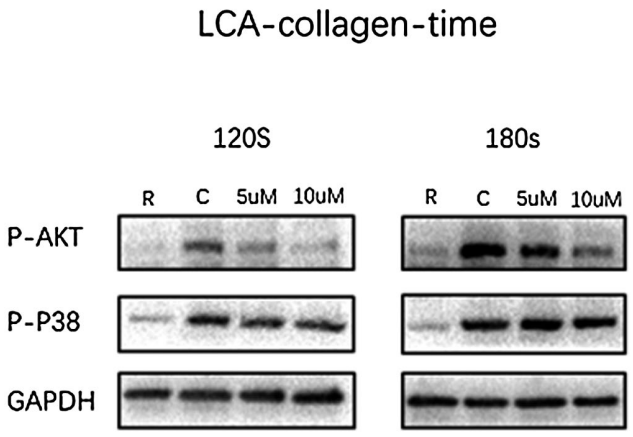

FIGURE 2 LCA suppress platelet activation via p38 and Akt pathways in time-concentration dependence

Results: Among bile acids, LCA significantly inhibitory the collagen induced platelet aggregation. Phosphorylated proteomics analysis revealed that p38, MAPK3, JNK etc. may take part in inhibiting platelet activation by LCA (Fig. 1). Western blot assays revealed that LCA regulate platelet activation via p38 and Akt pathway (Fig. 2). In addition, in the process of platelet activation, the inhibition of LCA on Ca^{2+} internal flow exhibited a dose-dependent effect. The average time to first occlusion for the GPBAR1$^{-/-}$ mice was 5.417 ± 0.538 minutes, in contrast to 6.825 ± 0.492 minutes (P<0.01) in the wild type mice.

Conclusions: LCA controls platelet activation via p38 and Akt pathway. Bile acids affect Ca^{2+} internal flow as well as in arterial thrombus formation in vivo. Therefore, bile acids take part in maintenance of platelet homeostasis and may reduce the risk of thrombotic disease. Anti-platelet studies of bile acids provide theoretical basis for the development of new antiplatelet agents.

PB1706 | The Platelet Indices in Patients with Obesity in Najran Area, Saudi Arabia

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Background: Nowadays, increasing prevalence of obesity is health concern. Obesity is associated with cardiovascular disease, atherosclerosis, chronic proinflammatory and prothrombotic state. Platelet count, Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) give information about platelet function and activity. MPV used to measure the platelet size and the PDW measures the variability in platelet size. Increased MPV and PDW indicator of platelet activation.

Aims: To evaluate platelet count, MPV and PDW in obese patients compared with non-obese control.

Methods: The study included 63 obesity patients (28 women and 35 men) and 63 healthy volunteer (28 women and 35 men). Platelet indices including platelet count, MPV and PDW were assayed using complete blood count (CBC).

Results: The mean body mass index (BMI) of the patients was 41.68 kg/m2. The mean Platelet count of the male obese patients was 259.9 ± 56.23 x 10^3/ul and in female obese patients was 261 ± 55.22 x 10^3/ul. The mean MPV level of the male obese patients was 10.74 ± 0.96 fl and in female obese patients was 10.30 ± 3.10 fl. The mean PDW of the male obese patients was 13.19 ± 2.23 fl and in female obese patients was12.66 ± 4.29 fl. The maen of Platelet count in male non obese was 259.1 ± 54.71 x 10^3/ul and in female non obese was 282.8 ± 55.36 x 10^3/ul. The mean of MPV level in male non obese was 10.35 ± 0.96 fl and in female non obese was 10.87 ± 3.10 fl. The mean of PDW in male obese was 12.06. ± 1.79 fl and in female non obese was13.53 ± 2.14 fl.

Conclusions: No significant difference was observed between obese patients and non-obese control group. In addition platelet count, MPV and PDW were within normal range and not significantly high or low.

PB1707 | Migraine in Patent Foramen Ovale Patients: Role of Platelet Activation

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Background: Migraine is a chronic neurovascular disorder with a multifactorial aetiology. Association with patent foramen ovale (PFO) has been reported, with symptom regression after PFO closure. Increased platelet aggregation has been documented in migraineurs who partially benefit from treatment with antiplatelet drugs. To

date, no further insights into platelet activation in migraineurs with PFO have been reported.

Aims: To characterize platelet activation, platelet thrombin generation capacity (pTGC) and microvesicle (MVs) profile in PFO patients with migraine on aspirin before and after PFO closure.

Methods: Platelet activation markers (Pselectin, activated glycoprotein IIb/IIIa, Tissue Factor [TF]) and MV profile were evaluated by whole blood flow cytometry in 61 patients the day before (T0) and six-months after (T1) PFO closure. pTGC was also assessed. Eight healthy subjects (HS), treated with aspirin for 7 days, were recruited as controls.

Results: The expression of Pselectin and activated glycoprotein IIb/IIIa was similar in PFO patients before and after PFO closure and in HS on the same pharmacological treatment. Conversely, platelet-associated TF expression was significantly higher in PFO patients at T0 compared to HS (3.3%±1.9 *vs* 2.3%±1.1, respectively; p = 0.014) as well as pTGC. After PFO closure, platelet TF expression and pTGC significantly decreased, returning to the levels found in HS. The total number of circulating MVs decreased after PFO closure (108450 ± 49099 at T0 *vs* 79777 ± 32591 at T1 *vs* 65647 ± 26506 MVs/µl in HS; p = 0.0106) as well as the TF-positive MVs (23253 ± 13083 at T0 *vs* 10644 ± 5960 at T1 *vs* 13610 ± 7180 MVs/µl in HS; p < 0.0001).

Conclusions: Classical markers of platelet activation behave similarly in PFO patients and HS both on aspirin. However, PFO patients show a significantly higher prothrombotic phenotype that may be involved in the clinical events and that is reverted only after foramen ovale closure.

PB1709 | The Effect of Ceramic Materials on Platelet Aggregation

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Background: Ceramic bone substituting materials are used for bone tissue repair. One of the most important characteristics of these materials is the size of pores in them. While interacting with implant platelets not only form osteoconductive matrix but also induce regeneration process.

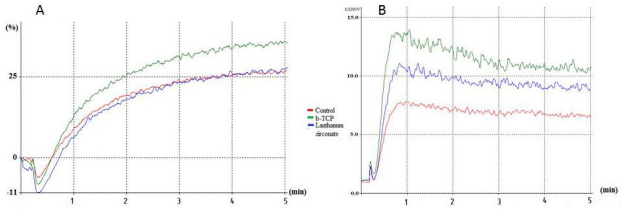

FIGURE 1 ADF-induced platelet aggregation. (A) Maximum platelet aggregation (%). (B) Mean aggregate size (conventional units)

Aims: To study aggregation activity of platelets after interacting with bone substituting materials with different physical and chemical properties.

Methods: We used Husky breed rats in the experiments. To get platelets-rich plasma (PRP) blood was centrifuged for 7 minutes with 1500 rmp. PRP was deluted to 360*1000/µL using platelets-poor plasma. As bone substituting materials we used: 1) $Ca_3(PO_4)_2$ (b-TCP), material with open cell porous 10-50 µm, pore fraction ~35%; 2) $La_{1.95}Ca_{0.05}Zr_2O_7$, (lanthanum zirconate), close cell porous material, pore size 2-30 µm, pore fraction ~15%). Materials were granule-shaped, overall volume of each material was 80 mm^3. Interaction between bone substituting materials and PRP conducted at 37°C, stirring for 30 minutes. Control samples underwent the same procedure but without bone substituting materials. Parameters of ADP-induced Bourne aggregation were measured. Final ADP concentration in samples was 20 µM. Nonparametric statistics were used. Kruskal-Wallis test and further multiple analysis were held. Research was approved by local ethics committee. All experiments were conducted in accordance with Helsinki Declaration.

Results: ADP induced the aggregation of platelets in all examined PRP samples. After interaction with lanthanum zirconate granules, the level of maximum platelet aggregation and the mean aggregate size did not exceed these indicators in the control group. After interacting with b-TCP granules maximum aggregation of platelets and mean size of aggregates were significantly larger then with lanthanum zirconate and control (table, picture).

Conclusions: Physical and chemical properties of bone substituting ceramic materials influence significantly on platelets activity, which can be of great importance in the process of osseointegration in the treatment of bone defects.

TABLE 1 ADF-induced platelet aggregation after interacting with ceramic bone substituting materials. Results are presented as Med [IQR]

	Control	b-TCP	Lanthanum zirconate
	1	2	3
Maximum platelet aggregation (%)	26,53 [26,26; 27,06]	33,29 [30,47; 35,78]	27,45 [27,23; 27,93]
		P 2-1 < 0,05 P 2-3 < 0,05	
Mean aggregate size (conventional units)	7,73 [7,57; 7,76]	13,95 [13,72; 14,06]	10,86 [10,81; 11,08]
		P 2-1 < 0,05 P 2-3 < 0,05	

PB1710 | The Effect of γ-Irradiation at a Dose of 1 Gy on Calcium Metabolism in Rat Platelets

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Background: Under the action of external γ-radiation on the mammals in doses of 1-10 Gy, the hematopoietic system is affected, which is accompanied by the death of radiosensitive bone marrow cells and peripheral blood. A special role in the mechanisms of cell damage and death belongs to universal systems of intracellular regulation - Ca2 + systems, which leads to changes in various biochemical processes in the cells found in animals.
Aims: The aim of this study was to investigate changes in calcium homeostasis in platelets of animals irradiated at a dose of 1 Gy, to study the possibility of using Ap4A as a radioprotective agent for normalizing the hemostasis system.
Methods: Rats were irradiated in a gamma setting at a dose of 1 Gy. Intracellular Ca^{2+} concentration in the platelets was determined using the fura 2-AM.
Results: It was found that in the cytoplasm of platelets after irradiation in a calcium-free medium an increased basal level of Ca2 + is formed, which indicates a decrease in the activity of Ca^{2+}-ATPases. The ADP-stimulated intake of Ca^{2+} into the cytoplasm of platelets of irradiated animals increased by 2.3 times on the 3rd day, and by 1.5 times on the 10th day due to an increase in transmembrane intake of these ions from the outside. Under the action of thrombin, a more intense release of calcium ions from intracellular depots is also stimulated during all the studied periods of the postradiation period. Ap4A, probably through indirect mechanisms, reduced thrombin-induced intake of calcium ions into the cytoplasm from the depositing structures of platelets of control and irradiated rats.
Conclusions: We conclude that post-radiation changes in the transmembrane mechanisms of calcium metabolism in platelets will be formed the basis for identifying methods for the targeted correction of these disorders, which reduce the risk of developing pathological conditions in the cardiovascular system.

PB1711 | Platelet Alpha-granule Secretion in Total Hip Replacement

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Background: Platelet activation promotes blood clot formation meanwhile alpha granules secretion stimulates repair process during surgery. β-thromboglobulin (β-TG) released from alpha-granules can be used as a marker of platelet activation.
Aims: To study β-TG levels and its correlation with platelets number and blood clot formation parameters after total hip replacement (THR).
Methods: The study included 54 patients (59.2 ± 10.4 years (mean±SD); 20 men) underwent THR. Concentration of β-TG, D-dimer, platelets number and thromboelastography parameters (R, Ang, MA, CI) were determined. Blood samples were taken 1 day before surgery, 30 minutes, 1, 3, 7, 14 days after surgery. Statistical analysis was performed using nonparametric criteria (Friedman ANOVA, Wilcoxon matched pairs test, Spearman rank order correlations). Study was approved by the local ethics committee, informed consent was obtained from all patients included in the study.
Results: At the end of the operation, β-TG concentration in blood increased to maximum and after that decrease gradually. (Figure 1). β-TG concentration in blood remained increased relative to the preoperative level on 7 and 14 days after the operation (Figure 1). Dynamics of platelets number differed from β-TG dynamics in postoperative period

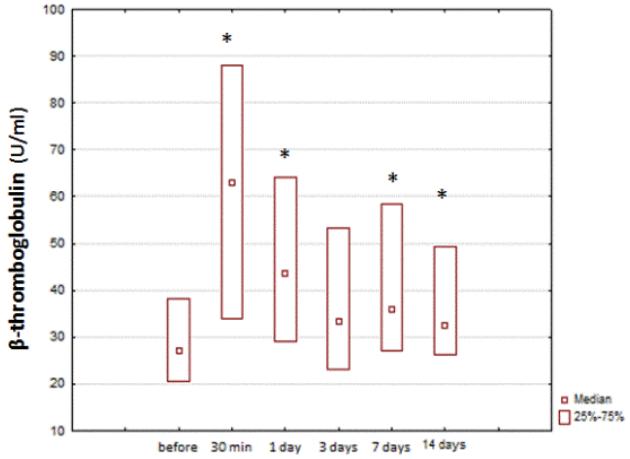

FIGURE 1 β-TG in the blood before and after hip replacement. Friedman ANOVA p < 0.001; *-differences with the preoperative level are significant, p < 0.05

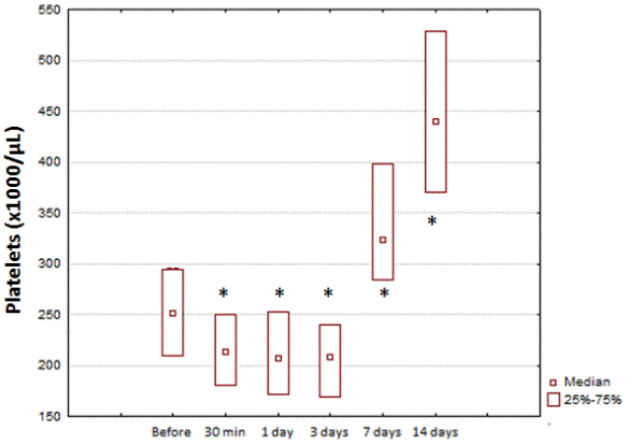

FIGURE 2 Platelets in the blood before and after hip replacement. Friedman ANOVA p < 0.001; * - differences with the preoperative level are significant, p < 0.05

(Figure 2). Analysis did not reveal significant correlation between platelet number and β-G concentration in blood throughout the study period. Significant correlation was found between maximum concentration of β-G in blood and D-dimer level (ρ=0.49, $p < 0.05$), as well as between β-TG and thromboelastogram parameters: R, Ang and CI (ρ=-0.46; ρ=0.39; ρ=0.45 respectively; $p < 0.05$ for all).

Conclusions: Blood clot as primary matrix for tissue repair has a significant correlation with alpha granules secretion level in the early postoperative period. Increased β-G level in blood persists for at least 2 weeks after surgery. The duration of alpha granule secretion increased level after THR seem to be important for successful reparative regeneration.

PB1712 | Platelet Force-Producing Subpopulations Predicted by a Stochastic Model

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Background: Platelets are sensitive to their local mechanical environment created by flow, substrate stiffness, and others.

Aims: Quantify and predict the dynamic force response from a platelet based on substrate stiffness.

Methods: Isolated platelets were allowed to spread on a fibrinogen substrates of varied stiffness (polyacrylamide gel at 4.47 ± 1.19 and 8.73 ± 0.79 kPa). Spreading rates, contractile forces, and total spread area, were quantified. Force production was quantified by lacing polyacrylamide with 40 nm Fluorosphere fluorescent beads. To identify processes involved in force production and platelet spreading, we applied a computational stochastic model based on the buildup of force through myosin IIa. The model was trained by applying inhibitors and through a series of additional experiments.

Results: The final platelet spread area increased with substrate stiffness, as predicted by the stochastic model, Fig. 1, with a similar trend seen for force production. The largest forces were found around the outer edge of the spreading platelet, reaching 20 nN for our stiffest substrate of 8.73 kPa, but with various subpopulations, i.e. oscillatory vs sustained force production. Substrate stiffness-dependent force production and subpopulations were predicted by the stochastic model, based on the defined myosin IIA content in the platelet.

Conclusions: The force response from platelets demonstrates multiple subpopulations, in agreement with Hanke et al. These subpopulations can be predicted through models based on the variability of myosin IIA content in a platelet. This work builds upon earlier work from Qui et al. who initially demonstrate the sensitivity of platelets to substrate stiffness. This work is a first step in identifying unexplained bleeding or prothrombotic platelet phenotypes. It may also be leveraged in blood-contacting medical device design.

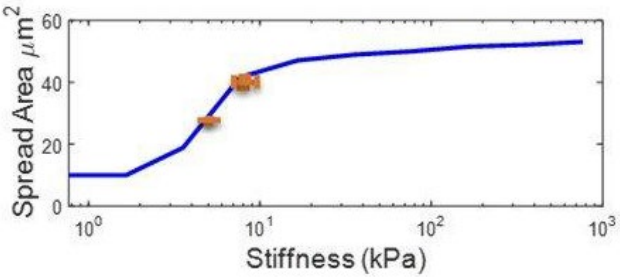

FIGURE 1 Platelet spread area relative to stiffness. Line: stochastic model. Markers: experimental measurements

PB1713 | Maternal Platelet Function Altered by Cigarette Smoking in Early Pregnancy

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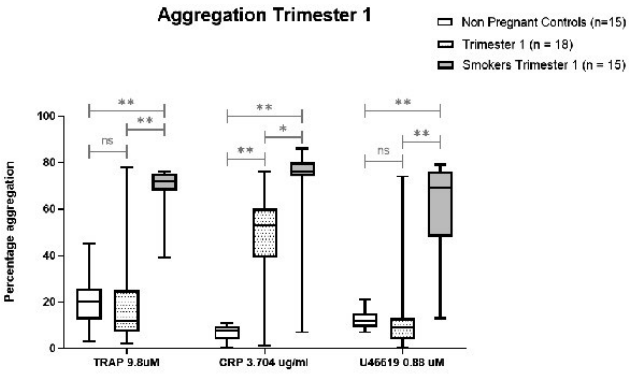
Background: Significant alterations in platelet activity occur during normal pregnancy and have been linked to placental disorders such as pre-eclampsia and fetal growth restriction (FGR). In recent years aspirin has been advocated to prevent such platelet and placenta mediated complications. Cigarette smoking increases risk of arterial thrombosis through platelet activation and is a leading cause of FGR in pregnancy.

Aims: In this study we examine platelet function in the first trimester of pregnancy in healthy pregnant women, pregnant smokers and non-pregnant controls.

Methods: Following informed consent, blood was obtained from 18 pregnant non-smokers, 15 pregnant smokers and 15 non-smoking volunteers. Platelet aggregation was measured in response to multiple doses of a thrombin-receptor activating peptide (TRAP), collagen



Box and whisker plot of platelet aggregation in non-pregnant controls(white), Pregnant women (speckled white), and pregnant smokers (grey) at 11-14 weeks gestation (trimester 1). Aggregation is assessed in PRP by Light Transmission Aggregometry, in response to threshold doses of TRAP (9.8μM), CRP (3.7μg/ml), and U46619(0.9μM).
Data is analysed using 2 way ANOVA testing in Graphpad Prism 8. Significance is shown as P ≤0.0001 (**), P ≤0.001(*), and not significant (ns).

FIGURE 1

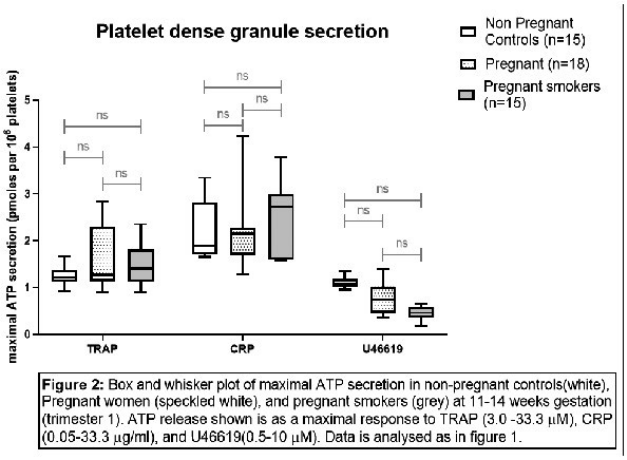


Figure 2: Box and whisker plot of maximal ATP secretion in non-pregnant controls(white), Pregnant women (speckled white), and pregnant smokers (grey) at 11-14 weeks gestation (trimester 1). ATP release shown is as a maximal response to TRAP (3.0 -33.3 μM), CRP (0.05-33.3 μg/ml), and U46619(0.5-10 μM). Data is analysed as in figure 1.

FIGURE 2

related peptide (CRP), and a thromboxane mimic (U46619). In parallel, platelet ATP secretion from dense granules was assessed using a dose range for each agonist, in order to quantify changes in ATP secretion from dense granules.

Results: Significant differences in platelet aggregation are observed at threshold doses of each agonist (TRAP: 9.8uM, CRP: 3.7ug/ml, U46619: 0.9uM) in early pregnancy compared with non-pregnant controls. (figure 1). Platelets from pregnant smokers have significantly higher aggregation to threshold doses of CRP than non-smoking mothers and controls (70.7 ± 6.5%, 45 ± 5.9%, and 6.5 ± 1.2% respectively; $P \leq 0.0001$). Similar patterns are seen with all agonists. In contrast, dense granule secretion is unchanged between subgroups in response to all agonists (figure 2).

Conclusions: Smoking leads to higher levels of platelet aggregation in response to low doses of all agonists, in early pregnancy. This is the first study to demonstrate enhanced platelet activation in pregnant women who smoke. Further work is planned to confirm this trend in middle and late pregnancy and may suggest a role for aspirin in mothers who continue to smoke in pregnancy.

PB1715 | SHARPIN Restrains αIIbβ3 Activation in Platelets in Three Dimensions

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Background: Platelets are heavily reliant on integrin αIIbβ3 and its regulator, talin, for functions in hemostasis, immunity and inflammation. Recently, we demonstrated that human platelets express the adapter molecule, SHARPIN, that can associate with αIIb, restrain αIIbβ3 activation, and function within the NF-κB pathway as a member of the linear ubiquitination activation complex, LUBAC.

Aims: We sought to further examine SHARPIN's impact on αIIbβ3 function.

Methods: SHARPIN flox/flox (fl/fl) mice were crossed with PF4-Cre mice to delete SHARPIN in platelets.

Results: At sub-maximal stimulatory concentrations, ADP induced a significant increase in fibrinogen binding to αIIbβ3 in SHARPIN knockout platelets compared to littermate control, SHARPIN$^{fl/fl}$-PF4Cre-negative platelets ($p < 0.05$). Furthermore, SHARPIN knockout platelets adherent to immobilized fibrinogen spread more extensively ($P < 0.01$), contained unusually long and occasionally barbed αIIb-positive filopodia, and showed increased colocalization of αIIbβ3 with talin. Feedback stimulation of platelets through ADP released from dense-granules, and through PI-3 kinase were likely contributors to the SHARPIN knockout phenotype because apyrase or wortmannin reverted the phenotype to normal. STED microscopy with a resolution of 30-50 nm enabled further insight into the three-dimensional platelet architecture of spread platelets. In all platelets, αIIbβ3 distributed as a thin, basal carpet forming an interface with fibrinogen and localized to the periphery. In addition, αIIbβ3 was organized into central pillars that traversed the platelet vertically. SHARPIN knockout platelets produced larger αIIbβ3 pillars and assembled denser talin patches with enhanced dorsal-apical Z-distribution.

Conclusions: These results are consistent with a restraining role for SHARPIN in the αIIbβ3 activation process, with implications for SHARPIN regulation of platelet adhesion and aggregation, and potentially thrombus growth through control of dorsal-apical transmission of integrin signals.

PLATELET PROTEOMICS AND GENOMICS

PB1716 | In-Depth Platelet Releasate Proteome Profiling: A Novel Source of Biomarkers for Early-Onset Preeclampsia

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Background: Upon activation, platelets release soluble and vesicular signals, termed the platelet releasate (PR), which plays critical role in haemostasis, wound healing and inflammatory responses. We have previously shown the stability of the PR proteome across healthy populations and reported fundamental PR changes in high-stress physiologic conditions, including pregnancy. We hypothesised that the PR could contribute novel biomarkers in early-onset preeclampsia (EOP), a potentially dangerous pregnancy-associated condition in which clinical prediction tools are urgently required to aid decision-making when facing challenging competing maternal and fetal risks.

Aims: 1. To characterise PR changes in EOP.

2. Identify candidate biomarker proteins through data-dependent mass spectrometry analysis (DDA).

3. Assess and confirm these differences through a data-independent mass spectrometry (DIA) approach.

Methods: Thrombin-induced PRs from 22 EOP patients (onset < 34 gestational weeks) and matched healthy controls, were obtained with informed consent. After trypsin/lys-C double digest, samples were subjected to DDA and DIA analysis. Bioinformatic and statistical analysis was carried out using Perseus, Skyline, mapDIA and RStudio.

Results: Utilising a DDA approach we quantified > 700 PR proteins and discovered that 69 and 14 proteins (including condition-specific proteins) were abnormally released in pregnancy and EOP patients respectively, compared to controls. Moreover 8/14 differential EOP proteins could be used to identify patients at high-risk of developing severe EOP with 90% accuracy. Subsequently, samples were subjected to DIA analysis and peptides from identified candidate biomarker-proteins (69 + 14) were further assessed. 59 peptides were confirmed to be abnormally released in EOP. Random forest analysis revealed that the combination of 6 of these peptides can diagnose EOP with 100% accuracy. Furthermore, a combination of 11/59 peptides improved our stratification of high-risk EOP patients (100% accuracy).

Conclusions: The PR proteome is dynamic and changes significantly in pathologic conditions such as EOP. The PR is a potential source of novel diagnostic and clinically-predictive biomarkers in EOP.

PB1717 | Finding the "Switch" in Platelet Activation - Prediction of Key Mediators Involved in the Reversal of Platelet Activation Using a Network Biology Approach

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Background: Current antiplatelet therapies focus mainly on inhibiting platelet activation. The cyclic adenosine monophosphate-protein kinase A signalling (cAMP-PKA) pathway is the main physiological route for platelet inhibition, yet many downstream mediators of PKA remain to be elucidated.

Aims: We aim to use a network biology approach to find molecular "switches" downstream of the cAMP-PKA pathway that are involved in the reversal of platelet activation.

Methods: We used a publicly available phosphoproteomics dataset to create a selection of proteins involved in the reversal of platelet activation. We selected proteins based on existing knowledge of platelet activation pathways. Proteins downstream of prostacyclin were included and proteins downstream of adenosine diphosphate were omitted from our selection. Using the STRING database, a protein-protein interaction network of the 30 selected proteins with potential involvement in platelet deactivation plus 20 additional relevant interaction partners was constructed. The likelihood of the proteins being phosphorylated by PKA was predicted using a Group-based prediction system (GPS 5.0) and visualized in the network. Moreover, the degree of phosphopeptide regulation following platelet deactivation by Iloprost, a stable prostacyclin analogue, was also visualized on the nodes.

Results: Using a comprehensive quantitative platelet proteome dataset, we were able to show that all original proteins and 60% of the additional proteins are present in human platelets under normal conditions. Furthermore, Gene Ontology analysis revealed enrichment of biological processes related to platelet activation, such as cytoskeletal reorganization and vesicle secretion, collectively confirming the validity of our network.

Conclusions: Our method describes a versatile approach to analyse large datasets in a coherent fashion. Currently, we are supplementing our network with existing pathway information from Reactome or WikiPathways. Additionally, enriching our network with drug interactions obtained from the DrugBank database can help us select the most promising targets to explore further in microfluidic models that mimic the vascular microenvironment.

PB1718 | Phosphoproteomic Fingerprint of Platelets in Obesity: Insights into Platelet Reactivity

M. Nuñez Barrachina[1]; L. Hermida-Nogueira[1]; L. A. Morán[1]; V. Casas[2]; S. M. Hicks[3]; A. M. Sueiro[4]; Y. Din[5]; R. K. Andrews[3]; E. E. Gardiner[3]; J. Abián[2]; M. Carrascal[2]; M. Pardo[6]; Á. García[1]

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Background: Obesity is associated with a pro-inflammatory and pro-thrombotic state that supports atherosclerosis progression.

Aims: The main goal of this study was to gain insights into the phosphorylation events related to platelet reactivity in obesity and identify platelet biomarkers and altered activation pathways in this clinical condition.

Methods: We recently performed a comparative phosphoproteomic analysis of resting platelets from obese patients and their age- and sex-matched lean controls. In the present study, we validate the phosphoproteomics data by targeted proteomics, mechanistic, functional and biochemical assays.

Results: Most of the altered phosphoproteins were involved in immunoreceptor tyrosine-based activation motif (ITAM)-based signalling pathways, cytoskeleton reorganization and vesicle transport. Interestingly, Src^{Y419}, VAMP8^{S55}, PKCθ^{S695}, LAT$^{S40/S43}$ were validated by targeted mass spectrometry in an additional cohort of patients showing increased levels of these phosphorylation sites in obesity.

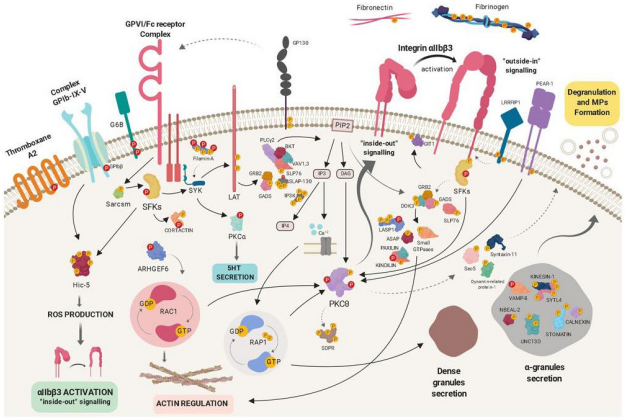

FIGURE 1 Phosphoproteomic fingerprint of resting platelets in obesity: the main up-regulated phosphoproteins

To confirm an alteration of ITAM-related signalling pathways, flow cytometry assays were performed in whole blood indicating higher surface levels of GPVI ($p < 0.0001$) and CLEC-2 ($p < 0.05$) in platelets from obese patients correlating positively with BMI. ROC curves analysis suggested a much higher sensitivity for GPVI (area = 0.84) to discriminate between obese and lean individuals. Indeed, we also found that obese platelets displayed more adhesion to collagen-coated plates ($p < 0.05$). In line with the above data, sGPVI levels - indicative of higher GPVI signalling activation - were almost double in plasma from obese patients ($p < 0.05$).

Conclusions: In conclusion, our results provide novel information on platelet phosphorylation changes related to obesity, revealing the impact of this chronic pathology on platelet reactivity and pointing towards the main signalling pathways deregulated.

PB1719 | DOAC Pharmacogenomics: Personalized Therapeutics to Avoid Bleeding and Thrombotic Complications

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Background: The direct oral anticoagulants (DOACs) have growing utility in patients with atrial fibrillation (AF) and venous thromboembolic disorders. Major bleeding and thrombotic complications occur in 1-3% of patients. A number of enzymes including CYP450 contribute to the absorption and metabolism of DOACs. CYP450 activity varies among individuals and is susceptible to inhibition and induction via drug-drug interactions. Utilizing pharmacogenomics (PGx) in DOACs dosing may lead to lower bleeding and thrombotic complications.

Aims: We evaluated the association of bleeding and thrombosis complications in subjects on DOACs with specific pharmacogenomics variations.

Methods: We analyze pharmacogenomics data of subjects enrolled in the "Right 10k Study" and on one of the DOACs.

Results: There are 10,036 patients with baseline PGx data enrolled in the Mayo Clinic "Right10k Study". 294 patients used DOACs, mean age was 73, and 46.9% were female. 66.3% (195/294) used apixaban, 33.7% (99/294) used rivaroxaban, and 7.1% (21/294) used dabigatran. 21 patients used more than 1 DOAC over the course of treatment for the qualifying condition. AF was the most common indication for DOAC use (71.8%). There were (46) subjects experienced bleeding and (39) have thrombotic complications. Among bleeding complications, 19 developed GI bleeding, 6 developed intracranial hemorrhage, and the remainder had clinically relevant nonmajor bleeding. Among thrombotic complications, 20 developed DVTs/PEs, 4 superficial thromboses, and the remainder were strokes/TIAs.

TABLE 1 DOACs Pharmacogenomics phenotype

Phenotype	No complications	Bleeding and Thrombotic complications	Total	Chi-Square P value
CYP3A4_Phenotype Normal (extensive) metabolizer	192 (86.9%)	68 (93.2%)	260 (88.4%)	0.1712
Warfarin sensitivity Phenotype	68 (30.8%)	25 (34.2%)	93 (31.6%)	0.9287
CYP2C19_Phenotype Decreased function	44 (19.9%)	17 (23.3%)	61 (20.7%)	0.2526
CYP2C9_Phenotype Normal (extensive) metabolizer	133 (60.2%)	43 (58.9%)	176 (59.9%)	0.4721
CYP1A2_Phenotype Rapid metabolizer	194 (87.8%)	66 (90.4%)	260 (88.4%)	0.6011
CYP3A5_Phenotype Poor metabolizer	192 (86.9%)	63 (86.3%)	255 (86.7%)	0.9423
CYP2D6_Phenotype Normal (extensive) metabolizer	76 (34.4%)	23 (31.5%)	99 (33.7%)	0.5665
SLCO1B1_Phenotype Decreased function	44 (19.9%)	17 (23.3%)	61 (20.7%)	0.1185
UGT1A1_Phenotype Normal (extensive) metabolizer	109 (49.3%)	32 (43.8%)	141 (48.0%)	0.0819

No significant differences with respect to age, gender, DOAC, cancer history were seen between those without complication while on therapy. Chronic kidney disease (CKD) stage 3 or greater was associated with significantly higher rates of bleeding (p = 0.018).

Conclusions: Patient genotype was not associated with increased risk of bleeding or thrombotic complications while on DOACs. Patients with CKD were significantly more likely to have major bleeding on DOACs.

PB1720 | Quantitative Phosphoproteomic Profiling and Causal Analysis Reveal Signaling Relations in GPVI/ITAM-Mediated Platelet Activation Programs

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Background: Platelets engage cues of pending endothelial dysfunction through coordinated adhesion, secretion and aggregation responses initiated by glycoprotein and immunoreceptor tyrosine-based activation motif (ITAM) receptor complexes (i.e., GPVI-FcγR). Despite knowledge of key intracellular mediators critical to platelet function, it remains unclear how signals from multiple platelet glycoprotein, ITAM, purinergic and other receptors coordinate platelet hemostatic as well as thrombotic responses.

Aims: We aim to systematically measure and map site-specific protein phosphorylation events associated with the initiation and progression of GPVI/ITAM-mediated activation programs.

Methods: We profile GPVI/ITAM-mediated platelet activation programs through a combination of anti-pTyr and TiO_2 phosphopeptide enrichment, 10-plex tandem mass tag (TMT) labeling and synchronous precursor selection (SPS)-based mass spectrometry (MS3) methods. Differential phosphorylation events are analyzed with algorithms that infer cause-effect relations using pathway information accumulated in Pathway Commons to map signaling events in platelet GPVI/ITAM activation pathways for iterative hypothesis generation and testing efforts.

Results: We reproducibly measure >5,000 phosphorylation sites in multiplexed (n=5) human platelet samples and detail >3,000 significant (FDR< 0.05) phosphorylation events on >1,300 proteins associated with the progression of GPVI/ITAM-mediated activation programs. With literature-guided causal inference tools, >300 site-specific signaling relations are mapped from phosphoproteomics data among key GPVI effectors (i.e., Syk, PLCγ2, PKCδ) and less characterized targets, including Ras/MAPK axis proteins (i.e., KSR1, SOS1) and several proteins associated with Rab GTPase systems.

Conclusions: Through a combination of quantitative proteomics methods and computational causal inference analyses, we measure >3,000 phosphorylation events in activating platelets and map >300 signaling relations to provide testable explanations of, and, validate how signaling events take place in GPVI-mediated platelet

activation. In addition to serving as a model for generating and testing hypotheses from omics datasets, this study puts forth a means to identify hemostatic effectors and biomarkers relevant to thrombosis, vascular inflammation and other platelet-associated disease states.

PB1721 | Lipidome Profile in Platelets from Obese Patients: New Assistances of Platelet Dysfunction in Obesity

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Background: Obesity is associated with a pro-inflammatory and pro-thrombotic state that supports atherosclerosis progression and platelet hyperreactivity. During the last decade, the platelet lipidome has been consider a treasure trove as a source of biomarkers for preventing and treating different pathologies.

Aims: The main goal of the present study was to determinate the lipid profile of platelets from morbid obese patients compared with their age- and sex-matched controls.

Methods: Lipids from washed platelets were extracted and major phospholipids, sphingolipids and neutral lipids were analyzed by LC and GS couple to mass spectrometry.

Results: According to the data, the platelet membrane cholesterol levels were significantly downregulated in the obese group and triglycerides increased. Cholesterol and triglycerides levels are known to impact on platelet membrane fluidity, which may lead to microviscosity alterations, and in turn on platelet activation and aggregation. Furthermore, the levels of ceramide and sphingomyelin were decreased in platelets from obese individuals.

Conclusions: We will discuss our lipid profiling results in the context of the hyperactivate platelet state in obese individuals and the atherothrombotic risk in obesity.

PB1722 | Towards Complete Assignment of the Full Platelet Proteome and Transcriptome

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Background: Recent advances in genome-wide platelet transcriptomics allow a first documentation of the complete proteome.

Aims: Compare platelet transcriptome and proteome.

Methods: Bottom-up identified human platelet proteomes of five cohorts (5,457 unique proteins). Genome-wide quantified transcripts (57,849 RNAs) in platelets and megakaryocytes from BLUEPRINT data; cut-off values for relevant expression. Supervised assignment to 21 platelet function classes (PFC) for 18,529 protein plus RNA genes.

Results: *Proteome.* Integrative analysis of human platelet proteomes (5 cohorts) gave a total of 5,457 unique proteins (74% present in ≥2 databases), 3,692 of which with estimated copy numbers. Guided by UniProt-KB, the proteins were assigned over 21 PFC. Of these, 0.6% were not present in the transcriptome, whereas 6.7% did not have relevant expression (in majority secretory/extracellular proteins).

Transcriptome. The total relevant transcriptome of platelets and megakaryocytes was assessed at 15,356 and 17,205 RNA species, respectively. Of these, 12,239 RNA species were present in either cell type. Transcript levels in platelets and megakaryocytes were strongly correlated ($r=0.8374$, $p< 0.0001$). Relevant protein-coding transcripts were 12,863 (platelets) and 12,016 (megakaryocytes); the remainder being non-coding RNAs and pseudogenes. Levels of protein-encoding transcripts again were correlated ($r=0.6852$, $p< 0.0001$). Distribution profiles over PFC of these transcripts were highly similar ($p< 0.01$).

Comparison. Transcript levels maximised but did not otherwise determine protein abundance. Profiles of PFC were compared of the identified and the remaining expected platelets proteomes. This revealed over-representation of the following classes: actin cytoskeletal, membrane receptors/channels, metabolic and signalling proteins. However, under-representation of: mitochondrial, microtubular system, nuclear, proteasome and transcription protein classes. Equally present were: endoplasmic reticulum and other vesicular proteins.

Conclusions: (i) The full platelet proteome is expected at 12-13k, containing only part of the nuclear, proteasomal and microtubular proteins from megakaryocytes.

(ii) Protein-encoding transcriptomes of platelets and megakaryocytes are quantitatively similar, indicating free distribution of RNA species during proplatelet shedding.

PB1723 | Platelet Proteomics to Understand the Pathophysiology of Immune Thrombocytopenia: Studies in Mouse Models

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Background: Immune thrombocytopenia (ITP) is an autoimmune disease whereby platelets become excessively cleared from the circulation. Diagnosis and treatment are complicated in these patients. While the existence of different pathophysiological entities in ITP is acknowledged, we are still far from being able of stratifying and understanding the disease. Recent research has pointed out a platelet production defect concomitant to the disease, as well as the potential relevance of the main platelet receptor target for autoantibodies (*i.e.* vWF R or GPIIbIIIa). We think that the platelet proteome might give insight on the pathophysiology of the disease, as a reflection of different or conditioned megakaryocyte differentiation programs.

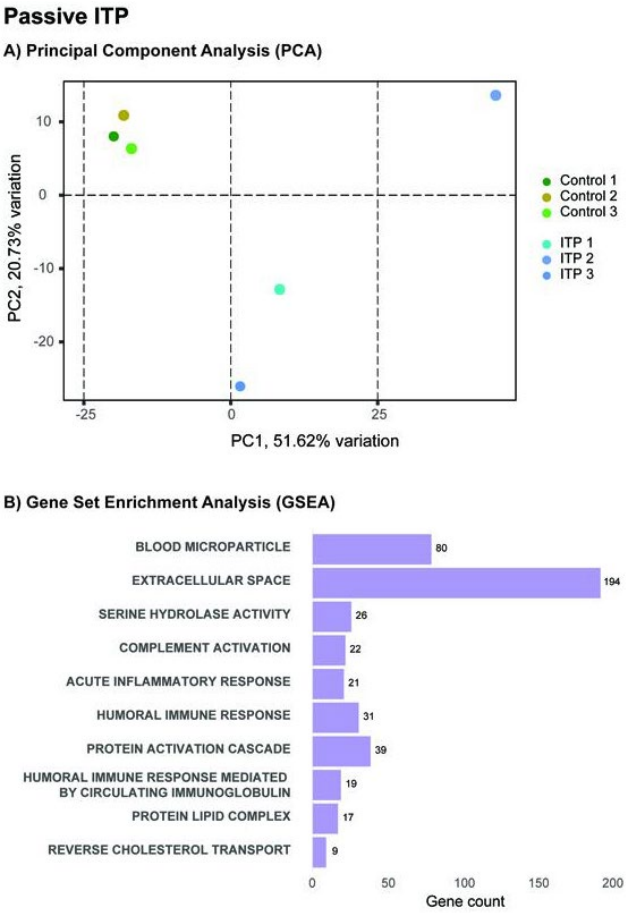

FIGURE 1 PCA and GSEA corresponding to passive ITP

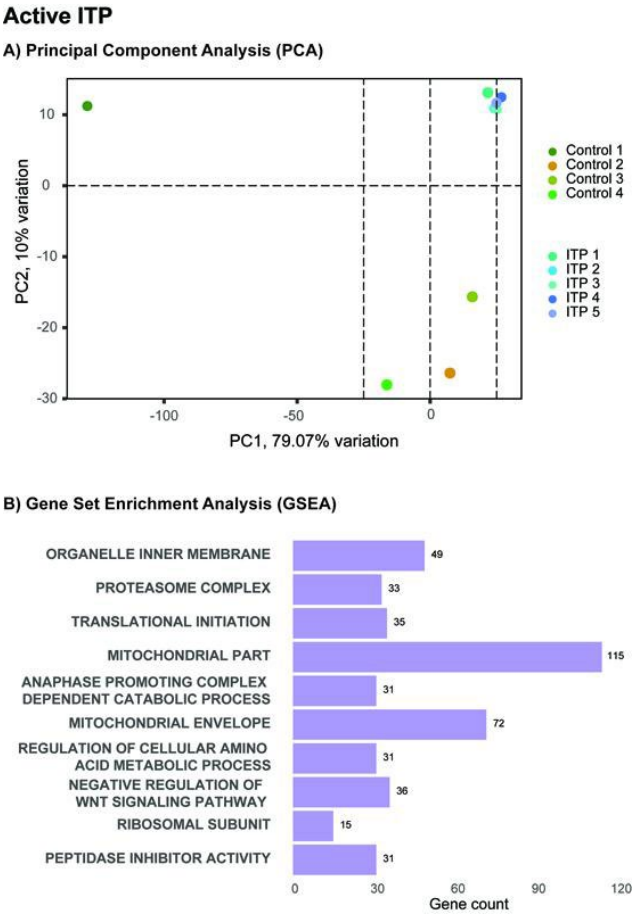

FIGURE 2 PCA and GSEA corresponding to active ITP

Aims: To study the potential alterations in the platelet proteome after induction of passive or active ITP in mice.

Methods: Platelets from passive and active ITP and the respective controls were collected and washed free of plasma. The passive ITP model consists on platelet depletion induced with anti-CD42b (vWF R subunit) antibodies. Platelets were collected at day 3 (severe thrombocytopenia) and at day 7 (platelet counts recovered). The active ITP model consists on transferring splenocytes from CD61 (GPIIIa) KO mice previously immunized with wildtype platelet transfusion into severe combined immunodeficient (SCID) mice. Platelets were collected after 3 weeks, and mice were severely thrombocytopenic. The platelet proteome was subject to label-free, liquid chromatography tandem-mass spectrometry (LC-MS/MS) and was further analyzed with MaxQuant and R software.

Results: Proteomic profiling shows that there were more pronounced differences in the platelet proteome in the active ITP mouse model, compared to the passive ITP mouse model. Gene set enrichment analysis (GSEA) revealed a marked enrichment of metabolic processes and mitochondria-related terms in the active ITP model, while inflammatory and immune responses were enriched in the passive ITP model (Figures 1 and 2).

Conclusions: The platelet proteome can be used for comparative analysis in ITP.

PB1724 | High Efficiency Transfection and Preservation of Platelets with Tumor Suppressing Short RNA

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Background: Platelets are ideal therapeutic carriers given systemic circulation and concentration at sites of injury. Platelet microparticle (PMP) generation and transfer of endogenous miRNA to nucleated cells via PMPs are well documented. However, investigators have had limited success introducing exogenous, potentially therapeutic miRNA to platelet carriers using common methods.
Aims: To assess rapid, high efficiency cationic lipid transfection of the tumor suppressor miR-34a and an siRNA control into platelets; and to assess the impact of transfection on platelet activation and function; and to define methods for preserving transfected platelets.
Methods: Platelet transfection with miR-34a and an siRNA control was performed using a cationic lipid formulation and proprietary transfection medium over 30 minutes. Platelets were cryopreserved by rapid freezing and maintained at -80°C until thawed. Lyopreserved platelets were frozen and dried under vacuum, and stored at room temperature until reconstituted. Fresh and preserved transfected platelets were evaluated by cell count, flow cytometry, light transmission aggregometry, fluorescence microscopy, and occlusion of a thromboplastin-collagen coated microcapillary under shear.
Results: There were no observed differences on platelet physiology with transfection. Both control and transfected platelets maintained low P-selectin expression (Table 1). Nearly all platelet events were transfected and showed intracellular accumulation of labeled RNA. Transfected platelets responded normally to common platelet agonists. Cryopreserved platelets exhibited quantitative cell recovery and high retention of transfected cargo. Lyopreserved platelets maintained high cell recovery (75%) but poor retention of transfected material. All transfected platelet products occluded a coated microcapillary within 15 minutes.
Conclusions: Platelets may be transfected with high efficiency and low toxicity using a tumor suppressing short RNA. Transfected platelets preserved by cryopreservation retain a substantial portion of transfected RNA. All transfected platelet products retain hemostatic function. Our results provide support for the investigation of platelets and preserved platelet products as vehicles for the delivery of short RNA therapeutics.

PLATELET RECEPTORS

PB1725 | P2Y12 Receptor Gene Polymorphisms and Platelet Aggregation at Patients with Arterial Hypertension in Bukovinian Region

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Background: P2YT2 receptor plays a key role in platelet activation. In different researches was presented association between H1 and H2 haplotypes of P2RY12 gene and platelet aggregation (Fontana P. et al.), although, usually, it was demonstrated changes in ADP-induced level, without including spontaneous aggregation, ADP-induced time and ADP-induced speed. So, the role of H1 and H2 haplotypes at patients with arterial hypertension isn`t finally defined. Also, investigations of regional features P2Y12 gene polymorphisms and their role in cardiovascular diseases progression is still actual.
Aims: The aim of this study was to investigate association of P2Y12 polymorphisms and platelet aggregation at patients with arterial hypertension in Bukovinian region.
Methods: 101 persons with arterial hypertension were genotyped according to H1 (744T) and H2 (744C) haplotypes of P2Y12 receptor. Arterial hypertension was determined due to AHA/ESC Guidelines. Platelet aggregation was investigated with method of J. Born.
Results: According to genotype presentation (H1/H1: H1/H2: H2/H2) we divided patients into the groups: homozygotes with H1/H1 genotype (34.6%), homozygotes with H2/H2 mutation (8.9%) and heterozygotes H1/H2 (56.5%).

TABLE 1 Evaluation of Transfected and Control Platelets

Parameter	Transfected Platelets	Control Platelets
Platelet P-selectin Expression (%)	5.51	4.14
Aggregation with Collagen 10 μg/mL (%)	66.0	88.9
Aggregation with Arachidonic Acid 50 μg/mL (%)	68.5	89.2
Aggregation with Thrombin 2.5 U/mL (%)	83.0	92.0
Cryopreserved Platelet siRNA Retention (%)	90	n/a
Lyophilized Platelet siRNA Retention (%)	15	n/a
Capillary Occlusion Time: Fresh PRP (mm:ss)	15:16	10:07
Capillary Occlusion Time: Cryopreserved Platelets (mm:ss)	09:32	10:55
Capillary Occlusion Time: Lyophilized Platelets (mm:ss)	14:52	12:54

Spontaneous aggregation of platelets was significantly lower in H1/H1 genotype (63.7±4.7%) vs. genotypes H1/H2 (72.5±8.4%) and H2/H2 (77.9±6.4%), $p,p_1 < 0.05$. The time of aggregation significantly decreased only in mutant genotype H2/H2 (55.0±35.6 sec.) vs. genotypes H1/H1 (106.7±70.2 sec.) and H1/H2 (94.5±62.0 sec.), $p < 0.05$. The similar feature was evaluated for ADP-depending speed of aggregation with significantly increasing in H2/H2 homozygote patients (42.6±15.6%/min.) vs. H1/H1 (29,1±14.5%/min.) and H1/H2 (34.8±9.8%/min.), $p < 0.05$.

Conclusions: It was set association of P2RY12 gene polymorphisms, genotypes H1/H2 and H2/H2, with increased level of spontaneous platelet aggregation; we estimated higher ADP-induced speed and reduced time of aggregation at H2/H2 genotype in hypertensive patients from Bukovinian region.

PB1728 | Platelets as Immune Cells: Lateral Mobility Dynamics of FcγRIIA on Human Platelets is Directly Linked to Integrity of Platelet Cytoskeleton

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Background: Human platelet FcγRIIA recognizes IgG immune complexes with high affinity. This specificity plays a central role in heparin-induced thrombocytopenia and during interaction of platelets with IgG opsonized bacteria.

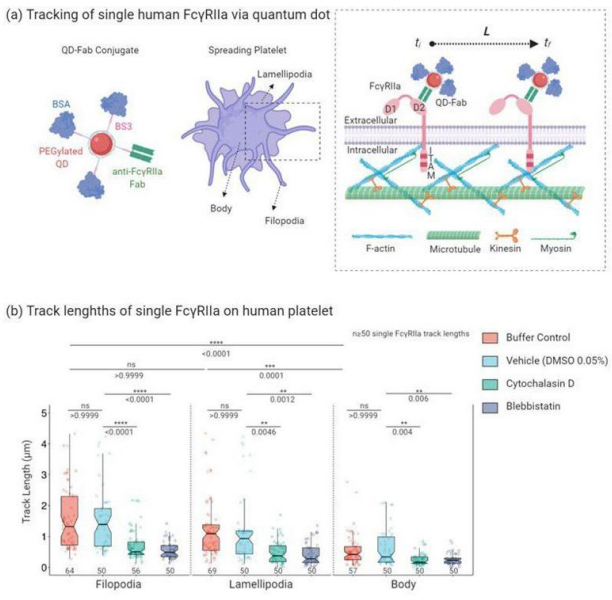

Figure 1. Mobility of FcγRIIA single molecules on human platelets. (a) Schematics of labelling and visualization of single FcγRIIA on platelets using QD conjugated to anti- FcγRIIA Fab. (b) Quantification of single FcγRIIA track length on platelet filopodia, lamellipodia and platelet body in the absence and presence of actin destabilizing agents cytoclalasin D and latrunculin B. Statistical tests were performed by One-Way ANOVA followed by Kruskal-Wallis multiple comparisons test. ns = not significant, $p \geq 0.05$; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$.

FIGURE 1 Lateral Mobility Dynamics of Single FcγRIIA on Human Platelets

Aims: To quantitatively assess the lateral mobility dynamics of FcγRIIA on human platelets and its regulation.

Methods: Total internal reflection fluorescence microscopy (TIRFm) was used for imaging and tracking of single FcγRIIA on human platelets by photoluminescent quantum dot (QD) nanoparticles (5nm diameter) conjugated to monoclonal anti-FcγRIIA Fab (Figure 1a). Quantification of FcγRIIA mobility dynamics (e.g. single receptor trajectories, diffusion coefficients, and track length) on live spreading platelet filopodia, lamellipodia and platelet body was performed. To assess the role of FcγRIIA mobility for sensing of IgG opsonized particles by platelets, we used micropatterned arrays coated with aggregated IgG, or IgG coated bacteria-mimetic beads, or IgG opsonized live bacteria. The relevance of platelet cytoskeletal integrity was assessed by blocking cytoskeletal functions with cytochalasin D and blebbistatin.

Results: We report for the first time diffusion dynamics of single FcγRIIA molecules on human platelets (Fig. 1 A). The single FcγRIIA molecules moved longer distances on filopodia (Mean ± SD 1.53 µm 0.98)) and lamellipodia (1.22 ± 0.96) compared to the FcγRIIA molecules on platelet body (0.55 ± 0.51; P=0.0001) (Figure 1 B). Disruption of F-actin assembly using cytoclalasin D and blebbistatin decreased the diffusion and track length of single FcγRIIA dynamics in the platelet membrane. Cytoskeletal integrity was indispensable for FcγRIIA mediated ligand sensing, platelet adhesion, spreading and activation on aggregated IgG, IgG coated bacteria-mimetic beads, and IgG opsonized live bacteria.

Conclusions: Continuous actin-based "treadmilling" mechanism controls the dynamics of FcγRIIA lateral mobility on the platelets. This facilitates rapid ligand engagement by FcγRIIA and may have broad implications for the response of platelets to opsonized bacteria and IgG coated autologous cells, e.g. in immune thrombocytopenia or the anti-phospholipid syndrome.

PB1729 | An Artificial Lipid Membrane Platform as an Approach to Study CLEC-2 Receptor Clustering

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Background: Activation of numerous surface receptors, including the C-type lectin-like receptor 2 (CLEC-2), depends on the formation micrometer- or submicrometer-sized clusters. However, the regulation and effects of such clustering on signalling molecule recruitment, distribution and signal transduction is unclear. Reconstitution

of the signalling pathway of human CLEC-2 using supported lipid bilayers (SLBs) may be a powerful tool to understand the dynamics of macromolecular assemblies and molecular behaviour of signalling components in ways that cannot be achieved using intact platelets.

Aims: Develop a reconstituted artificial lipid membrane platform as an experimental approach to test and validate signal manipulation of human CLEC-2 and understand the behaviour and dynamics of proteins and molecules involved in clustering.

Methods: Density of hPodoplanin molecules incorporated into the upper leaflet of the bilayer was estimated using flow cytometry. Lateral mobility of SLBs of defined lipid composition with tethered monobiotinylated and fluorescently labelled hPodoplanin was determined using Fluorescence recovery after photobleaching method (FRAP). Total internal reflection fluorescence (TIRf) was used to investigate the dynamics of platelet-mediated clustering of hPodoplanin-Fc.

Results: 1,558 ± 131 molecules/μm^2 was estimated as a density of hPodoplanin within SLBs composed of 2% of biotinylated lipids. FRAP experiments showed that bilayers with tethered hPodoplanin are highly mobile with a mobile fraction of 82% when assembled in an FCS2 imaging chamber and 90% when assembled in a 96-well glass bottom plate. Live-cell imaging showed that once a human platelet contacts the supported lipid membrane, hPodoplanin molecules migrate within the SLBs forming a single bright central cluster within 10 minutes.

Conclusions: The data demonstrates that CLEC-2 on platelets induces the dynamic cluster formation of its ligand hPodoplanin within SLBs. Therefore, the SLB platform may be further used as a high-throughput screening platform to identify novel inhibitors, and to study the interactions and dynamics between signalling pathway components and inhibitors using advanced microscopy.

PB1730 | First novel Pathogenic Variant in ITGA2B in a Ecuatorian Patient with Glanzmann Thrombasthenia

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Background: Glanzmann thrombasthenia (GT) is an autosomal recessive platelet disorder, caused by defects of the platelet integrin αIIbβ3 (GPIIb/IIIa) resulting from pathogenic mutations in either ITGA2B or ITGB3. It is characterized by spontaneous mucocutaneous bleeding. The molecular features of GT in equatorian population have not been identified so far.

Aims: We describe the clinical and molecular features of a ecuatorian patient with GT.

Methods: A ecuatorian woman thirty years old, with clinically suspected bleeding disorder, was derived at the Hematology department, San Pedro de Alcántara Hospital, Cáceres, Spain. GT diagnosis was done based on clinical and hematological parameters as well as genetic analysis by means of HTS (high-throughput sequencing) targeted gene panel, realized in Hematology department, Universitario Salamanca Hospital.

Results: The patient presented with severe bleeding symptoms (ISTH-BAT bleeding score 12). Flow cytometry to assess the surface GPIIb/IIIa complex showed absent expression, consistent with the severe type I phenotype. By HTS, we successfully identified a mutant alleles in ITGA2B: the patient was homozygous for the c.C1229A (p.P410H). This variant has never been previously described.

Conclusions: We report a novel variant expanding the genotypic spectrum of ITGA2B causing GT. In our knowledge, this is the first variant in ITGA2B identified in equatorian population.

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PB1731 | A Humanised CLEC-2 Mouse Model to Study Anti-Human CLEC-2 Biologics *in vivo*

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Background: Platelet C-type lectin-like receptor (CLEC) 2 has been proposed as a potential anti-thrombotic target, based on animal studies suggesting a role in thrombosis. Moreover, it could also be an anti-tumour target as its endogenous ligand, podoplanin, is involved in metastasis. However, there are currently no ways of testing potential human therapeutics in *in vivo* models of tumour metastasis or thrombosis. Furthermore, there is clear data that mouse CLEC-2 can be immunodepleted *in vivo* but it is unknown whether this is also possible with human CLEC-2.

Aims: We aimed to generate a humanised CLEC-2 (hCLEC-2^{KI}) mouse model in which receptor depletion and potential therapeutics could be tested *in vivo*.

Methods: The extracellular domain of murine CLEC-2 was replaced with the human variant and the mice, as well as their platelets, characterised *in vitro* and *in vivo*. CLEC-2 regulation was assessed using the anti-hCLEC-2 monoclonal antibody AYP1.

Results: hCLEC-2^{KI} mice were viable, fertile and born at Mendelian ratio, without evidence of blood-lymphatic mixing. Based on flow cytometric analyses, CLEC-2 surface expression was comparable on wildtype and hCLEC-2^{KI} murine platelets but roughly two times higher than for human platelets. Expression of other platelet glycoprotein receptors was also unaltered, and platelet activation responses were normal except for a slight delay with rhodocytin.

Furthermore, platelet spreading on fibrinogen was also unaltered whereas spreading was reduced on mouse podoplanin compared to wildtypes. Together these data suggest that only subtle differences exist between mouse and human CLEC-2. AYP1 treatment induced a transient thrombocytopenia and CLEC-2 downregulation in hCLEC-2^{KI} but not wildtype mice.

Conclusions: We have generated a humanised mouse model in which potential anti-human CLEC-2 biologics can be tested *in vivo*. Furthermore, we have shown that human, like mouse CLEC-2 can be immunodepleted which could be a potential therapeutic intervention.

PB1732 | Heme Activates Platelets and Exacerbates Rhabdomyolysis-Induced Acute Kidney Injury via CLEC-2 and GPVI/FcRγ

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Background: Rhabdomyolysis can lead to severe acute kidney injury (AKI); however, there is no effective treatment at present. According to a recent study, heme (hemin) released from damaged muscles activates platelets, and the activated platelets induce macrophage extracellular traps (METs), resulting in exacerbation of AKI. We hypothesized that C-type lectin-like receptor-2 (CLEC-2) and glycoprotein VI (GPVI) on platelets are involved in hemin-induced platelet activation, and we have reported in the last ISTH that both CLEC-2 and GPVI contributes to hemin-induced platelet aggregation in mice.

Aims: The first aim is to reveal the detailed mechanism of hemin-induced platelet activation in human platelets. The second is to investigate the role of CLEC-2 and GPVI in rhabdomyolysis-induced AKI by using a mouse model.

Methods: We performed human platelet aggregation assay and western blotting to identify the signaling cascade activated by hemin stimulation. We next evaluated the direct binding between hemin and recombinant CLEC-2 or GPVI by surface plasmon resonance. Using a mouse model of rhabdomyolysis, we biochemically and histologically compared the renal function of CLEC-2-depleted

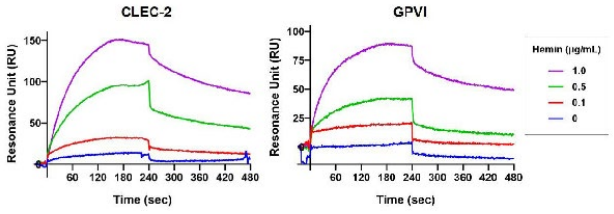

FIGURE 1 Direct binding of hemin to immobilized human CLEC-2 (left) and human GPVI (right)

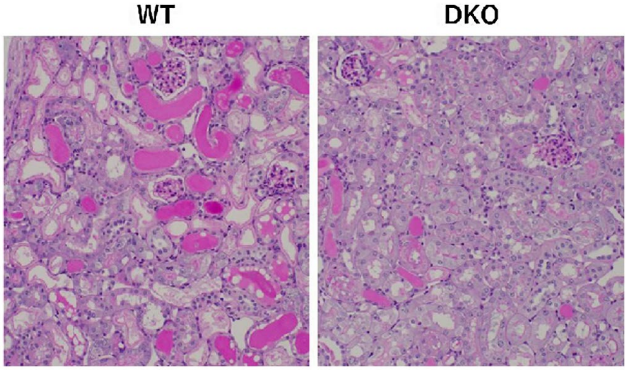

FIGURE 2 Renal tubular injury evaluated by PAS staining

FcRγ-deficient (equivalent to CLEC-2/GPVI double knockout and referred to as DKO) mice with that of wild type (WT) mice.

Results: Hemin induced human platelet aggregation in a dose-dependent manner with increasing phosphorylation of SYK and PLCγ2, which was blocked by SFK inhibitor. In addition, surface plasmon resonance analysis revealed that hemin directly binds to both CLEC-2 and GPVI (Figure 1). Lower serum creatinine level and less injured renal tubules (Figure 2) and METs-like structures were observed in DKO mice compared with WT mice.

Conclusions: Hemin is a novel ligand of both CLEC-2 and GPVI and activates platelets through the SFK-SYK-PLCγ2 pathway. Less prominent renal dysfunction in DKO mice suggested that CLEC-2 and/or GPVI may be new therapeutic targets for rhabdomyolysis-induced AKI patients.

PB1733 | Targeting of a Conserved Epitope in Mouse and Human GPVI Differently Affects Receptor Function and in vivo Depletion

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Background: Platelet glycoprotein (GP) VI is the major platelet collagen receptor and a promising antithrombotic target since its functional inhibition protects from arterial thrombosis without affecting haemostasis. This was first demonstrated in mice using the monoclonal antibody JAQ1, which completely blocks the collagen/CRP binding site on mouse GPVI. Injection of JAQ1-IgG induces transient thrombocytopenia and depletion of GPVI from platelets, whereas its F(ab′) fragment only blocks GPVI without affecting platelet count or GPVI expression in vivo. A possible in vivo downregulation of human GPVI (hGPVI) by antibodies has not been studied systematically.

Aims: To test the effects of JAQ1 on human GPVI function in vitro and the targeted downregulation of the receptor in newly generated hGPVI-knockin ($hGPVI^{KI}$) mice in vivo.

Methods: We used flow cytometry, aggregometry, spreading assays and ex vivo flow adhesion studies to characterize the effect of JAQ1 on human platelets and hGPVI^{KI} mouse platelets. The effects of JAQ1 treatment on hGPVI expression in vivo was analyzed in hGPVI^{KI} mice.

Results: Flow cytometry revealed binding of JAQ1 to human GPVI, but not other species, including rat, hamster, rabbit, swine, cat, dog, horse, and cow. In human or hGPVI^{KI} mouse platelets, JAQ1 binding did not cause detectable activation unless cross-linked, comparable to the effect on wild-type platelets. Remarkably, in contrast to mouse GPVI, JAQ1 did not inhibit CRP or collagen binding of human GPVI. In vivo, JAQ1-treatment induced complete depletion of mGPVI in wild-type mice, but only partially downregulated the receptor in hGPVI^{KI} mice, which was in both groups accompanied by a comparable transient thrombocytopenia.

Conclusions: The binding site of JAQ1 on GPVI is conserved between mouse and human, but differs in its functional significance. In vivo targeting of this site with JAQ1 IgG results in different immunodepletion efficacies, indicating that epitope-specific effects could be involved in this process.

PB1734 | Tetraspanin CD9 Regulates GPIIb-IIIa-Mediated Platelet Function: Identification of Unique Protein Interactomes

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Background: Tetraspanins are transmembrane proteins involved in cellular events such as adhesion, motility, and proliferation. CD9 (Tspan-29) is highly expressed on the platelet surface. Previously, it was determined that CD9 and the platelet integrin, GPIIb-IIIa form noncovalent complexes that are dependent upon the GPIIb-IIIa conformation state.

Aims: The aim of this study was to further define how CD9 and its integrin partner, GPIIb-IIIa, regulate platelet function via common and unique protein interactomes.

Methods: We characterized CD9-dependent platelet phenotypes using Fab fragments of a well-characterized anti-CD9 mAb (mFab7). Platelet adhesion and spreading were examined on matrices fibrinogen (FG), fibronectin (FN), fibrin (Fb) or Fb-FN. Platelet activation, GPIIb-IIIa -mediated aggregation response and aggregate stability (LTA) were measured after mFab7 ligation or addition of a recombinant CD9 protein fragment. Protein interactomes were characterized by immunoprecipitation (IP) by 10E5 (anti-GPIIb-IIIa) or mAb7 (n=3) followed by shotgun mass spectrometry.

Results: Confocal microscopy showed that CD9 was highly localized in platelet-platelet contact regions. mFab7 pre-treatment increased platelet spreading and LTA responses to threshold levels of agonists ADP or collagen by 40% ($p< 0.01$) and 20% ($p< 0.05$), respectively. Consistent with platelet activation, fibrinogen binding, CD62p and CD63 expression were significantly increased
($p< 0.01$). Interactome analyses identified 24 proteins common to both CD9 and GPIIb-IIIa. Six unique protein interaction partners for CD9 included tetraspanin-9, CD63 and Rho family GTPase-activating proteins ($p \leq 0.001$) whereas eight unique GPIIb-IIIa partners included cytoskeleton organization regulators ($p \leq 0.01$) and complexes involved in early stage hemostasis ($p \leq 0.001$).

Conclusions: CD9 localizes to platelet-platelet contacts, modulates GPIIb-IIIa functional status and facilitates platelet responses to ligands upon platelet activation. Such interactions are likely controlled by co-complex members that serve as hubs for the crosstalk of different signaling events. Our findings provide new molecular insights contributing toward a better understanding of platelet signaling and function that might improve therapeutic strategies.

PB1735 | Differential Regulation of the Platelet GPIb-IX Complex by Anti-GPIbβ Antibodies

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Background: GPIb-IX is a highly expressed platelet receptor complex composed of one GPIbα, one GPIX, and two GPIbβ subunits. Once immobilized to VWF, shear generated by blood flow unfolds a membrane-proximal mechanosensory domain (MSD) in GPIbα, exposing a conserved trigger sequence and activating the receptor. Although it is established that all three subunits of the complex are required for robust surface expression, only GPIbα appears to facilitate activation, containing both the MSD and the binding sites for all known ligands to GPIb-IX.

Aims: Despite being positioned directly adjacent to (and potentially in contact with) the MSD, the roles of GPIbβ and GPIX in signal transduction remain murky. We aim to investigate the effects of antibodies targeting these subunits in activating or allosterically modulating GPIb-IX.

Methods: Using a host of activating ligands for GPIb-IX, we tested the effects of antibodies targeting GPIbβ on shear-induced platelet activation via flow cytometry. We also assessed the modulatory effects of these antibodies on GPIb-IX-induced changes in the morphology of platelets and Chinese hamster ovary (CHO) cells expressing human GPIb-IX via confocal microscopy.

Results: We report the effects of anti-GPIbβ monoclonal antibodies RAM.1 and the novel 3G6. Both antibodies bound to the extracellular domain of GPIbβ with low nanomolar affinity. 3G6 amplified morphology changes in platelets induced by VWF or anti-GPIbα antibodies, leading to a greater proportion of platelets exhibiting

a "spread" morphology. 3G6 also amplified platelet degranulation and desialylation downstream of GPIb-IX activation. In contrast, RAM.1 inhibited GPIb-IX signaling, blocking both platelet membrane spreading as well as surface markers of activation triggered by GPIb-IX activation.

Conclusions: These data represent the first report of a positive modulator of GPIb-IX activation, and strongly suggest that changes in the conformation of GPIbβ underlie mechano-transduction via GPIb-IX.

PB1736 | Non-redundant Roles of Platelet Glycoprotein VI and IIb/IIIa via Syk Kinase in Fibrin-dependent Thrombus Shielding under Flow

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Background: Fibrin in the thrombus core strengthens platelet aggregation by binding to integrin aIIbb3 (glycoprotein (GP) IIb/IIIa). Lately GPVI has been shown to bind also to fibrin(ogen).

Aims: To determine how fibrin-GPVI interaction contribute to and consolidate thrombus formation.

Methods: Fibrin-coated surfaces were generated from fibrinogen and thrombin, containing or not transglutaminase factor XIIIa and von Willebrand factor-binding peptide (VWF-BP). The thrombus-forming potential of all surfaces was assessed in the presence or absence of GPVI, GPIIb/IIIa or GPIba activity. Whole-blood flow was performed using the Maastricht microfluidics device under coagulant conditions at arterial wall-shear rate.

Results: Fibrin fibres with(out) FXIIIa moderately supported single platelet adhesion and activation in flowed whole blood. Thrombus build-up on fibrin surfaces was more delayed than that on collagen-III, and aggregated platelets were positive for secretion markers, but not for phosphatidylserine expression. Coated fibrinogen only supported platelet adhesion. On fibrin surfaces, VWF-BP enhanced adhesion but not thrombus build-up. Blocking GPVI with 9O12 antibody (50 mg/ml) substantially but incompletely reduced aggregation and secretion, with limited effect on adhesion. Inhibition of Syk (PRT-060318, 10 mM) nearly abolished thrombus parameters, with residual adhesion remaining. Absence of GPIIb/IIIa (Glanzmann blood) dramatically suppressed platelet adhesion regardless of arterial/venous shear. Both 9O12 and PRT-060318 suppressed fibrin-induced platelet aggregation in suspension and reduced Ca^{2+} signals under flow. Inhibition of GPIba with RAG35 antibody affected platelet adhesion with minor effect on platelet secretion.

Conclusions: With or without FXIIIa, fibrin surfaces moderately support thrombus formation, when compared to collagens. Formation of the shielding thrombus relies on GPIIb/IIIa and Syk activation, and on essential but low GPVI activity. These results indicate that fibrin triggers a low-level activation of GPVI, which is dependent on GPIIb/IIIa for inducing Syk-mediated platelet activation. This work provides new insights into the potential of targeting GPVI-fibrin for treatment of thrombosis.

PB1737 | Role of Receptor for Advanced Glycation End-products (RAGE) in Human Platelets

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Background: Cardiovascular diseases (CVD) are the main causes of mortality in the world. A common element in CVD is thrombus formation, mostly composed of platelets.

Platelets participating in hemostasis and the innate immune system. In particular, platelets release cytokines and express pro-inflammatory receptors.

In this context, the presence of the Receptor for Advanced Glycation End-products (RAGE) during the development of CVD has been recently described. RAGE is a pattern recognition receptor and is capable of potentiating the progression of atherothrombosis.

However, it is not yet clear what is the role of RAGE in platelet functionality.

Aims: To evaluate the role of RAGE activation in human platelet functionality.

Methods: Human platelets were collected from healthy volunteers. We analyze the presence of RAGE by flow cytometry. The platelet function was evaluated *in vitro* by lumiagregometry and P-selectin exposure. In addition, RAGE-mediated intra-platelet signaling was evaluated by western blot. Finally, the agonist and inhibitor interaction with RAGE was determined *in silico*. The statistical analysis T-Test was carried out with Prism 6.0c.

Results: We found a mean fluorescence intensity (MFI) of RAGE on resting platelet was 2236±481 and increased significantly in stimulated platelets (4390±1087).

In addition, RAGE stimulation with AGE-BSA activated NFkB signaling and increase the platelet aggregation (58.50±7.8) and exposure of P-selectin (3402±844.1 of MFI). This effects are blocked by RAGE inhibitor.

Finally, we were able to predict that the region stimulated by AGE-BSA is located in region V of RAGE and also 13 amino acids are critical for its binding.

Conclusions: In conclusion, we confirmed the presence of RAGE in human platelets. The activation of RAGE causes changes in platelet functionality such as increased aggregation and activation by NFkB signaling. In addition, we predicted the critical region for its stimulation. This information is crucial for in the future to propose possible treatments for CVD.

PB1739 | Prediction of Dynamic Structural Changes in the Complex of Platelet Glycoprotein (GP) Ibα Bound with von Willebrand Factor in the Absence and Presence of Coagulation Factor XI using Molecular Dynamics Simulations

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Background: Platelet membrane glycoprotein (GP) Ibα binding with von Willebrand factor (VWF) exclusively mediates initial platelet adhesion at site of endothelial damage under blood flow conditions. Coagulation factor XI (FXI) is known to bind with GPIbα through its A3 domain. However, the role of FXI binding to GPIbα on GPIbα-VWF binding and platelet adhesion is unknown.
Aims: To evaluate the impact of FXI binding to GPIbα on GPIbα interaction with VWF using molecular dynamic simulation calculation.
Methods: All atoms and water molecules constructing the N-terminus GPIbα domain containing leucine rich repeat (residues HSE^{1}-THR^{284}), A1 domain of VWF (residues ASP^{506}-PRO^{703}), and FXI (residues CYS^{2}-THR^{604}) were targeted for Molecular Dynamics (MD) calculation. Positions and velocities of all atoms were calculated in each 2×10^{-15} second with NAnoMolecular Dynamics (NAMD) software using Chemistry at Harvard Macromolecular Mechanics (CHARMM)-36 force field. Dynamic binding structure of GPIbα binding with VWF was calculated both in the absence and presence of FXI.
Results: The root mean square deviation (RMSD) of all protein atoms of GPIbα bound to VWF in the presence or absence of FXI, starting from the initial structure was stabilized after 53.4 nano seconds (approximately 1×10^{9} steps) and 105.0 nano seconds of calculation, respectively. The distance between the center of mass of GPIbαN-terminal and VWF A1 domain in the absence and presence of FXI was 31.5 Å and 41.7 Å, respectively (Fig. 1).

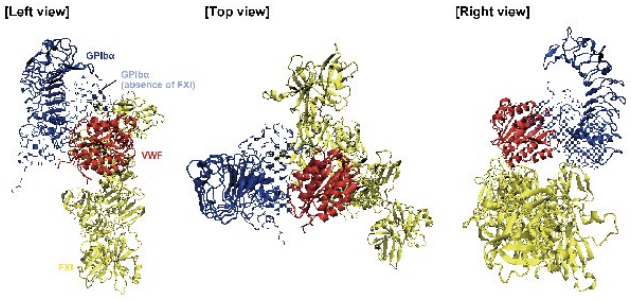

FIGURE 1 The predicted stable structures of GPIbα bound to VWF in the absence and presence of FXI

Conclusions: Stable structure of GPIbα bound to VWF is markedly influenced by FXI binding to GPIbα, which may influence binding characteristics of platelet at vessel wall under blood flow conditions.

PB1740 | Physiological Significance of Lysophosphatidylinositol/G Protein-coupled Receptor 55 System in the Regulation of Platelet Function

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Background: L-α-lysophosphatidylinositols (LPIs) are bioactive lipid substances involved in the regulation of numerous physiological functions including reproduction, angiogenesis, apoptosis, and inflammation[1]. LPIs normally persist at nanomolar concentrations in circulating plasma. LPIs have been identified as the first, and currently the only, known endogenous ligands for G protein-coupled receptor 55 (GPR55)[2]. However, the expression of GPR55 in platelets, and its impact on the modulation of platelet reactivity, thrombosis and haemostasis upon ligation with LPIs under physiological conditions has not yet been investigated.
Aims: This study aims to determine the significance of LPI/GPR55 system in the regulation of platelet function, thrombosis and haemostasis.

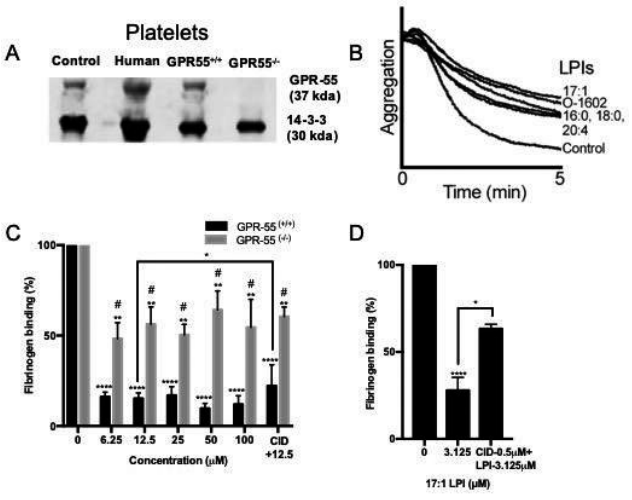

*Figure 1;(A) The presence of GPR55 in human and mouse platelets was confirmed by immunoblot analysis. (B) Various concentrations (0.78 μM-50 μM) of LPIs (16:0, 17:1, 18:0, 20:4 and soy LPI), as well as O-1602, inhibited CRP-XL-induced human platelet activation as measured by optical aggregation. Data representative of n=3 at 0.78 μM. (C) The effects of different concentrations of LPIs on fibrinogen binding [a marker for inside-out signalling] in both GPR55+/+ and GPR55-/- mice upon platelet activation by CRP-XL (0.5 μg/mL). Data represent mean ± S.D. (n=5). *p<0.05, **p<0.01 and ****p<0.0001 when compared with their respective control (0 μM) by one-way Anova; #p<0.05 when compared between the effects of LPI at their respective concentrations on GPR55+/+ and GPR55-/- mice platelets by two-tailed t-test. (D)The inhibitory effects of LPI (17:1 LPI) on ADP (10 μM)-induced activation in human platelets (platelet-rich plasma) was reversed by the pre-treatment with a selective GPR55 antagonist, CID16020046 (0.5 μM) as determined by the extent of fibrinogen binding using flow cytometer. Data represent mean ± S.D. (n=3). *p<0.05, **p<0.01 and ****p<0.0001 by one-way Anova.*

FIGURE 1

Methods: Different species of LPIs (16:0, 17:1, 18:0, 20:4, liver and soy LPIs) and O-1602 (a synthetic ligand for GPR55) were used to investigate their impact on the modulation of platelet reactivity. Diverse concentrations of these molecules were incubated with human and mouse (control and *GPR55*$^{-/-}$ mice) platelets as either isolated platelets, platelet-rich plasma (PRP) or whole blood, and various platelet functions were analysed.

Results: We have confirmed the presence of GPR55 in human and mouse platelets using immunoblot analysis (Fig 1A). All the LPIs used inhibited platelet activation to various extent (with a minimum concentration < 1 μM) indicating their varying levels of interactions with GPR55 (Fig 1B). Using platelets obtained from GPR55-deficient (*Gpr55*$^{-/-}$) mice, we confirmed the absence of this receptor, and the inability of LPIs to inhibit a cross-linked collagen-related peptide (CRP-XL)- induced platelet activation in comparison to controls as measured by the levels of fibrinogen binding (Fig 1C) and p-selectin exposure using flow cytometry. The effects of LPIs were reversed by a GRP55 selective antagonist, CID16020046 (Fig 1D).

Conclusions: LPI/GPR55 system offers novel insights into their potential roles in modulating haemostatic functions of platelets and their significance in the control of platelet reactivity under diverse pathological conditions.

PB1741 | The Recombinant Disintegrins Jarastatin and Jararacin and their Inhibitory Activity on Platelet and Endothelial Adhesion

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Background: Integrins are transmembrane heterodimeric glycoproteins, present in almost all cell types, binding extracellular matrix proteins to cytoskeleton. The activation of the integrin by the interaction with its bind promotes the activation of pathways that regulate adhesion, proliferation, differentiation, migration and apoptosis, thus modulating physiological and pathological processes. For this reason, the integrins has been the target of new antithrombotic drugs. The disintegrins from snake venoms are peptides capable of modulating the activity of integrins, among them the αIIbβ3, responsible for the platelet aggregation and αvβ3, related to angiogenesis.

Aims: The aim of this study was to express and analyse the activity toward platelets of recombinant disintegrins jarastatin (rJast) and jararacin (rJarc).

Methods: The secondary structure and their biological activities were analyzed. We performed the expression of these disintegrins in *Pichia pastoris*, using synthetic gene in the vector pPIC9. They were expressed and secreted in the cultured media and were purified using molecular exclusion chromatography. We confirmed the molecular mass and internal sequence the by mass spectrometry and confirmed protein folding by Circular Dichroism and ^{1}H Nuclear Magnetic Resonance spectra.

Results: The yield of the rJast and rJarc were approximately 40 and 30 mg/ 1L of culture, respectively. rJast and rJarc inhibited platelet aggregation induced by ADP, collagen and thrombin. Both disintegrins inhibited the adhesion of platelets to collagen under continuous flow. We also evaluated effect of rJast on HMEC-1 cells. The viability of the cells was not altered, even with 10 μM of disintegrin. rJast significantly inhibited the adhesion of these cells to vitronectin as well as HMEC-1 migration.

Conclusions: We expressed two RGD disintegrins that showed correct folding and inhibition of platelet aggregation similar to the natives proteins. Finally, these proteins can be used as tools to understand the role of integrins in various physiological and pathological systems including thrombosis and angiogenesis.

PLATELET SIGNALING

PB1742 | Role of HIF-2α in Promoting Thrombogenicity through PAI-1 Synthesis & EV-Releae during Adaptive Signalling in Human Platelets

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Background: Thrombus exists as meshwork of polymerized fibrin clot. It holds the aggregated platelets which remain cut-off from supplies of O_2 and nutrients. As we understand in this aspect, access to O_2 drops from periphery of a mass of thrombus to its inner core. It, therefore, exposes these cells to differential hypoxic stress. In spite of it, platelets perform several energy-intensive tasks such as protein synthesis, retraction of fibrin clot and extracellular vesicle release.

Aims: As little is known about signalling dynamics in platelets exposed to hypoxia, we aim to explore:

- Nature of hypoxia signalling in human platelets,
- Mechanistic basis of platelet responses to hypoxia and
- Possibility for evolution towards pro-thrombotic phenotype

Methods:

1. Isolation of platelets from fresh human blood by differential centrifugation
2. Separation of platelet proteins by SDS-PAGE followed by Western Analysis

3. Extraction of total RNA from platelets and reverse-transcription to cDNA followed by qRT-PCR
4. Hypoxic stimulation of platelets in automatically controlled hypoxia chamber
5. Isolation and analysis of platelet-derived extracellular vesicles (EV) using a Nanoparticle Tracking Analyzer

Results:

1. Human platelets express HIF-2α. Here, we observed that circulating platelets express HIF-2α and its expression is increased in hypoxia.
2. Either hypoxic stress or physiological agonists such as thrombin & collagen augmented expression of HIF-2α.
3. Hypoxia had increased the shedding of EVs and synthesis of PAI-1 in human platelets-indicative to inducing the prothrombotic phenotype

Conclusions: Our study elucidates adaptive signalling of human platelets in response to challenges from hypoxic environments & agonist stimulation that closely resemble to environment within thrombus. Under these situations, platelets synthesize HIF-2α and thrombogenic polypeptide PAI-1 as well as EV release which correlate towards prothrombotic state.
As hypoxic adaptation in platelets contribute to prothrombotic phenotype, the strategies to target platelet hypoxia signalling could be an effective, new generation anti-thrombotic strategy.

PB1743 | Reelin Modulates Glycoprotein VI and Integrin Outside-in Signaling of Platelets

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Background: Reelin is known as an extracellular matrix protein mediating cell migration upon brain development. Recent studies provide evidence of reelin being expressed in platelets, co-localizing to F-actin, binding to amyloid precursor protein (APP) and apolipoprotein E receptor 2 (ApoER2) and being important for glycoprotein Ib (GPIb) mediated platelet adhesion and thrombus formation under high shear. Consequently, defective thrombus formation protects reelin deficient mice (reeler) against arterial thrombosis.
Aims: Defining the role and signaling mechanisms of reelin in platelet cytoskeletal reorganization and glycoprotein VI (GPVI) mediated platelet activation and aggregation.
Methods: *In vitro* and *in vivo* analysis of reeler mice.
Results: Reelin activates the small GTPases RhoA and Rac1 in platelets and was shown to be important for PAK1/2 phosphorylation thereby supporting lamellipodia formation upon spreading on fibrinogen. Experiments revealed strongly reduced clot retraction using platelet-rich plasma (PRP) from reeler mice, emphasizing the role of reelin for integrin outside-in signaling. Furthermore, adhesion and cytoskeletal reorganization of reeler platelets were found to be reduced on collagen-related peptide (CRP) and collagen. Correspondingly, reduced phosphorylation of PLCγ2 and Syk as well as platelet aggregation and ATP release were detected using reeler platelets after selective GPVI stimulation. A significant reduction was observed by antibody inhibition of GPVI examining the adhesion of platelets to immobilized reelin. Additionally, immunoprecipitation of reelin using recombinant GPVI clearly showed a direct interaction of GPVI and reelin. It was also observed, that *in vivo* thrombus formation was completely abrogated in reeler mice treated with a GPVI deleting antibody.
Conclusions: Taken together these data provide the first evidence for reelin modulating GPVI signaling and integrin outside-in signaling of platelets. Thus, reelin affects platelet activation via different signaling pathways involving GPIb, GPVI and the classical reelin receptors APP and ApoER2 pointing to a promising role of reelin as a therapeutic target for antithrombotic therapy.

PB1744 | Plasma Fibrinogen Interaction with Prion Peptide Mitigates Prion-Mediated Neuronal Cell Toxicity And Human Platelet Activation

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Background: When prion, gets misfolded into infectious scrapie isoform (PrPs), primarily a β-pleated insoluble fibril, it forms aggregates in the extracellular matrix that leads to non-treatable neurodegenerative disease. We demonstrated earlier that human plasma fibrinogen interacts with the amyloid β (Aβ), whose structure resembles PrPs, and prevents Aβ-induced human platelet activation and neurotoxicity. We have also shown that PrPs induces a prothrombotic state *in vitro* by stimulating platelets. This led us to explore whether fibrinogen interaction with the prion peptide also attenuates PrPs-induced toxicity to neuronal cells and human platelets.
Aims: To explore interaction of fibrinogen with PrPs and to study the effects of this interaction on prion-mediated neuronal cell toxicity and human platelet activation.
Methods: Neuroblastoma SH-SY5Y cells were cultured in DMEM/F12 media. Platelets were isolated from fresh human blood with

consent from healthy volunteers, as approved by the Institutional Ethical Committee of IMS, BHU, Varanasi, India. The study complied with the Declaration of Helsinki. Surface plasmon resonance, confocal microscopy, and scanning electron microscopy were used to study the interaction between PrPs and fibrinogen. To assess cell viability and cell signaling in human platelets and neuronal cells, cell viability assay, measurement of cytosolic free Ca^{2+}, calpain activity assay, immunoblotting, extracellular vesicle (EVs) release, measurement of mitochondrial membrane potential, were carried out. Two-tailed Student's *t*-test was employed for the evaluation of significance.

Results: A significant interaction was found between plasma fibrinogen and PrPs. Fibrinogen upon interaction with PrPs attenuated prion peptide-induced rise in intracellular Ca^{2+}, shedding of EVs, calpain activity and proteolysis of talin in platelets. Fibrinogen also prevented toxic effects of PrPs on viability, morphology and mitochondrial function of neuronal cells.

Conclusions: Plasma fibrinogen acts as a natural deterrent for prion-induced Neurotoxicity and human platelet activation.

PB1745 | NAADP/SERCA3-Dependent Ca2+ Stores Pathway Specifically Controls Early Autocrine ADP Secretion Potentiating Platelet Activation

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Background: Ca^{2+} signaling is key to cell organization, and its modulation controls many cellular responses. Sarco-endoplasmic reticulum Ca^{2+}-ATPases (SERCAs) pump cytosolic Ca^{2+} into internal stores that in turn regulate cytosolic Ca^{2+} concentration rise upon cell activation. Platelets exhibit 2 types of SERCAs, SERCA2b and SERCA3, which may exert specific roles, yet ill-defined. We have recently shown that Ca^{2+} mobilization from SERCA3-dependent stores was required for full platelet activation in weak stimulation conditions.

Aims: The aim of this study was to uncover the signaling mechanism involved in the mobilization of Ca^{2+} from SERCA3-dependent stores leading to an early ADP secretion that acts as an autocrine loop to potentiate platelet activation.

Methods: Mouse wild-type or SERCA3-deficient platelets, or human platelets were tested for calcium mobilization, secretion and aggregation in presence of different inhibitors.

Results: We demonstrate that an early (5 to 10 seconds following stimulation) ADP secretion is specifically dependent on SERCA3 stored Ca^{2+} exclusively mobilized by nicotinic acid adenosine dinucleotide phosphate (NAADP), both being blocked by the NAADP receptor antagonist Ned-19, and conversely stimulated by permeant NAADP. In contrast, Ca^{2+} mobilization from SERCA3-dependent stores and primary ADP secretion were unaffected by inhibition of the production of inositol-1,4,5-trisphosphate (IP3) by phospholipase-C, and accordingly were not stimulated by permeant IP3. On the contrary, late ADP secretion and SERCA2b-dependent Ca^{2+} stores mobilization were blocked by phospholipase C inhibition or conversely stimulated by permeant IP3 and not by NAADP. Finally, platelet incubation with NAADP and IP3 triggered ADP secretion to a level comparable to thrombin stimulation.

Conclusions: An early ADP secretion, specifically driven by an NAADP/SERCA3 Ca^{2+} mobilization pathway, potentiates platelet activation via a secondary wave of ADP secretion driven by an IP3/SERCA2b-dependent Ca^{2+} stores pathway. Because of its modulating effect on platelet activation, this NAADP-SERCA3 pathway may be a relevant target for future anti-thrombotic therapy.

PB1746 | The Inhibitory Mechanism of Adiporon during Platelet Activation

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Background: The occurrence of thrombotic disease involves many factors, including vascular endothelial injury, hemodynamic changes and changes in blood fluid composition. Abnormal activation of platelets is the core of thrombotic disease [1]. Clinical studies have shown that obesity increases an individual's risk to suffer potentially lethal atherothrombotic complications such as myocardial infarction or stroke [2] In this regard, Adiponectin, an adipocytokine that is abundantly present in plasma [3,4] but is down-regulated in association with obesity-linked diseases including coronary artery diseases [5,6] Adiponectin function is mediated through its receptors: AdipoR1 and AdipoR2, Whether adiponectin can affect arterial thrombosis by affecting platelet activation remains unknown. AdipoRon is an orally active synthetic molecule which can bind to AdipoR1, AdipoR2 [7].

Aims: By studying the effect of AdipoRon on human platelets, we investigate the mechanism of adiponectin regulation of thrombotic diseases.

Methods: We explore the role of AdipoRon in platelet activation through platelet aggregation, adhesion experiment and flow

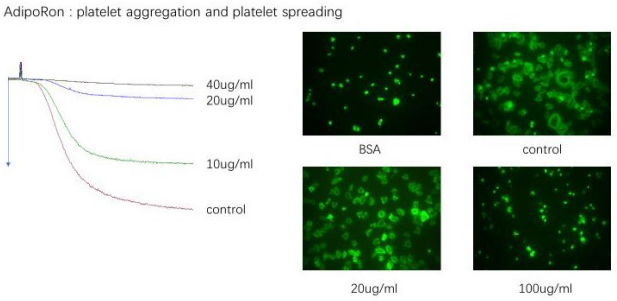

FIGURE 1

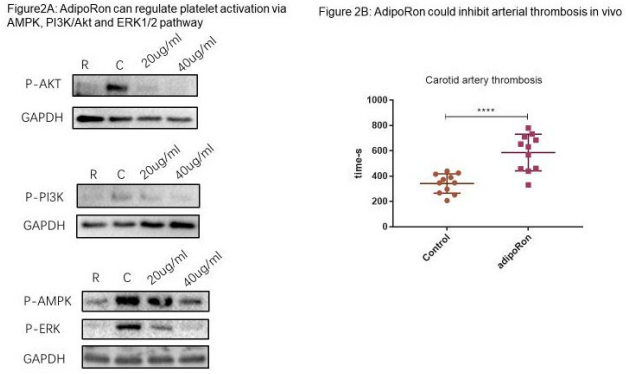

FIGURE 2A & FIGURE 2B

cytometric analysis; Western blots were used to explore signaling pathway of AdipoRon; Ferric chloride-induced carotid injury model was used to establish the influence of AdipoRon on thrombus formation in vivo.

Results: AdipoRon inhibited the platelet agonist (U46619, Thrombin, Collagen) induced platelet aggregation. In addition, AdipoRon incubation markedly decreased the surface area of spread platelets (Fig. 1). Western blot assays revealed that AdipoRon regulated platelet activation via AMPK, PI3K/Akt and ERK1/2 pathway (Fig. 2A). The occlusion time in mice that received AdipoRon was 586.8± 43.73s which was remarkably different from that of the vehicle-treated group 242.5± 23.09s (Fig. 2B).

Conclusions: AdipoRon inhibited platelet activation mainly through the adiponectin receptors AdipoR1, ultimately inhibiting platelet activation via PI3K/Akt and ERK1/2 pathway, AdipoRon also inhibit arterial thrombosis in vivo. Further study of the signaling pathway of AdipRon is conducive to the development of anti-platelet drugs.

PB1748 | Fibrillar Amyloid Peptides Promote Platelet Aggregation through the Coordinated Action of ITAM- and ROS-Dependent Pathways

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Background: Abnormal deposition of amyloid peptides Aβ40 and Aβ42 in brain is correlated with the onset of Alzheimer's Disease. Aβ40 and Aβ42 are also present in platelets and deposit in cerebral vessels. Aβ40 and Aβ42 promote platelet adhesion and activation and generate a pro-inflammatory state. The molecular mechanism of amyloid peptide-induced platelet activation is not clearly defined.

Aims: In this study we have investigated the signalling pathway triggered by fibrillar amyloid peptides to promote platelet aggregation and ROS formation.

Methods: Amyloid peptides Aβ40 and Aβ42, and their respective scrambled peptides, were dissolved in DMSO and then diluted in PBS for 48 hours. Fibril formation was evaluated by ThioflavinT and Congo Red staining. Aggregation of washed platelets was evaluated in parallel with the analysis of the activation of signalling effectors in immunoblotting. Generation of intracellular Reactive Oxygen Species (ROS) was analysed in flow cytometry.

Results: Aβ40 and Aβ42 dilution in PBS for 48 hours resulted in the formation of amyloid fibrils with β-sheet structure. Fibrillar Aβ40 and Aβ42, but not their scrambled peptides or the synthetic Aβ25-35 peptide, promoted platelet aggregation with comparable efficacy by interacting with GPIbα and triggering the activation of associated FcγRIIA. Stimulation of PLC, PKC and PI3K by fibrillary Aβ peptides was ITAM- and Src family kinase-dependent and was required for platelet aggregation. Fibrillar Aβ also induced Src family kinase-dependent ROS generation and NOX inhibitors, as well as ROS scavengers, prevented platelet aggregation. However, Aβ peptide-induced ROS production did not require binding to GPIbα or activation of FcγRIIA, but was initiated by CD36, which was also shown to be necessary for full platelet aggregation induced by fibrillar Aβ peptides.

Conclusions: Differently from soluble forms of amyloid peptides, fibrillary amyloid Aβ40 and Aβ42 induce platelet aggregation through the recruitment of GPIb-IX-V and CD36 and the convergence of ITAM- and ROS-dependent pathways.

PB1750 | Phosphorylation and Activation of Pannexin-1 upon Platelet Activation and Thrombus Formation

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Background: Pannexin-1 (Panx1) is a single membrane channel, which functions as an ion channel for small molecules. Recently, it was shown that platelet activation induces opening of Panx1 channels, amplifies $[Ca^{2+}]_{int}$ and induces platelet aggregation. Panx1-deficient platelets show defects in hemostasis in *in vitro* and *in vivo* models.

Aims: To study Panx1 activation mechanisms in platelets and its impact on hemostasis and thrombosis.

Methods: Analysis of activation patterns of Panx1 phosphorylation after platelet activation, Panx1 inhibition by the specific inhibitor probenecid and *ex vivo* analysis of thrombus formation on collagen under flow.

Results: Western blot analysis demonstrated increased phosphorylation of tyrosine residue 198 (Tyr198) of Panx1 following platelet activation with classical platelet agonists. Tyrosine phosphorylation was shown to be fully dependent on Src - and partially dependent on PKC kinases. Interestingly, ADP does not phosphorylate Panx1 at Tyr198 but at Tyr308; another well-known phosphorylation site of Panx1. Here, Src kinases are only partially involved in phosphorylating Tyr308, which indicates other activation mechanisms of Panx1 channels. Blockage of Panx1 with probenecid led to reduced $[ATP]_{ex}$ after platelet activation with collagen-related peptide (CRP), Par4 peptide and thromboxane, but

not with ADP. Panx1 inhibition leads to reduced thrombus formation on collagen at low (450 s^{-1}) and moderate (1000 s^{-1}) arterial shear rates *ex vivo*. Moreover, initial experiments provided evidence that low agonist activation of platelets lead to extracellular cleavage of Panx1 channels, which is known to irreversibly open the channel in inflammatory cells.

Conclusions: Panx1 channels function as ATP-release channels in platelets to support arterial thrombus formation. Panx1 activation is regulated by phosphorylation at different tyrosine residues following platelet activation. These results suggest an important role of Panx1 in hemostasis by releasing extracellular ATP to enable cell communication and to support thrombus formation.

PB1752 | Feedback Inhibition of Human Platelet Syk by PKC-caused Syk S297 Phosphorylation

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Background: The spleen tyrosine kinase Syk plays an essential role in platelet proximal signaling mediated by ITAM-dependent receptor activation. Recently, we showed that the crosstalk between GPIbα and platelet inhibitory pathways (cAMP/PKA, cGMP/PKG) results in significant inhibition of platelet aggregation but surprisingly induces prolonged Syk activation mediated by hyperphosphorylation of $Syk^{Y525/526}$ (kinase domain) and Syk^{Y352} (interdomain-B) but downregulation of Syk^{S297} (interdomain-B).

Aims: To investigate the role of Syk^{S297} phosphorylation in relation to Syk activation and its regulation by serine/threonine (S/T) protein kinases and phosphatases.

Methods: Echicetin beads (EB) and convulxin (cvx) were used as specific GPIbα and GPVI agonists, respectively. Platelet aggregation was performed by light transmission aggregometry. Phosphorylated proteins were analyzed by immunoblotting using standard and phos-tag SDS-PAGE. Intracellular messengers inositol monophosphate (InsP1) and Ca^{2+}i were quantified by ELISA/IPOne assay and flow cytometry, respectively.

Results: Mass spectrometry analysis revealed upregulation of Syk^{S297} induced by ADP, thrombin and Ca^{2+}-ionophore, which was significantly downregulated by cAMP/cGMP-elevating agents. Upon ITAM-mediated platelet signaling induced by EB or cvx, Syk^{S297} was significantly upregulated in a transient, SFK and Syk-dependent manner. Interestingly, the cAMP-elevating agent iloprost and the global PKC inhibitor GF109203X reduced EB/cvx-induced Syk^{S297}, but mediated $Syk^{Y525/526,Y352}$ hyperphosphorylation and prolonged Syk activation. The hyperphosphorylation profiles of Syk Y-sites led to a significant increase of InsP1/InsP3 production and Ca^{2+}i-mobilization, but only partial inhibition of platelet aggregation in GPIbα/GPVI-activated platelets. Furthermore, inhibition of the S/T-protein phosphatase 1 and 2A by calyculin A and okadaic acid resulted in time-dependent stoichiometric increase of global Syk phosphorylation and increase of Syk^{S297} but not of $Syk^{Y525/526,Y352}$.

Conclusions: Activated PKC stimulates Syk^{S297} phosphorylation, which is also observed when S/T-phosphatases are inhibited. Reduced Syk^{S297} phosphorylation coincides with enhanced Syk activation, suggesting that Syk phosphorylation at S297 represents a mechanism for feedback inhibition in human platelets and a potential diagnostic/therapeutic target.

PB1753 | Dysfunctional CLEC-2- and GPVI-mediated Signaling in Syk Y342F Knock-in Mouse Platelets

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Background: Platelet activation is essential for hemostasis. Central to platelet activation are the signals transmitted through surface receptors such as GPVI and C-type lectin-like receptor 2 (CLEC-2). Both GPVI and CLEC-2 signal through spleen tyrosine kinase (Syk). Syk activity is dependent on phosphorylation of several residues including Y342, Y346, and Y519/520 (Y348, Y352, and Y525/526 in human). However, the importance of each residue on either phosphorylation of other residues or activation of Syk is unknown.

Aims: To determine the relative contribution of Syk Y342 on ITAM and hemITAM signaling, and platelet functional response.

Methods: We produced a Syk Y342F knock-in mouse line to investigate the importance of Y342 on Syk-mediated signaling using a platelet system.

Results: We were unable to detect any Syk Y342 phosphorylation following stimulation of Y342F platelets with the GPVI agonist

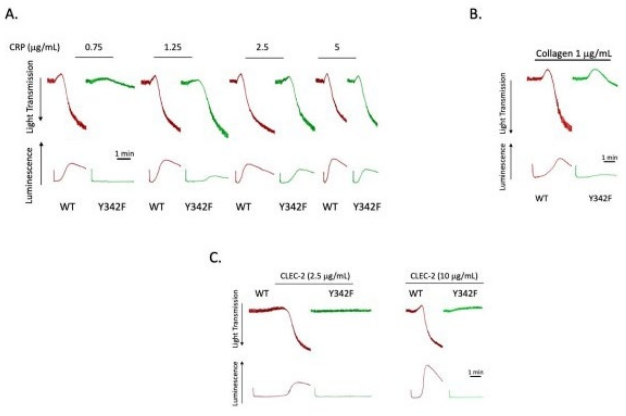

FIGURE 1 ITAM-mediated function is reduced while hemITAM-mediated function is abolished in Syk Y342F knock-in mouse platelets

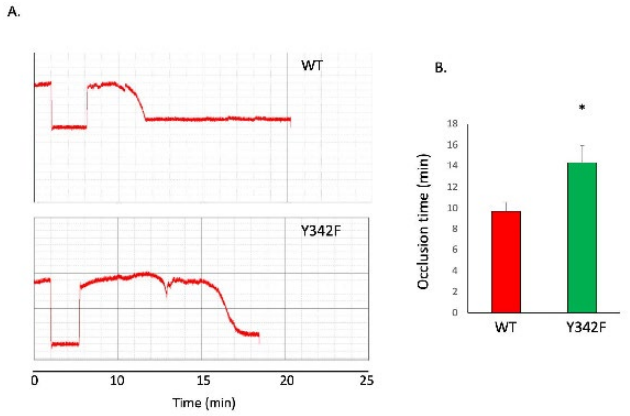

FIGURE 2 In vivo thrombus formation is hindered in Syk Y342F knock-in mice

collagen-related peptide (CRP). Platelet reactivity was reduced in response to the GPVI agonists CRP and collagen (Figure 1 A-B). When using CLEC-2 antibody as an agonist, no response was observed in Syk Y342F mouse platelets though control mouse platelets responded well (Figure 1C). Signaling initiated by either GPVI or CLEC-2 was also inhibited, as Syk Y519/520 phosphorylation is undetectable. Furthermore, SLP-76 phosphorylation was reduced in Syk Y342F platelets compared to platelets from littermate control mice following stimulation of GPVI. Hemostasis, as measured by tail bleeding time, was not altered in Syk Y342F mice, but thrombus formation in response to $FeCl_3$ injury was prolonged in Syk Y342F mice (Figure 2 A-B).

Conclusions: These data demonstrate that phosphorylation of Y342 on Syk following stimulation of either ITAM or hemITAM-bearing receptors is important for the ability of Syk to transduce a signal.

PB1754 | Acetyl-CoA Carboxylase Inhibition Alters Tubulin Acetylation and Aggregation in Thrombin-stimulated Platelets

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Background: We previously showed that constitutively activated acetyl-CoA carboxylase (ACC) promotes platelet activation and thrombus formation by increasing platelet phospholipid content. ACC carboxylates acetyl-CoA into malonyl-CoA, the precursor of de novo lipogenesis. Inhibition of its activity decreases lipogenesis. The concomitant increase in acetyl-CoA can serve as a substrate for protein acetylation. This posttranslational modification plays a key role in the regulation of platelet functions, especially on platelet aggregation, shape change and spreading kinetics via tubulin acetylation.

Aims: We postulate that ACC inhibition might affect platelet functions via an alteration of lipid content and/or tubulin acetylation.

Methods: Platelets were incubated for 2 hours with CP640.186, an ACC inhibitor, before thrombin stimulation. Platelet functions were assessed by aggregometry, flow cytometry and adhesion studies. Lipogenesis was measured via 14C-acetate incorporation into fatty acids. Lipidomics analysis was carried out on the commercial Lipidyzer platform. Protein phosphorylation and acetylation were evaluated by western blot.

Results: Treatment with CP640.186 drastically decreases platelet lipogenesis. Surprisingly, the quantitative lipidomics analyses showed that a 2 hours preincubation with the compound did not affect global platelet lipid content. However, the short-term ACC inhibition (and potential acetyl-CoA accumulation) was sufficient to modify the level of tubulin acetylation, at the basal state or after thrombin stimulation. It is associated with a default of platelet aggregation in response to low concentration of thrombin, and a faster platelet spreading on fibrinogen-coated surface. Mechanistically, we highlighted a decrease in PAK2 phosphorylation, a key regulator of actin and microtubule remodeling.

Conclusions: Pharmacological ACC inhibition by CP640.186 decreases platelet aggregation and affects the kinetics of platelet spreading upon thrombin stimulation. The underlying mechanism does not involve a change in lipid content but may depend on tubulin acetylation, subsequent alteration of PAK2 signaling and downstream actin and/or microtubule remodeling.

PB1756 | NADPH Oxidase 1 and Protein Disulphide Isomerase Synergize to Modulate Platelet Function

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Background: Reactive oxygen species (ROS) levels are often elevated in cardiovascular disease and thought to be both cause and consequence of damage induced by thrombosis. NADPH oxidase 1 (Nox-1) and protein disulphide isomerase (PDI) are key regulators of ROS production in cardiovascular cells. Both of these proteins contribute to platelet activation independently of one another. Understanding how platelets produce ROS and ultimately if this involves Nox-1 and PDI can provide better tools to treat and prevent thrombotic events.

Aims: To assess if and how PDI and Nox-1 regulate platelet function.

Methods: Nox-1 and PDI were visualized in resting and activated human washed platelets (WP) through immunofluorescence. WP

were co-incubated with Nox-1 inhibitor ML171 and/or different PDI inhibitors and assays carried to assess ROS production, reductase activity, as well as platelet function through aggregometry, fibrinogen binding and P-selectin exposure. Similar experiments were performed in Nox-1 -/- mice in the presence or absence of PDI inhibitor. Phosphorylation of key proteins was investigated through immunoblot analysis in both human and mouse platelets.

Results: PDI and Nox-1 colocalization increased after activation with CRP. Likewise, ROS production was decreased by both PDI and Nox-1 inhibitors, whereas platelet membrane reductase activity increased in the presence of the Nox-1 inhibitor. These data indicate PDI may co-localize and modulate Nox-1 function and vice-versa. Co-incubation of PDI and Nox-1 inhibitors synergized to decrease platelet function only in collagen or CRP-activated platelets. A similar synergistic effect was seen using Nox-1 -/- platelets incubated with PDI inhibitor. Immunoblots of human and mouse platelets suggest Nox-1 and PDI co-inhibition induce a steep decrease in p47phox phosphorylation, whereas upstream molecules were not affected.

Conclusions: This is the first report of a synergistic effect amongst redox proteins in platelets, and has implications for the development of more effective ways to prevent thrombotic events.

PB1757 | Role of RXRbeta in Platelet Function and Arterial Thrombosis

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Background: Retinoid X receptors (RXR) are a family of nuclear receptors which play critical roles in the regulation of numerous fundamental biological processes including cell proliferation, differentiation and death. Earlier studies suggested that treatment with RXR agonists attenuate platelet activation in men and mice, however, the underlying molecular mechanisms have remained insufficiently understood.

Aims: To elaborate on the role of RXR in megakaryocytes and platelet.

Methods: We generated a megakaryocyte and platelet specific PF4Cre+;RXRbflox/flox conditional knock-out mice. We studies platelet activation and thrombopoiesis in the presence or absence of RXR ligands. Arterial thrombus was analysed in a Fe(III)Cl model in vivo.

Results: First, we identified RXRb as the dominant RXR receptor in murine platelets, prompting us to generate a megakaryocyte and platelet specific $PF4^{Cre}$;$RXRb^{flox/flox}$ mouse. Second, we studied activation, spreading and aggregation of platelets from C57Bl/6 wild type mice (WT), $PF4^{Cre+}$;$RXRb^{flox/flox}$ mice and $PF4^{Cre-}$;$RXRb^{flox/flox}$ littermate controls in the presence or absence of RXR ligands, i.e. 9-cis-retinoic acid (9cRA) and methoprene acid (MA). We found that in vitro treatment with RXR ligands attenuates platelet spreading and aggregation response. In addition, an increase in megakaryocyte (MK) proplatelet particle formation was shown. However, these effects were also observed in RXRb-deficient platelets and MKs and thus are independent of RXRb. Next, we investigated arterial thrombus formation in a FeCl3-induced vascular injury model in vivo, which was not affected by the absence of RXRβ in platelets as well.

Conclusions: We found that absence of the most abundant RXR receptor in murine platelets, RXRβ, does not affect platelet function in vitro and thrombus formation in vivo. Furthermore, we showed that RXR agonists' mediated effects on platelet function are independent of RXRβ expression. Hence, our data do not support a significant contribution of RXRβ to arterial thrombosis in mice.

PB1759 | Acetylation of α-Tubulin by Aspirin and Histone Deacetylase 6 in Human Platelets

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Background: Acetylation of non-histone proteins is an emerging mechanism of signal transduction in nucleated cells. The best-established function for cytoplasmic acetylation is regulation of microtubule stability by a-tubulin acetylation, a process controlled by the action of histone deacetylases (HDACs) HDAC6 and sirt2. However, the role of acetylation of proteins in anucleated platelets is relatively unknown.

Aims: Our goal was to study: (1) the presence of acetylated proteins in human platelets, (2) the regulation of a-tubulin acetylation by aspirin and HDACs, and (3) the effect of a-tubulin acetylation on platelet reactivity.

Methods: Washed human platelets were treated with inhibitors previously to activation with indicated agonists.

Results: We found that in resting platelets, several proteins are acetylated, including a-tubulin. Aspirin treatment or inhibition of HDACs with trichostatin A (TSA) 15 μmol/L, but not inhibition of sirt2, strongly increased α-tubulin acetylation, suggesting that HDAC6 could be responsible for α-tubulin deacetylation. Immunoblotting experiments demonstrated the presence of HDAC6 in platelet lysates, and the agonist-dependent incorporation of HDAC6 into the reorganized cytoskeleton together with acetylated α-tubulin. Platelet aggregation induced α-tubulin deacetylation, a process inhibited by blocking the $\alpha_{IIb}\beta_3$ receptor. TSA-induced acetylation of α-tubulin was associated with reduced platelet aggregation, dense granule release, and calcium increase in response to collagen and U46619. To confirm the implication of HDAC6 in these processes, we used Tubastatin A, an specific inhibitor of HDAC6, and found that incubation with this agent increased α-tubulin acetylation and inhibited platelet aggregation.

Conclusions: These results indicated that aspirin and HDAC6 regulate the acetylation/deacetylation of α-tubulin a process strikingly related to platelet responses.

PB1760 | Interplay between the Tyrosine Kinases Chk, Csk and Phosphatase CD148 Is Critical for Regulating Platelets in Mice

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Background: The Src family kinases (SFKs) Src, Lyn and Fyn are critical regulators of platelet activation and have also been implicated in megakaryocyte (MK) development and platelet production. Platelet SFKs are inhibited by C-terminal Src kinase (Csk), which phosphorylates a conserved tyrosine residue in their C-terminal tail. The receptor-type tyrosine phosphatase CD148 (PTPRJ) serves a dual function, dephosphorylating the same C-terminal inhibitory tyrosine residue, as well as a conserved tyrosine residue in the activation loop. Deletion of Csk and CD148 in the MK lineage in mice results in increased SFK activity, but paradoxically hypoactive platelets due to negative feedback mechanisms, including upregulation of Csk homologous kinase (Chk) expression.
Aims: To investigate functional redundancy between Chk and Csk in the MK lineage in mice.
Methods: Mice lacking Chk (*Chk* knockout [KO]), Chk and Csk (*Chk;Csk* double KO, DKO) and Chk, Csk and CD148 (*Chk;Csk;CD148* triple KO, TKO) were generated and analysed for defects in platelet production and function using standard assays.
Results: Platelet count was normal in *Chk* KO mice, reduced by 92% in DKO mice, and partially rescued in TKO mice. MK numbers were significantly increased in the spleen of DKO mice, and bone marrow and spleen of TKO mice, with accompanying myelofibrosis (MF). Phosphorylation of the inhibitory tyrosine residue of SFKs was significantly reduced in DKO platelets, and partially rescued in TKO platelets. Residual phosphorylation was blocked by the Src inhibitors PP1 and Dasatinib, revealing a novel auto-inhibitory mechanism of SFKs.
Conclusions: We demonstrate that reduced inhibitory phosphorylation of SFKs leads to thrombocytopenia, expansion of the MK population and MF, with Csk being the dominant inhibitor in platelets, and Chk having an auxiliary role. Deletion of CD148 in addition to Chk and Csk ameliorates thrombocytopenia, but exacerbates MK counts and MF, establishing critical functions in platelet homeostasis.

PB1761 | Influence of Bile Acids on Hemostasis and Arterial Thrombosis

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Background: Patients with liver disease suffer from bleeding complications and thrombocytopenia but also exhibit hypercoagulability suggesting a bivalent role of platelets in liver disease. Induced cholestatic liver disease through bile duct ligation in mice leads to impaired platelet activation and thrombus formation *in vitro* as well as bleeding complications *in vivo*. It is hypothesized that increased levels of bile acids in plasma affect platelet activation and hemostasis, whereas the underlying mechanisms are not well understood.
Aims: In this study we investigated the effect of bile acids on platelet activation and hemostasis
Methods: Human and murine platelets were analyzed in *in vitro* and *ex vivo* experiments regarding the effect of a variety of common bile acids on platelet activation.
Results: The incubation of isolated murine and human platelets with bile acids resulted in reduced adhesion on a fibrinogen matrix 5, 20 and 60 minutes after adhesion as well as reduced amount of fully spreaded platelets. Flow cytometric analysis of murine and human platelets revealed a reduced P-selectin expression as well as integrin $\alpha_{IIb}\beta_3$ activation following preincubation with different bile acids whereas bile acids alone had no effect on platelet activation. In aggregation experiments no significant differences in platelet aggregation could be detected. However, diminished platelet activation resulted in reduced thrombus formation on a collagen matrix under arterial flow conditions using human and murine whole blood which were preincubated with different bile acids. Regarding the underlying mechanism, bile acids lead to platelet inhibition through phosphorylation of vasodilator activating phosphoprotein (VASP) on serine residue 157 in a time dependent manner as shown in western blot analysis of isolated murine and human platelets.
Conclusions: These results reveal that bile acids affect platelet activation negatively and impair thrombus formation, indicating a new class of negative regulators of primary hemostasis

PB1762 | Loss of the Exocyst Complex Component, EXOC3, Accelerates Arterial Thrombosis

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Background: The exocyst is an octameric complex comprising eight distinct protein subunits, exocyst complex components (EXOC) 1-8,

and has an established role in regulating the tethering of secretory vesicles to the plasma membrane.

Aims: Proteomic studies demonstrate the expression of the exocyst in platelets, but its relevance to platelet granule secretion and function remains to be determined.

Methods: EXOC3 conditional knockout mice in the megakaryocyte/platelet lineage were generated to assess exocyst function in platelets.

Results: Significant defects in GPVI-induced platelet aggregation, integrin activation, α- (P-selectin and platelet factor 4), dense and lysosomal granule secretion were detected in EXOC3$^{-/-}$ platelets following treatment with collagen related peptide (CRP). With the exception of P-selectin exposure, these defects were completely recovered by maximal concentrations of CRP. Notably, FACS analysis revealed a significant reduction in resting GPVI surface levels in EXOC3$^{-/-}$ platelets. Analysis of the GPVI pathway revealed significant defects in activation of proximal signalling molecules, Syk and LAT, while calcium responses were also reduced, implying an indirect mechanism for the majority of these defects in platelet activation due to a decrease in surface GPVI levels. In contrast to GPVI driven platelet responses, platelet dense granule secretion, integrin activation and changes in surface expression of CD41 were significantly increased in EXOC3$^{-/-}$ platelets in response to PAR4-AP- and thrombin treatment. These elevated integrin activation responses were completely suppressed with a P2Y12 receptor antagonist suggesting enhanced secretion of ADP was a critical mediator of these responses in EXOC3$^{-/-}$ platelets. However, calcium responses to PAR4-AP or thrombin were unaltered suggesting that the respective receptor signalling events were normal in EXOC3$^{-/-}$ platelets. Finally, an *in vivo* injury model of arterial thrombosis revealed a significantly accelerated rate of thrombosis in EXOC3$^{-/-}$ mice.

Conclusions: Overall, the exocyst complex appears to negatively regulate platelet dense granule secretion which constrains arterial thrombosis.

PB1763 | Omega-6 DPA and its 12-LOX Oxylipins Regulate Platelet Reactivity in a Non-genomic PPARa-dependent Manner

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Background: Arterial thrombosis is the underlying cause for several cardiovascular-related events, such as myocardial infarction, ischemia, and stroke. While dietary supplementation that includes polyunsaturated fatty acids (PUFAs) have been proposed to elicit a cardiovascular protective effect, several conflicting clinical trials have reported an overall inconclusive anti-thrombotic role. The underlying mechanism by which omega-6 PUFAs impart antiplatelet properties remains unknown.

Aims: The current study sought to investigate whether fatty acids produced in the body following administration of the omega-6 PUFA docosapentaenoic acid (DPA), and its oxylipin metabolites provide direct cardiovascular protection and the mechanisms mediating their antiplatelet effects.

Methods: Human and mouse blood and isolated platelets were tested with omega-6 DPA and its 12-LOX-derived oxylipins, 11-HpDPA and 14-HpDPA, in their ability to inhibit platelet activation. In human and mouse studies, pharmacological and genetic approaches were utilized to investigate and verify the underlying mechanism by which DPA and their oxylipins evoke anti-platelet effects.

Results: DPA_{n-6} was demonstrated to potently inhibit platelet activation through its 12-LOX-derived oxylipins, 11-$HpDPA_{n-6}$ and 14-$HpDPA_{n-6}$, and the inhibitory effects were selectively reversed through inhibition of PPARα. PPARα binding was confirmed using a PPARα transcription reporter assay and PPARα-deficient mice. These approaches confirmed selectivity of platelet inhibition was due to effects of 11-$HpDPA_{n-6}$ and 14-$HpDPA_{n-6}$ acting through PPARα. Finally, mice administered DPA_{n-6}, 11-$HpDPA_{n-6}$, or 14-$HpDPA_{n-6}$ exhibited reduced thrombus formation in the arteriole following a laser-induced injury of the cremaster, while thrombus growth was not further affected by the oxylipins in the PPARα-deficient mice.

Conclusions: The current study demonstrated that DPA_{n-6} and its oxylipins potently and effectively inhibits platelet activation and thrombosis. Platelet function is regulated in part through and oxylipin-induced PPARα-dependent manner, suggesting targeting PPARα may represent a new strategy to treat thrombotic-related diseases.

PB1764 | Important Role for SHARPIN in Supporting Platelet Linear Ubiquitination and Thrombus Formation

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Background: SHARPIN (Src homology 3 and multiple ankyrin repeat domains protein (SHANK)-associated RH domain-interacting protein) as part of the linear ubiquitin chain assembly complex (LUBAC) catalyses the addition of linear (Met1-linked) ubiquitin chains to substrates. As part of this complex SHARPIN acts as a multi-functional modulator of immune and inflammatory responses through regulation of NfkB activation. In addition, SHARPIN can act as a negative regulator of integrin function. Despite platelets being anucleate cells several studies have determined potential roles for both ubiquitination and NfkB in regulating platelet function, however little is known about either linear ubiquitination and/or SHARPIN in platelets.

Aims: To determine the role of SHARPIN in platelet linear ubiquitination and platelet function.

Methods: Platelet functional responses (integrin activation, P-selectin expression, ATP secretion and in vitro thrombus formation) and platelet ubiquitination were determined in platelets from mice homozygous for SHARPIN^{cpdm} allele (cpdm/cpdm), which lack SHARPIN expression.

Results: We confirmed that SHARPIN was expressed in platelets from wild-type mice but not mice homozygous for SHARPIN^{cpdm} allele (cpdm/cpdm) and that this correlated with a reduction in linear ubiquitination. Platelet function in response to thrombin was unaffected. In

contrast, CRP-XL- and U46619- mediated platelet responses were significantly reduced and thrombus formation under flow on a collagen-coated surface was impaired. Despite the reported role for NfkB in regulating platelet function we were unable to recapitulate the cpdm/cpdm phenotype by inhibiting IκBα phosphorylation.

Conclusions: The absence of SHARPIN in platelets from cpdm/cpdm mice results in impaired linear ubiquitination, functional responses to CRP and U46619 and thrombus formation, indicating an important role for SHARPIN in platelet functional responses.

ROLE OF HEMOSTATIC SYSTEM IN CANCER, INFLAMMATION AND IMMUNITY

COAGULATION PROTEINS BEYOND HEMOSTASIS

PB1768 | Cross-Talk of Activated Protein C(aPC) with Integrin and its Effect on T-Cell Signaling

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Background: The serine protease activated protein C (aPC) plays a role in innate immunity through both its anticoagulant and signaling properties via protease activated receptors (PARs). We recently established a function of aPC and the signaling incompetent receptor (PAR3) in adaptive immunity (GvHD). aPC has been shown to interact with RGD-binding-integrins as co-receptors. However, whether aPC and PAR3 can modulate T-cell integrin signaling, a key mechanism regulating T-cell activation, adhesion and migration, is unknown.

Aims: We aim to investigate whether aPC regulates T-cell activation, adhesion and migration via integrins on T-cells.

Methods: GvHD was induced in APC^{high}, $APC^{high/RGE}$, and C57BL/6 (WT) mice. Disease severity was assessed by studying survival, clinical scores and cell death in gastrointestinal tract. *In-vivo* migration of aPC pre-incubated T-cells was studied in a murine DTH model by two-photon-microscopy. T-cell activation and adhesion were studied in-vitro. The effect of aPC on integrin signaling was studied using immunoblotting and FACS. A T-cell(Jurkat)-B-cell(Raji) conjugation assay was performed using dual color flow cytometry to study the effect of aPC on immunological synapse.

Results: Mice with endogenous high levels of aPC (APC^{high}), but not mice expressing a mutant aPC lacking the integrin binding site ($APC^{high/RGE}$), were protected from GvHD in comparison to WT mice. Surprisingly, we observed *in-vitro* that aPC additionally regulates the activation of non-RGD integrin. aPC modulated global T-cell phosphorylation. aPC reduced T-cell activation, and adhesion to ICAM1 and fibronectin while increased migration (*in-vivo*) and phosphorylation of p-PLCγ-1 along with a decline of active-Rap1. A reduction in T-cell B-cell conjugation was observed upon aPC incubation of T-cells.

Conclusions: aPC reduces global phosphorylation and thus modulates inside-out signaling of integrins. In addition, aPC binds via its RGD domain to RGD-binding integrins, affecting T-cell function via outside-in signaling. In future studies we will dissect these independent molecular pathways and evaluate the pathophysiological relevance of these findings.

PB1769 | Chronically Elevated Interleukin-6 Levels in Mice Lead to a Disturbance in the Coagulation System

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Background: Pro-inflammatory cytokines like Interleukin-6 (IL-6) play an important role as activators of the hemostatic system and in the downregulation of physiological antithrombotic mechanisms. IL-6 has been described to influence platelet production and activation. It was associated with accelerated clotting and intravascular coagulation in tissue factor (TF)-driven murine thrombosis models.

Aims: Our goal was to analyze the precise role of myeloid cell-derived IL-6 on thrombosis formation.

Methods: We generated a new mouse model with Cre-recombinase driven constitutive overexpression of IL-6 in myeloid cells (LysM-IL-$6^{OE/+}$, control mice: IL-$6^{OE/+}$) and performed subtotal vena cava inferior ligation to analyze the development of deep vein thrombosis (DVT) via ultrasound analysis and histology. Thrombin generation, coagulation parameters and coagulation factors, as well as cytokine levels were measured.

Results: LysM-IL-$6^{OE/+}$ mice displayed no DVT 3 days post inferior vena cava ligation compared to normal thrombus formation in controls. Platelets were more reactive in LysM-IL-$6^{OE/+}$ mice compared to control mice. Platelet number was unaltered in LysM-IL-$6^{OE/+}$ mice and there were no differences in D-Dimer levels. We found unstoppable post-operative bleedings in LysM-IL-$6^{OE/+}$. They displayed a prolonged aPTT (LysM-IL-$6^{OE/+}$: 104 s, IL-$6^{ind/+}$: 29 s) and a significantly delayed INR (LysM-IL-$6^{OE/+}$: 0.72, IL-$6^{OE/+}$: 0.65) as well as moderately

reduced levels of coagulation factors V and IX. An increase of von Willebrand factor in LysM-IL-6$^{OE/+}$ mice as well as elevated levels of antithrombin. Besides, we detected increased levels of fibrinogen in LysM-IL-6$^{OE/+}$ (LysM-IL-6$^{OE/+}$: 536 mg/dl, IL-6$^{ind/+}$: 211 mg/dl). Most importantly, hepatic levels of proteinase inhibitor α2 macroglobulin (α2M) mRNA and protein were highly increased in LysM-IL-6$^{OE/+}$ mice. There was a significantly elevated erythrocyte sedimentation rate in line with agglutinating erythrocytes, which seemed to be at least partly mediated by IL-6 and α2M. Platelet-erythrocyte interaction seemed to be essential in this severe bleeding phenotype.

Conclusions: Chronic myeloid overexpression of IL-6 provokes a bleeding phenotype in mice.

PB1770 | Human Plasma Kallikrein (PKa) Potentiates ADP-Induced Platelet Activation

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Background: PKa contributes to contact-activated thrombin formation and, in turn, hemostasis by acceleration the activation of factor XII to XIIa when exposed to negatively charged surfaces on medical devices or biologic substances such as polyP, RNA, DNA, and collagen. However, PKa's contribution to platelet activation has not been studied.

Aims: To examine the mechanism(s) and significance by which PKa potentiates platelet aggregation induced by ADP.

Methods: Human platelet: blood was collected into 3.8% sodium citrate (w/v) 1:10, centrifuged 141 x *g*/12 min (PRP, platelet rich plasma) and 350 x *g*/15 min (PPP, platelet poor plasma).

Platelet activation: ADP-induced platelet activation was measured in a lumi-aggregometer (Chronolog). PRP also were incubated with Fluo-4/AM and calcium mobilization was measured in the confocal microscope.

Immunoblot studies: platelets were lysed, separated by SDS-PAGE and transferred onto nitrocellulose membrane and immunoblotted with anti-phospho-p38, anti-p38 MAPK antibody, anti-phospho-ERK or anti-ERK antibody or anti-AKT monoclonal antibody.

Results: PKa potentiates platelet responses to subthreshold doses of ADP (2 μM) by proteolysis of the G-protein-coupled receptor PAR-1, although PKa itself does not induce platelet aggregation. The potentiation is mediated by the integrin αIIbβ3 through interactions with the KGD/KGE sequence motif in PKa. PKa binds integrin αIIbβ3 to position it for PAR-1 hydrolysis and leads to the amplification of the phosphorylation of Src, AkTS473, ERK1/2, and p38 MAPK, and to Ca^{2+} mobilization initiated with subthreshold doses of ADP. The effect of PKa is blocked by specific antagonists of PAR-1 (SCH 19197) and αIIbβ3 (abciximab) and by synthetic peptides containing the KGD and KGE sequence motifs of PKa. Further, recombinant plasma kallikrein inhibitor, rBbKI, blocks this entire mechanism.

Conclusions: These findings indicate a new function for PKa. Formation of PKa lowers the threshold for ADP-induced platelet activation. The present observations are consistent with the notion that PKa is a risk factor for cardiovascular disease.

PB1774 | The Fc and C1/C2 Domains of Recombinant Factor VIII Fc Fusion Protein Engage Monocytes in an FcgammaRII-Dependent Manner to Reduce Their Differentiation Potential into Osteoclasts

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Background: Procoagulant factor VIII (FVIII) is an essential cofactor in the coagulation cascade. Deficiency of FVIII activity leads to hemophilia A. Recombinant FVIII Fc fusion protein (rFVIIIFc) was developed to extend factor half-life, thus decreasing treatment burden and increasing protection in patients. We have previously reported that rFVIIIFc, but not rFVIII, inhibits monocyte-derived osteoclast formation *in vitro*.

Aims: To further investigate the mechanisms by which rFVIIIFc interacts with blood monocytes to reduce their differentiation potential.

Methods: Primary human blood monocytes were treated with rFVIIIFc or rFVIII plus human IgG at various concentrations and then cultured for osteoclast differentiation *in vitro*. Multiple myeloid lineage markers were used to immunophenotype and distinguish differentiated monocytes and osteoclasts. The involvement of Fc or FVIII domains in mediating rFVIIIFc interaction with monocytes was probed using antibodies blocking each type of FcγRs, or anti-FVIII antibodies and von Willebrand factor (VWF) binding to various FVIII domains.

Results: We report a novel immune-regulatory effect of rFVIIIFc on inhibiting monocyte differentiation into osteoclasts through Fc and FVIII domains with a mean IC50 of 8 nM. The cells differentiated from rFVIIIFc-treated monocytes were phenotypically distinct from osteoclasts and remained largely monocytic. As for the interactions between rFVIIIFc and monocytes modulating this phenotype, the Fc domain most effectively engaged FcγRII on the cell surface; C1 and C2 domains of FVIII were mapped to be required for interacting with monocytes, also evidenced by loss of the immune-regulatory effects of VWF-complexed rFVIIIFc.

Conclusions: These data suggest a "dual-touchpoints" model for rFVIIIFc interacting with monocytes. The FVIII portion interacts with monocytes via C1 and C2 domains and, in parallel, the Fc domain predominantly engages FcγRII on the same cell, subsequently reducing monocyte differentiation potential into osteoclasts. Therefore, rFVIIIFc may possess a biological activity unique from rFVIII which may reduce joint bone erosion and bone mass loss in hemophilia A patients.

PB1776 | Coagulation Factor XII Signaling Triggers Inflammasome-associated Renal Damage in Diabetic Nephropathy

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Background: Diabetic nephropathy (dNP) is the leading cause of end stage renal disease worldwide. Diabetes is associated with elevated levels of FXII and other contact coagulation pathway proteins. FXII signaling is linked to the pathogenesis of several inflammatory conditions including pulmonary fibrosis, atherosclerosis and cutaneous wounds. Whether FXII plays a role in dNP is currently not known.

Aims: To identify the role of coagulation FXII in dNP and to elucidate the involved signaling pathways.

Methods: Wild type (Wt) and FXII knockout mice ($FXII^{-/-}$) were used in this study. Persistent type-1 diabetes was induced using streptozotocin and was maintained for 24 weeks, followed by *ex-vivo* and *in vitro* studies to obtain mechanistic insights. Additionally, human samples were studied to determine translational relevance.

Results: In diabetic $FXII^{-/-}$ mice albuminuria, tubulointerstitial fibrosis and glomerular damage were ameliorated compared to diabetic Wt mice. Increased tubular expression of FXII was observed in human and mice diabetic kidney sections. Activated FXII was detected in urine samples from human and diabetic mice. Markers of inflammasome activation (cleaved Casp-1 and IL-1β, NLRP3 expression) were reduced in kidney lysates of $FXII^{-/-}$ mice as compared to Wt mice. Renal expression of urokinase-type plasminogen activator receptor (uPAR), which is strongly induced in diabetic WT mice, was normalized in diabetic $FXII^{-/-}$ mice. Treatment of tubular cells in vitro with recombinant mouse FXII induced tubular injury markers and inflammasome activation. These effects were abolished by uPAR blockade. Treatment of Wt mice with established dNP with vivo morpholino against FXII stopped progression of the disease and improved kidney functions.

Conclusions: Coagulation FXII signaling via uPAR triggers inflammasome activation and renal damage in diabetic mice. Inhibition of FXII may be a promising therapeutic approach to prevent activated inflammasome signaling in dNP and to halt the progression of the disease.

PB1777 | A Validated Model for the Diagnosis of Periprosthetic Joint Infection with Coagulation-related Markers: TGT and NETs

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Background: Bacterial infection activates coagulation through immunothrombosis. Neutrophils are the first line of defense against bacterial infection, but its mechanism in periprosthetic joint infections (PJI) is unknown. Maybe through the release of neutrophil extracellular traps (NETs), since they are present in the bacterial biofilm of PJIs. There are no diagnostic tests of PJI with 100% sensitivity/specificity and often the diagnosis is uncertain due to lack of compliance with some criteria. We previously obtained a model to estimate the pre-surgical PJI risk using markers of NETs and thrombin generation test (TGT).

Aims: To validate the diagnostic capacity of PJI of our model.

Methods: We obtained citrated plasma, in the preoperative period of prosthetic surgery, from 32 patients prospectively recruited. The PJI was diagnosed in 9 of these patients. The diagnosis of 9 patients was uncertain, which is relatively frequent. We validate our predictive model of PJI that includes markers of NETs (DNA and calprotectin) and TGT variables (lagtime and starttail), with a multivariable logistic regression model with R (v3.5.0).

Results: All parameters of NETs and TGT were significantly increased in patients with PJI and with uncertain diagnosis compared with patients undergoing prosthetic surgery due to non-septic causes. The area under the ROC curve of our model was 0.79 (P = 0.019, 95% CI[0.58,1]). With our model, the presurgical probability of PJI for each patient was higher in patients with a confirmed diagnosis of PJI than without PJI (P = 0.009).

Conclusions: Markers of NETs and TGT seem to have diagnostic utility of PJI before surgery. Our model could reinforce the clinical criteria currently available in order to reduce the number of uncertain diagnoses and thus be able to make an early and effective diagnosis and treatment to minimize side effects of the PJI such as tissue damage, bone degradation and replacement surgery. ISCIII-FEDER(PI14/00079, PI14/00512, FI14/00269, CPII15/00002, PI17/00495), GV(ACIF/2017/138),SETH.

PB1778 | Coagulation Markers, Neutrophil Extracellular Traps and Thrombin Generation Test, in the Detection and Risk Stratification of Prostate Cancer

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Background: Cancer patients present coagulation abnormalities that provide the background for an increased tendency of these patients to both thrombosis and hemorrhage. Reciprocally, malignancy affects haemostasis and the hemostatic system affects the tumour biology. Patients with prostate cancer (PCa) paradoxically have both a hypercoagulable state and a bleeding diathesis, and this hypercoagulable state is a reflection of poor prognosis and represents a selective advantage for tumor cells.
Aims: To explore the usefulness of thrombotic markers, neutrophil activation markers and the thrombin generation test (TGT), for the diagnosis and stratification of PCa patients.
Methods: We obtained citrated plasma from 27 locally advanced PCa patients, 51 PCa patients, 18 benign prostatic hyperplasia (BPH) patients and 199 healthy controls. Plasma markers of neutrophil activation (cell-free DNA, myeloperoxidase, nucleosomes and calprotectin) were quantified in PCa and BPH patients and the TGT (CAT, Thrombinoscope) was performed in all samples. Statistical analysis was performed using R (v3.5.0).
Results: We adjusted two elastic net predictive models for PCa classification. The first model, able to discriminate different PCa degrees of severity from BPH patients, included cfDNA, myeloperoxidase, nucleosomes, starttail and lagtime as predictor variables with a validated AUC of 0.68. The second model, able to discriminate PCa and BPH patients from healthy controls, included lagtime, peak, ttPeak, velIndex and startTail as predictor variables with a validated AUC of 0.79.
Conclusions: We propose two models able to distinguish PCa patients of different degrees of severity (locally and non-locally advanced), BPH and healthy controls. Our study highlights the hypercoagulable state found in PCa patients and reinforce the idea of using therapeutic strategies targeting effector molecules of blood coagulation.
ISCIII-FEDER (PI14/00079, PI14/00512, FI14/00269, CPII15/00002, PI17/00495), Generalitat Valenciana (ACIF/2017/138) and SETH.

PB1780 | Plasma Concentration of Activated Factor VII-antithrombin, But Not Tissue Factor Pathway Inhibitor and Factor VII Levels, Predicts Mortality in Subjects with Liver and Colon Cancer Undergoing Curative Surgical Intervention

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Background: Tissue factor (TF), the main initiator of the coagulation cascade, plays a role in cancer progression. In preliminary analysis we showed that plasma concentration of activated factor FVII-antithrombin (FVIIa-AT), a biomarker reflecting FVIIa-TF interaction, may predict mortality in patients with liver and colon cancer.
Aims: To examine more in depth the potential prognostic role of FVIIa-AT by evaluating other hemostatic biomarkers related to FVIIa-TF pathway in a cohort of subjects with liver and colon cancer undergoing surgical intervention with a curative intent.
Methods: Out of the original study cohort of 138 neoplastic patients assessed for FVIIa-AT levels, plasma samples for FVII antigen (FVII_Ag), total and free tissue factor pathway inhibitor (TFPI) assays were available for 137 subjects (67.2% males, mean age 67.4 ± 9.6 years, 37.2% hepatocellular carcinoma, 31.4% cholangiocarcinoma, and 31.4% colon cancer). Samples of venous blood were drawn before surgery. FVIIa-AT, FVII_Ag, total and free TFPI levels were measured by ELISA.
Results: FVIIa-AT, FVII_Ag, total and free TFPI plasma levels were all reciprocally directly correlated. After a 34-month median follow-up, 75 subjects (54.7%) died. Stratifying the study population according plasma concentration of these hemostatic biomarkers, mortality rate increased progressively from the lowest to the highest quartile of FVIIa-AT (38.2%à54.3%à54.3%à70.6%, P = 0.003) and total TFPI (35.3%à61.8%à51.4%à70.6%, P = 0.023), while no significant association was found for either free TFPI or FVII_Ag. The association of FVIIa-AT and total TFPI with mortality was confirmed after adjustment for sex, age, and type of cancer. Including both FVIIa-AT and total TFPI in Cox regression models, only FVIIa-AT remained a predictor of mortality (the highest versus the lowest quartile: HR 2.95 with 95%CI 1.49-5.84), while the association was lost for total TFPI.
Conclusions: Plasma concentration of FVIIa-AT, rather than those of TFPI and FVII_Ag, may predict mortality in patients with liver and colon cancer undergoing curative surgical intervention.

PB1781 | Hemostatic and Thrombotic Profiles in Pre and Post Remission Induction Phase of Acute Leukemia

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Background: Hemostatic, fibrinolytic and thrombotic parameters are good diagnostic tools for hematological malignancies.Impairment of hemostatic, fibrinolytic and thrombotic activities are often associated with hematological malignancies.

Aims: Present study was designed to evaluate hemostatic, fibrinolytic and thrombotic changes in patients with de novo AML (acute myeloid leukemia) and ALL (acute lymphoid leukemia) prior to start and after the induction of treatment in following 4 weeks

Methods: A total of 150 subjects were enrolled and diagnosis of acute leukemia was made by a hemato-pathologist and verified by according to the WHO classification. Blood sample was collected from controls and patients at the time of diagnosis and at day 28 post chemotherapy. Platelet free plasma is used for blood coagulation analysis. Findings from the present study indicates the derangements of hemostatic, fibrinolytic and thrombotic parameters.

Results: Significant increased level of PT, APTT, prothrombin time, von Willebrand antigen, fibrinogen and factor VIII were found in AML and ALL pretreated patients. Fibrinolytic markers indicated a marked increased D- Dimer and plasminogen level while reduced level of alpha 2 antiplasmin in pretreated patients. Thrombotic markers showed increased level of antitrypsin III and Lupus anticoagulant while decreased level of Protein C in AML and ALL pretreated patients. Chemotherapy treatment showed significant results on PT, fibrinogen, D-Dimer,alpha 2 antiplasmin, Protein C, and anti-thrombin III.

Conclusions: The present investigations indicates that early detection of thrombotic and hemostatic defects will help to cope up the severity of leukemia and reducing morbidity and mortality rates. Early diagnosis and active intervention will help in reducing the bleeding complications and provide better survival with good quality of life. However post chemotherapy complications is still a big challenge.

COMPLEMENT AND HEMOSTATIC SYSTEM

PB1782 | The Role of Antithrombin in Complement Activation: Pilot Data from Patients with Inherited Antithrombin Deficiency

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Background: Antithrombin (AT) is a serine protease inhibitor (serpins) and plays a critical role in in the regulation of coagulation. Its major function is the inhibition of activated coagulation factors predominantly activated factor X (FXa) and thrombin (FIIa). Growing evidence demonstrates that antithrombin also exerts anti-inflammatory function via direct interactions with endothelium and indirectly by inhibiting coagulation factors that may be involved in complement activation. We hypothesized that patients with inherited antithrombin deficiency may have increased complement activation if the above was true.

Aims: To determine whether markers of complement activation are altered in patients with antithrombin deficiency compared to with healthy controls.

Methods: Twenty patients with AT deficiency and 20 healthy controls were bled using flawless venipuncture and plasma was prepared in the normal way and stored at -80C. Complement activation was determined by measuring C3a, iC3b and C5a with specific anti-human antibodies by ELISA (Hycult Biotech). The complement haemolytic activity (CH50) was also determined using Diamedix EZ Complement Cells. All samples tested in the same run to prevent inter-assay variability. Statistical analysis was conducted using IBM SPSS 25; the data were non-parametric and so the Mann Whitney test was used to compare results.

Results: Medians (minimum and maximum) and p values for all four complement activation makers are presented in table 1.

Conclusions: In this small study there was a trend for all markers of complement activation to be increased in those with antithrombin deficiency. This reached significance for C3a. This data suggests a novel observation that there is increased complement activation in patients with inherited AT deficiency compared to healthy individuals. Further studies are ongoing.

TABLE 1

PARAMETERS	CH50 [%]	C3a [ng/ml]	C5a [ng/ml]	iC3b [ng/ml]
AT deficient patients median (min and max)	134 (103-159)	201 (71-561)	3 (1-13)	2 (2-4)
Control group median (min and max)	130 (104-147)	113 (54-367)	2 (1-26)	2 (1-4)
P values	0.213	0.005	0.194	0.130

PB1783 | Complement Mannan-Binding-Lectin Is Associated with Activated Platelets during Clot Formation in a Microfluidic Bleeding Model

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Background: Even though the coagulation cascade is responsible for the formation of clots, other physiological systems, such as the complement system, can influence it. Mannan-binding-lectin (MBL), the initiator of the complement lectin pathway, has been shown to bind to fibrinogen. To investigate the clotting process under realistic physiological conditions, we have established a microvascular endothelialised whole blood flow model. This model allows the imaging of clot formation in real-time by confocal microscopy.
Aims: The aim of this project was to investigate the role of MBL during clot formation upon vascular injury in a microfluidic bleeding model.
Methods: Endothelial cells were used to create a synthetic vessel in a polydimethylsiloxane mould. Whole blood was then flown through the vessel and an injury was created by rupturing a collagen pouch adjacent to the vessel. The formation of the clot was monitored in real-time with a confocal microscope, fluorescently labelled fibrinogen and antibodies against MBL, fibrin crosslinks, CD41 (platelets), CD62P (activated platelets) and their corresponding secondary antibodies.
Results: Early experiments showed that, after an injury, MBL accumulated outside the vessel in a "wall-like" structure. To determine the exact location of this structure, several antibodies were tested: MBL did not completely co-localize with fibrinogen nor fibrin crosslinks. The same structure was clearly recognizable with platelets ($CD41^+$), but the MBL was not present to the same extent. The only exact co-localization was clearly recognizable with activated platelets (CD62P).
Conclusions: MBL seems to be interacting with activated platelets ($CD62P^+$) during the coagulation reaction, and not only with fibrin as demonstrated before. This discovery might indicate, that platelet activation could initiate the complement system by accumulating MBL when endothelial cells are injured. The microvascular bleeding model is thus an interesting tool to investigate the cellular components during the clotting process.

PB1784 | Anti-complement Effect of Heparin in Stimulated Whole Blood

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Background: Emerging *in vitro* studies have shown that heparin, an anticoagulant, possesses anti-inflammatory properties. Recently this was demonstrated by reduction of toxicity exhibited by neutrophil extracellular traps (NETs) in the presence of heparin and modified heparin, but the exact mechanism for this anti-inflammatory activity remains to be elucidated. It has also been established that negatively-charged heparin can bind to positively-charged histones, a major component of NETs which can cause complement activation.
Aims: To investigate the anti-complement effects of heparin on whole blood stimulated by histones.
Methods: Selectively desulfated heparins and molecular weight fractions of heparin were investigated. Histones (50 μg/mL) spiked in whole blood were incubated with 200 μg/mL of heparin for 6 hours and levels of C3a, a marker of complement activation, were then measured by ELISA. Data were analysed by Dunnett's multiple comparison (* = $p < 0.05$) relative to histones only, and across samples.
Results: Heparin reduced histone induced C3a by 90% (figure 1). 2-O-desulfated, N-acetyl and 6-O-desulfated heparins significantly lowered C3a levels by 83%, 71% and 70% respectively. Of these heparins there was no difference in reduction for 2-O-desulfated heparin when compared to heparin. N-desulfated and N-acetyl-O-desulfated heparin had no effect. Molecular weight heparin fractions significantly reduced C3a production (figure 2), with fractions ≤Mw1900 less effective than fractions ≥Mw3450. However, increase in Mw above 3450 did not further reduce observed C3a.

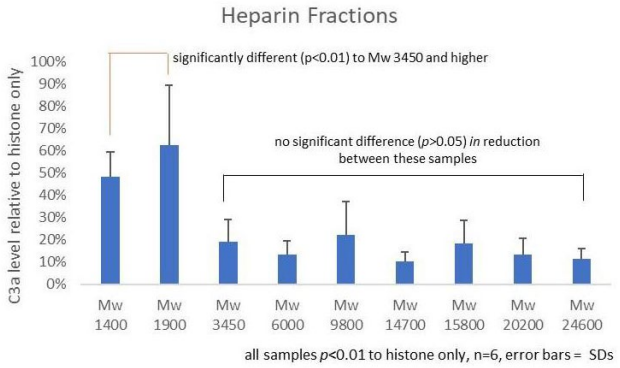

FIGURE 1 Residual C3a levels of histone stimulated whole blood following addition of modified heparins

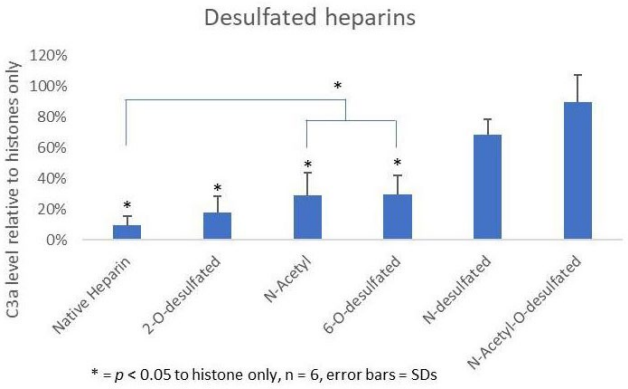

FIGURE 2 Residual C3a levels of histone stimulated whole blood following addition of heparin fractions

Conclusions: All heparin tested attenuated histone induced C3a production. This effect was charge dependent as both fully desulfated (N-acetyl-O-desulfated heparin with complete loss of negative-charge) and N-desulfated (which exposes positively charged amine groups) heparins had no effect, whilst selective desulfation retained some of heparin's activity. There was a molecular weight dependence, with a threshold of fractions > Mw3450 g/mole (~10 saccharides) showing no further decrease in C3a levels induced by histones.

PB1785 | Activated Factor XI Promotes Complement Activation by Neutralizing Complement Factor H

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Background: Complement factor H (CFH) is the major inhibitor of the alternative pathway of the complement system and is structurally related to B2-GPI, which itself is known to bind to ligands including factor XI (FXI). We observed reduced complement activation when FXI activation was inhibited in a baboon model of sepsis, suggesting crosstalk between FXI and the complement cascade. While interaction of the complement system with other contact activation enzymes, FXIIa or kallikrein, has been demonstrated, it is unknown whether FXIa directly interacts with complement.
Aims: FXIa promotes complement activation by neutralizing CFH.
Methods: Recombinant CFH was incubated with FXIa for increasing times before being analyzed by Western blot and aminoterminal sequencing. CFH cofactor activity was evaluated by measuring the cleavage of C3b by factor I (FI) and the lysis of sheep red blood cells (RBCs). Binding of CFH to endothelial cells (ECs) was measured using a cell surface immunoassay. FXI activation by FXIIa or thrombin was evaluated using a FXIa chromogenic assay.
Results: Incubation of CFH with FXIa reduced the capacity of CFH to enhance the cleavage of C3b by FI. FXIa enhanced hemolysis in the presence of CFH. The binding of CFH to ECs was reduced after incubating CFH with FXIa. FXIa neutralized CFH by cleavage of the Arg341/Arg342 bonds at the end of complement control protein (CCP) 6 domain. The addition of polyP enhanced the capacity of FXIa to cleave CFH. FXIa cleaved CFH that was bound to the surface of ECs, and inhibited FXI activation by either thrombin or FXIIa.
Conclusions: Our study provides a novel molecular link between the contact pathway of coagulation and the complement system. These results suggest that FXIa generation enhances the efficiency of the complement system, thus, may support the immune system.

INFECTION AND HEMOSTATIC FACTORS

PB1786 | Lung Vessels Thrombosis Induced by Severe CAP: 3D Model of Thrombus

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Background: High lethality of hospitalized patients with CAP is well known fact, but mortality because of lung thrombosis had not been investigated.
Aims: To study microscopically changes in lung autopsy in patients who died because of severe CAP.
Methods: Electronic microscopy of lung tissue in 5 patients died because of severe CAP, 3-D computer modeling.
Results: Microscopically were found mixed thrombi in the lumen of the arteries, more often in average caliber. These thrombus had a head (the structure of a white thrombus), a body (actually a mixed thrombus) and a tail (the structure of a red thrombus), which were isolated. The head was attached to the endothelial lining of the vessel, which distinguishes a thrombus from a posthumous blood clot or from an embolus.
On three-dimensional model of the lung vessel wall fragment (top view): gray color - the stroma of the vessel wall; brown - smooth myocytes; yellow - endothelial cells; red - erythrocytes; blue - lymphocytes, histiocytes, macrophages. Cytoplasmic endothelial cell membrane is intense; change in the strength of the contacts between the endothelial cells and the basal membrane (phenomenon of the "picket fence of endothelial cells"), signs of swelling in the places of defects; extension of subendothelial space.
Conclusions: Thrombus, which were found microscopically in the lumen of the arteries in patients with severe CAP, were differ from embolus or post-mortem blood clots. Therefore, these changes can reflect systemic thrombosis at patients with severe CAP and could be one of the reason of mortality in patients with severe CAP.

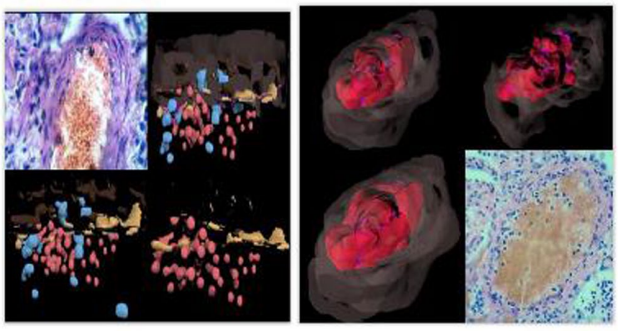

FIGURE 1 3D model of thrombi

PB1787 | Relationship between HAART treatment and Markers of Coagulation among People Living with HIV/AIDS (PLWHA) in Gombe, Nigeria

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Background: Human immunodeficiency viral infection is known to cause coagulopathies by various mechanisms, especially during its late course. Administration of highly active antiretroviral therapy (HAART) could positively or negatively affect people living with HIV/AIDS (PLWHA). Thus specific investigations and determination of effect of HAART on coagulation variables are required for initiation and monitoring early to prevent disease progression.

Aims: The aim of the study was to investigate the effect of HARRT on the viral load and some coagulation screening variables (prothrombin time (PT), INR, partial thromboplastin time with kaolin (PTTK), and d-dimer among PLWHA on HAART and treatment naïve patients in the Federal Teaching Hospital Gombe, Gombe state, Nigeria.

Methods: It is a case control study that compared coagulation markers between 120 PLWHA on HAART, and 60 treatment naïve consenting patients. Blood samples samples were analyzed using standard operating procedures. Means and standard deviations were compared between the two groups using Student's t-test. Level of statistical significance was set at 0.05. Ethical approval was obtained from the ethical committee of the Federal Teaching Hospital Gombe.

Results: People living with HIV/AIDS (PLWHA) on HAART had significantly ($p < 0.05$) lower viral load (316.2 ± 2.0 cp/ml), prothrombin time (16.02 ± 2.79 seconds), d-dimer (302.00 ± 2.69 ng/dl), and INR (1.15 ± 0.21) when compared with PLWHA treatment naïve; viral load (1584.9 ± 15.8 cp/ml), PT (17.15 ± 3.28 seconds), d-dimer (676.08 ± 3.02 ng/dl) and INR (1.26 ± 0.37). The PLWHA on HAART had significantly ($p < 0.05$) higher PTTK (45.06 ± 16.10 seconds) when compared with treatment naïve (40.53 ± 10.12 seconds). No significant differences observed among the groups based on the type of HAART regimen.

Conclusions: The study showed that, HAART has positive impact on some coagulation variables; thus have the possibility of improving treatment outcomes.

PB1788 | Coagulation Profile in Patients with Pneumonia Assessed by Thrombin Generation Procedures

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Background: Coagulation profile subsequent to pneumonia is hardly assessed by conventional coagulation tests or measurement of pro- and anticoagulant factors when taken in isolation. Conversely, thrombin generation assays (TGA) evaluating thrombin-generation and decay, is more suited to assess the balance between pro- and anticoagulants as it occurs in-vivo.

Aims: To assess the coagulation profile and detect hypercoagulability (if any) in patients with pneumonia at the onset and at recovery (one month later) of disease.

Methods: We investigated 20 consecutive patients admitted to hospital because of pneumonia and 20 healthy subjects (controls). Blood was collected into vacuum-tubes containing 1/10 volumes of 0.109M citrate at onset of the disease (T_0) and at recovery (T_1). TGA was assessed according to Hemker with a homemade method by addition to plasma of tissue-factor (1pM) and synthetic phospholipids (1 μM). TGA was performed with and without thrombomodulin (TM). Results were expressed as endogenous thrombin potential (ETP) and as ratio of ETP with/without TM (ETP-TM ratio). Increased ETP or ETP-TM ratio were taken as indexes of hypercoagulability. Factor VIII (FVIII), protein C (PC) and their ratio (FVIII/PC) were also evaluated. Comparison of results for ETP or ETP-TM ratio (patients vs controls) were performed and p values < 0.05 were considered statistically significant.

TABLE 1 Results [median (range)] * p-value, Mann-Withey test; § p-value, Wilcoxon test

	Controls	Patients at onset	Patients at recovery
ETP (nM*min)	1993 (1655-2617)	1950 (1359-2722)	1819 (1384-2380)§§
ETP-with-TM (nM*min)	1340 (827-1905)	1762 (973-2359)**	1304 (826-1928)§§
ETP-TM-ratio	0.65 (0.47-084)	0.87 (060-0.96)***	0.80 (0.44-0.88)**§
FVIII (%)	134 (87-227)	195 (103-364)***	143 (70-274)§
PC (%)	116 (80-137)	93 (44-136)**	114 (61-154)§§
FVIII/PC-ratio	1.16 (0.75-1.90)	2.02 (1.39-7.28)***	1.37 (0.62-2.31)§§§

Results: ETP with TM and ETP-TM ratio in patients at T_0 were significantly higher than controls ($p < 0.001$). ETP (regardless of TM) and ETP-TM ratio in patients at T_1 were significantly lower than T_0 ($p < 0.01$), but ETP-TM ratio was still higher than controls ($p < 0.05$). Changes of FVIII/PC ratio taken as an additional index of hypercoagulability paralleled those of ETP-TM ratio (table).

Conclusions: The hypercoagulability shown by ETP and ETP-TM ratio is likely due to the reduction of PC (anticoagulant driver) and increase of FVIII (procoagulant driver). The study contributes to define the mechanism of hypercoagulability associated with pneumonia.

PB1789 | M1 Protein from Group A Streptococcus Regulates Fibrin Clot Formation, Structure and Fibrinolytic Potential

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Background: The surface anchored M1 protein is a major virulence determinant of a highly prevalent and virulent Group A streptococcus (GAS) strain, M1T1. During early infection M1 is cleaved from the cell surface resulting in soluble M1 at the site of infection. Fibrin formation is an essential part of innate immunity and a protective fibrin biofilm covers the external surface of clots acting as the first line of defense. Within the clot the fibrin network traps bacteria to limit dissemination and reduce the severity of invasive infections. M1 is known to form a supramolecular complex with fibrinogen however the impact of this on fibrin formation and structure is unknown.

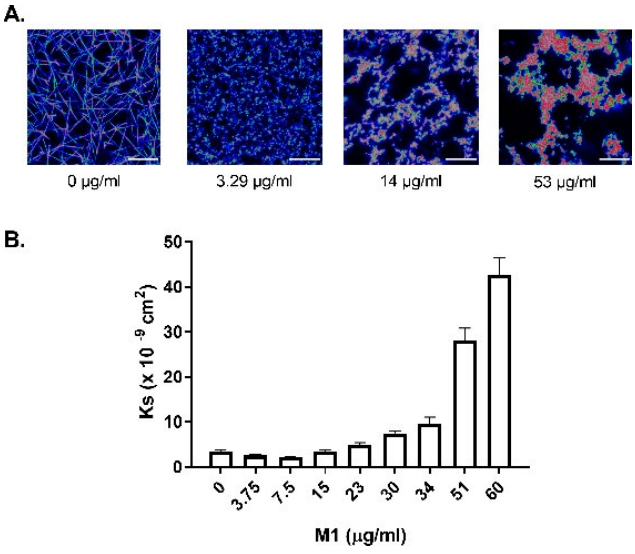

FIGURE 1 Confocal microscopy (A.) and porosity (Ks) (B.) of fibrin clots in the presence of varying concentrations of M1 (µg/ml). Scale bar: 20 µm

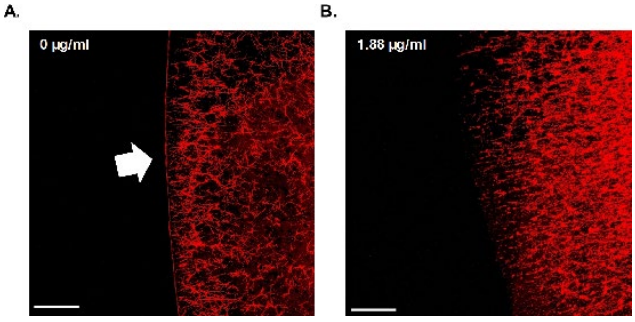

FIGURE 2 Confocal microscopy of protective fibrin biofilm formation (white arrow) in the absence (A.) and presence (B.) of M1. Scale bar: 100 µm

Aims: Investigate the impact of GAS M1 protein on fibrin clot formation, structure and fibrinolytic potential.

Methods: Fibrin clots incorporating recombinant M1 (rM1) were made by the addition of thrombin to human blood, plasma and purified fibrinogen. Fibrin structure was assessed using imaging techniques (confocal microscopy and scanning electron microscopy), permeation assays (gel porosity) and bacterial migration assays. Fibrin clot formation, mechanical strength and lysis rates were measured kinetically using thromboelastography (ROTEM).

Results: rM1 (0.47-60 µg/ml) increased clotting rates to produce heterogeneous clots with irregular fibre bundles and compacted fibrin (Figure 1A) and increased porosity (above 15 µg/ml rM1) up to 12-fold at 60 µg/ml (Figure 1B). rM1 disrupted formation of the protective fibrin biofilm (Figure 2) and reduced mechanical clot strength to undetectable levels above rM1 concentrations of 15 µg/ml. Lysis times for purified and plasma clots incorporating rM1 were reduced up to 2-fold.

Conclusions: GAS strains of M1-type are commonly associated with invasive infections. M1-bound fibrinogen at infection sites may contribute to the severity of infection by forming fibrin clots with a compromised protective film at the surface, that are mechanically weaker, more porous, and less resistant to lysis by plasmin.

PB1790 | Coagulation Factor XII Binds to the Opportunistic Pathogen, *Candida albicans* and Promotes Biofilm Formation

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Background: *Candida albicans* is an opportunistic pathogen that causes a wide variety of infections, including life-threatening systemic candidiasis. The virulence of this pathogen includes their ability to form hyphae and biofilms, protecting it from the host immune system and antifungal drugs. The surface of *C. albicans* is rich in *O*-mannan which, along with the hyphal protein Als3p, has

been shown to be crucial for biofilm formation. There is evidence to suggest that haemostatic proteins participate in biofilm formation. Factor XII (FXII) is a contact pathway protein which when activated can initiate coagulation, fibrinolysis and inflammation, however its role in biofilm formation is currently unclear.

Aims: Here we investigate the interaction between FXII and *C. albicans* to define whether the contact pathway influences virulence and biofilm formation.

Methods: Binding of FXII to *C. albicans* wild-type (WT), *als3*Δ/*als3*Δ and *O*-mannan *mnt1*Δ/*mnt2*Δ mutants was analysed by confocal microscopy with Dylight488/550-FXII(a). *C. albicans* biofilms were formed over 48 h on polymer squares ± FXII and quantified by colony forming units/ml.

Results: We have demonstrated that FXII and its activated form, FXIIa, bind to WT hyphae, but not yeast cells. Binding was not dependent on the presence of zinc. In marked contrast, FXII bound only to the germinating hyphal tip of the *als3*Δ/*als3*Δ mutant and did not bind *mnt1*Δ/*mnt2*Δ, implicating Als3p and *O*-mannan in the interaction of FXII with *C. albicans*. Our data show that biofilm formation is increased in the presence of purified FXII, and conversely is decreased in FXII-deficient plasma relative to normal plasma.

Conclusions: Our data show clear binding of FXII to the *C. albicans* hyphae. Use of mutant forms indicate potential roles for Als3p and *O*-mannosylated glycans in the interaction of FXII with *C. albicans*. Our studies indicate that FXII-hyphae binding promotes biofilm formation, thereby implicating this haemostatic protein in the virulence of this fungal pathogen.

PLATELETS AND CANCER

PB1791 | Successful Improvement Sexual Dysfunction After Pelvic Irradiation by Platelet Growth Factor

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Background: Sexual dysfunction is a common sequel of cancer treatment, affecting the quality of life in women treated with pelvic radiotherapy.

Aims: To evaluate the safety, symptom resolution and objective improvement of the autologous platelet released growth factor (APRGF) injection for treatment of Sexual Dysfunction (SDF) in cited patients.

Methods: This prospective *case series* study enrolled 10 cancer patients with SDF who underwent pelvic radiotherapy at least 3 years ago. Each patient received 1-2 cc PRFG within three-weeks (3 times injection) period. All patients were re-evaluated at eight weeks and six months.

Results: The mean age of participants was 46.90 ± 7.90 years. 70% of womens were multiparous and 30% were nuligravida. The type of cancer in participants include: rectum 20%, cervix 40%, bladder 20% and endometrial cancer 20%. Radiation type containing brachytherapy in 40% of cases and external radiotherapy in other 60%. 40% of patients had history of hormone replacement therapy. This study showed that *PRFG injection* was effective and symptoms had disappeared in all of the patients with significant objective improvements in vaginal diameter (mean of tranvaginal length before and after *PRFG injection* was 6.5 cm and 7.1 cm respectively (p- value = 0.001). In addition vaginal flexibility improved and mean of flexibility before treatment was 0.72 cm and it increased to 1.85 cm after PRGF injection (P-value = 0.026). Characteristics of discharge before the injection in 60% of patients include dry vagina and 40% had mild discharge and after PRFG injection 40% of patients had moderate and 60% had mild (P-value = 0.190). after the injection of *PRFG* all of the patients had sexual satisfaction, that was related to TVL, vaginal flexibility and dyscharge.

Conclusions: Overally, our patients reported a better sexual and vaginal function indexes after APRFG injection.

PB1793 | Platelet Function in Primary Myelofibrosis

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Background: Among all chronic myeloproliferative neoplasms, platelet hemostatic abnormalities in primary myelofibrosis (PMF) have been the least studied. Despite strong evidence of in vivo platelet activation, patients with PMF may present an increased incidence of thrombosis as well as bleeding complications. Nevertheless, the treatment options include aspirin alone as primary prevention for the low-risk patients, and specific therapy combined with aspirin for the other patients.

Aims: The aim of our study was to evaluate the platelet function in patients with PMF.

Methods: Light transmission aggregometry was utilized to characterize the platelet function in 47 patients with PMF and 30 healthy volunteers. 12 patients were treated with ruxolitinib, 5 of them also got an antiplatelet therapy; 35 patients didn't have a specific therapy, 9 of them got an antiaggregants. Platelet aggregation was

TABLE 1 Aggregation tests in patients with PMF and control group (MA, %)

Agonist	Final concentration	Patients, n = 47 (Me, 95% CI)	Controls, n = 30 (Me, 95% CI)
ADP	1 μM/L	8,5 *(2,4 - 26,9)	19,5 (12,1 - 26,6)
ADP	5 μM/L	40,0 *(16,6 - 75,7)	54,0 (36,2 - 77,4)
Collagen	2 μg/mL	6,2 *(0,7 - 76,3)	53,3 (38,2 - 84,8)
Ristocetin	1,2 μg/mL	78,5 (22,1 - 99,2)	73,7 (55,9 - 91,8)

TABLE 2 Aggregation tests in patients subject to ruxolitinib taking (MA, %)

Agonist	Final concentration	Patients with ruxolitinib, n = 12 (Me, 95% CI)	Patients without ruxolitinib, n = 35 (Me, 95% CI)
ADP	1 μM/L	11,9 (2,3 - 20,3)	8,0 (2,4 - 26,9)
ADP	5 μM/L	35,7 (4,4 - 63,5)	42,1 (16,6 - 79,7)
Collagen	2 μg/mL	5,0 (0,7 - 54,1)	6,6 (1,5 - 88,4)
Ristocetin	1,2 μg/mL	68,5* (9,3 - 92,0)	83,2 (22,9 - 99,4)

induced by adenosine diphosphate (ADP) in two concentrations, collagen and ristocetin.

Results: The maximum aggregation rate (MA, %) of ADP and collagen was significally lower in patients than in controls, the MA of ristocetin was the same in both groups (Table 1, *$p < 0.05$ compared to controls). But when we compared platelet function in patients with and without specific therapy, we found that the MA of ristocetin was significally lower in patients treated by ruxolitinib (Table 2). Surprisingly, there was no correlation between aggregation patterns and antiplatelet therapy in both groups of patients.

Conclusions: The reduced response to ADP and collagen stimulation in patients with PMF can be a consequence of disease - associated intravascular platelet activation and the pre-term devastation of the platelet granules. The impaired platelet aggregation response to ristocetin may reflect the effect of the specific therapy due to abnormalities in von Willebrand factor - platelet interactions. It could be concluded that the platelet function and antiplatelet therapy regimen in patients with PMF remains unclear and needs further investigation.

PB1794 | ML355 Inhibits Platelets via GC Activation and Blocks Caspase-Dependent Apoptosis in Platelets

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Background: Overexpression of 12-lypoxygenase (12-LOX) is a common feature of tumor cells. Inhibition of 12-LOX induces apoptosis in several cancer cell lines and appears to be a promising strategy in anticancer therapy. However, 12-LOX inhibition can impair platelet activation during therapy and cause bleeding in patients.

Aims: The aim of our study was to investigate effects of a novel selective 12-LOX inhibitor ML355 on platelet functions and apoptosis.

Methods: Washed platelets from healthy donors were incubated with ML355 for indicated time and analyzed by flow cytometry or Western blotting. Phosphatidylserine (PS) surface expression (Annexin V-PE) and αIIbβ3 integrins activation (PAC-1-FITC) were assessed by flow cytometry. Vasodilator-stimulated phosphoprotein (VASP), PKB, p38 phosphorylation and caspase-3 activation were analyzed by Western blotting.

Results: We showed that ML355 (10 μM) impaired platelet αIIbβ3 integrins activation induced by thrombin, and inhibited PKB and p38 phosphorylation. The major inhibition mechanisms in platelets are mediated by adenylate (AC) and guanylate cyclases (GC) activation and consequent synthesis of cyclic AMP and GMP, which activate protein kinases A and G respectively. Activation of the inhibitory pathways can be monitored by the phosphorylation of the substrate protein VASP. ML355 caused VASP phosphorylation starting from 20 μM meaning that inhibition of platelet activation in lower doses was mediated by 12-LOX inhibition and consequent dose elevation lead to PKA or PKG activation. VASP phosphorylation induced by ML355 was prevented by GC but not AC inhibition. NO-synthase (NOS) inhibition by LNAME did not affect ML355-mediated VASP phosphorylation. These data imply NO-independent mechanism of GC activation induced by ML355. Additionally, ML355 dose-dependently blocked PS exposure and caspase-3 activation, induced by a high affinity BCL-X_L inhibitor ABT-737.

Conclusions: In summary, we showed that ML355 inhibits platelet activation via NO-independent GC activation and blocks platelet apoptosis, induced by ABT-737.

The reported study was funded by RFBR, project number 19-315-90102.

PB1795 | Modulation of ABT-737 Induced Platelet Apoptosis by Curcumin

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Background: Platelets play an important role in cancer development, metastasis formation, and thrombosis frequently observed in cancer patients. Curcumin has been shown to have antithrombotic and anticancer properties and in combination therapy can enhance effects of anticancer drugs. In cancer cells curcumin can induce caspase-dependent apoptosis, however, it is not known whether this compound can activate caspase pathways and induce or modulate apoptosis in platelets.

Aims: The aim was to analyze whether curcumin can induce platelet apoptosis or modulate apoptosis mediated by BCL-2 protein inhibitor ABT-737.

Methods: Washed platelets were prepared by centrifugation from the whole blood of healthy donors. Phosphatidylserine externalization (PS), αIIbβ3 integrin activation, mitochondrial membrane potential (ΔΨm), and microparticle formation (MP) were analyzed by flow cytometry. Labeled Annexin V, fibrinogen and TMRE were used respectively for PS, integrin activation and ΔΨm measurements. Caspase3 activity was analyzed by Western blot.

Results: Curcumin at 50 μM did not activate caspase 3, however, it inhibited platelet αIIbβ3 integrin activation and induced PS externalization, a fall of ΔΨm, MP formation. Curcumin at lower concentrations (0.5 and 5 μM) had no effects at all measured parameters. The combination of BCL-2 protein inhibitor ABT-737 with curcumin (0.5 and 5 μM) potentiated caspase 3 cleavage, and PS externalization induced by ABT-737. Whereas at 50 μM curcumin inhibited apoptosis induced by ABT-737 (Fig 1.)

Conclusions: Curcumin itself did not induce platelet apoptosis, however it modulated ABT-737-induced apoptosis concentration-dependently by potentiation at low (0.5, 5 μM) doses and inhibition at high (50 μM) doses.

The reported study was funded by RFBR, project number №17-00-00141 (17-00-00139).

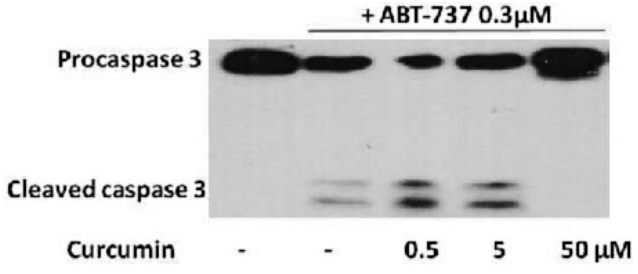

FIGURE 1 Curcumin at low concentraions potentiated, whereas at high concentration inhibited ABT-737 induced caspase 3 activation

PB1796 | Anti-Platelet Drug Ticagrelor Has a Direct Anticancer Activity

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Background: Ticagrelor is an inhibitor of P2Y12, a receptor primarily found on platelets. The drug is used to reduce the risk of thrombotic complications in patients with cardiovascular diseases. Recent clinical data suggest that ticagrelor is associated with a lower incidence of solid tumours.

Aims: To investigate direct anticancer activities of ticagrelor and the possible underlying mechanisms.

Methods: Anti-proliferative activity of ticagrelor *in vitro* was assessed in pancreatic cancer cells AsPC-1, BxPC-3, MiaPaCa-2, PANC1 and CFPAC-1 and the normal pancreatic duct cell line, hTERT-HPNE. The Caspase 3/7 activity, Annexin-V binding, and anti-apoptotic protein BCL-XL detection assays were used to investigate ticagrelor-induced apoptosis. P2Y12-mediated signal transduction was studied using siRNA knockdown strategies and pharmacological inhibitors. The benefits of ticagrelor as an additional treatment to chemotherapy was assessed *in vitro* and in a xenograft and syngeneic murine models of pancreatic cancer.

Results: Ticagrelor displayed anticancer activity *in vitro* with IC_{50s} less than 10 μM; the maximum tolerated plasma concentration in humans. Ticagrelor up to 20 μM did not affect the growth of non-malignant hTERT-HPNE. Ticagrelor at 2.5 and 5 μM significantly reduced Akt activation and activated apoptosis in pancreatic cancer cells, respectively ($N \geq 4$). Interestingly, P2Y12 expression was observed in pancreatic cancer cells and was important in mediating epidermal growth factor receptor-dependent and independent-Akt activation. Ticagrelor (2.5 μM) showed synergistic action with gemcitabine, paclitaxel, cisplatin, and erlotinib in pancreatic cancer cells *in vitro*. Combined with gemcitabine (25 mg/kg per week), ticagrelor (100 mg/kg per day) significantly reduced tumour growth *in vivo* ($N \geq 5$, $p < 0.033$), whereas gemcitabine alone had a non-statistically significant effect.

Conclusions: Our findings revealed a novel anticancer effect of ticagrelor and a critical role of P2Y12 signalling on cancer cells which may partly explain the reduced solid tumour incidence in patients receiving ticagrelor in clinical studies.

PB1797 | Platelet Function and Coagulation Abnormalities in Polycythemia Vera Patients: Effects of Low-Dose Aspirin

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Background: Polycyhemia vera (PV) patients have increased thromboxane (TX) and thrombotic risk. Low-dose aspirin (ASA) reduces the risk of major vascular events by 50 to 60%.

Aims: We evaluated platelets, hematocrit (HCT), β-thromboglobulin (β-TG) and platelet factor 4 (PF4), TXB2, the platelet function activity (PFA), the clotting time (CT), clot formation time (CFT).

Methods: We studied 60 patients (38 men, 22 women; mean age 51 years, range 32-70) with PV according to WHO. The mean duration of disease was 12 years. All patients were on ASA 100 mg once daily and on phlebotomy. None had inherited or acquired thrombotic risk. Of 60 patients, 30 had thrombosis (20 men, 10 women) and 30 had no thrombosis. Of 30 with thrombosis, 15 developed nonfatal myocardial infarction (10 men, 5 women) defined on electrocardiography, 10 had nonfatal stroke (8 men, 2 women) confirmed with the use of magnetic resonance imaging, and 5 (2 men, 3 women) had deep venous thrombosis confirmed by ultrasonography. Platelets and HCT were measured by automated analyzer, β-TG and PF4 by ELISA, TXB2 by radioimmunoassay, ASA platelet sensitivity by Platelet Function Analyzer (PFA-100) and CT and CFT by thomboelastometry.

Results: The platelets were 430 ± 170x109/L. The HCT was 42 ± 3%. The patients with thrombosis had high β-TG, PF4 and TXB2 (110 ± 45 IU/ml, 45 ± 21 IU/ml, and 1.700 ± 1.990 nmol/L, respectively), shortened C/EPI closure time (T, unit: s, n.v. 84-160 s) (55 ± 10 s), shortened CT (CT, unit: s. n.v. 100-240 s) (45 ± 20 s) and CFT (CFT, unit: s, n.v. 30-110 s) (15 ± 10 s)whereas the patients without thrombosis had normal β-TG, PF4 and TXB2 (20 ± 11 IU/ml, 6 ± 2 IU/ml, and 800 ± 280 nmpl/L, respectively), prolonged C/EPI closure time (249 ± 40 s) and normal CT (110 ± 20 s) and CFT (45 ± 5 s).

Conclusions: According to results, a PV patients subset may be ASA "low-responder".

PB1798 | Comparison of Platelet Parameters in the Patients with JAK2 V617F Mutated Essential Thrombocytosis and Reactive Thrombocytosis

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Background: Elevated platelet counts may be caused by two conditions. One is reactive thrombosis which is due to infection or inflammation. The other is caused by essential thrombocytosis which is one type of myeloproliferative neoplasm. Janus kinase 2 (JAK2) V617F mutation was found in around 50% of essential thrombosis patients. Several studies have showed that hemogram was changed in patients with JAK2 mutation. JAK2 V617F induced abnormal activation of thrombopoietin receptor (TPOR) and granulocyte colony-stimulating factor (G-CSFR) pathway, leading to hyperplasia of megakaryocytic progenitor cells.

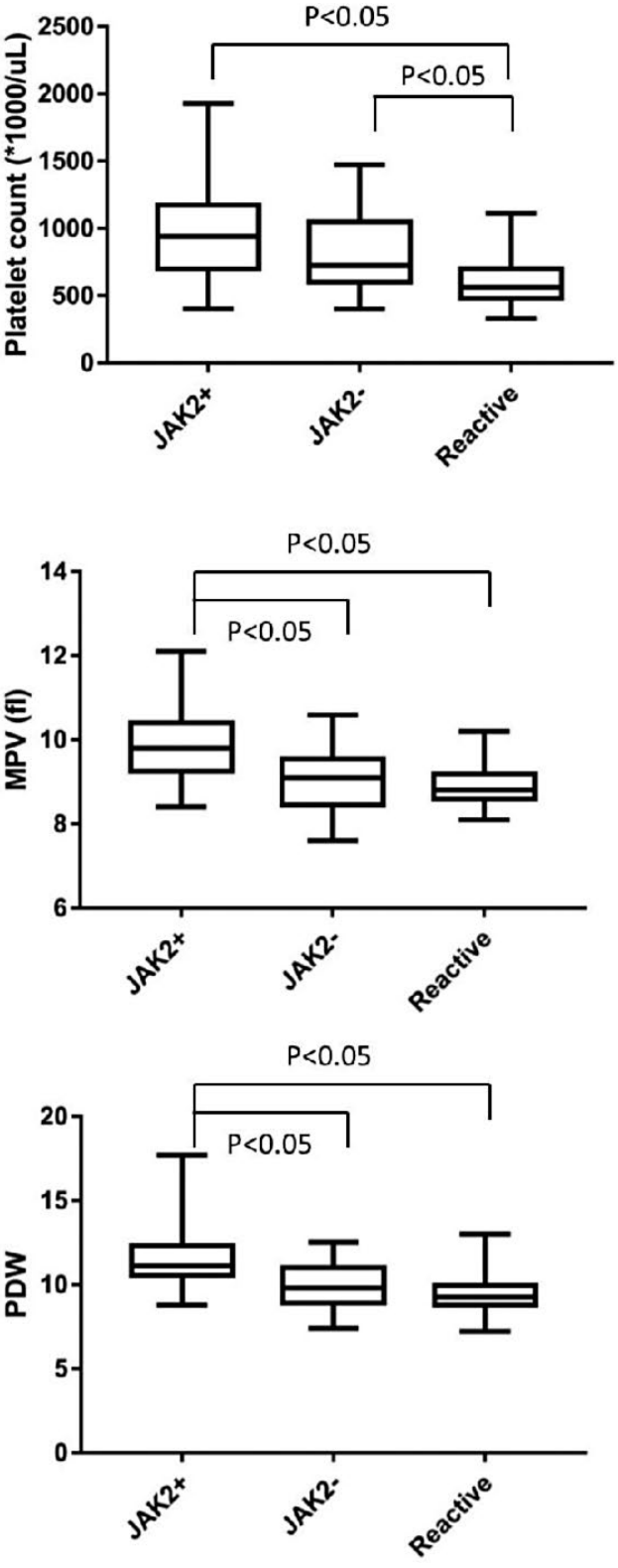

FIGURE 1 Platelet count, MPV and PDW in ET with JAK2 + , JAK2-, and reactive thrombocytosis

TABLE 1 Platelet counts, MPV, and PDW in three groups

	N	Age (years old)	PLT count (/uL)	MPV (fL)	PDW
Essential thrombocytosis					
_JAK2V617F(+)	37	67	968,000	9.8	11.5
_JAK2V617F(-)	21	55	829,000	9.1	9.8
Reactive thrombocytosis	58	55	614,000	8.8	9.3

Aims: We hypothesis that JAK2 V617F mutation may influence platelet proliferative and showed differences in platelet parameters, including platelet count, mean platelet volume (MPV) and platelet distribution width (PDW).

Methods: This retrospective study was approved by IRB, and was conducted for patients visiting our hospital (Hualien Tzu Chi Hospital, Taiwan) from 2010 to 2019. All patients had thrombocytosis (more than 400,000/uL) and JAK2 V617F mutation was detected by allele specific real-time quantitative fluorescence PCR. The platelet parameter, including platelet count, MPV and PDW were record. Data analysis with Mann-Whitney test was performed using GraphPad Prism (Version 7). A p-value of < 0.05 was considered significant.

Results: 58 patients with ET, including 37 with JAK2 positive and 21 with JAK2 negative and 58 patient with reactive thrombocytosis (all with JAK2 negative) were enrolled in this study. JAK2 V617F mutated ET patients are older (67, 55, vs 55 years old), and had higher platelet count (968,000, 829,000, vs 614,000/uL), larger size (high MPV, 9.8, 9.1, vs 8.8 fL), and higher PDW (11.5, 9.8, vs 9.3). All platelet parameters had significant difference. The data showed as table and figure.

Conclusions: The higher platelet count, MPV and PDW were found in essential thrombocytosis patients with JAK2 V617F mutation than JAK2-negative and reactive thrombocytosis.

PB1799 | Tumor Platelet Transcriptome Changes in Hyperthrombotic Mice

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Background: Activation of the coagulation cascade and platelets are hallmarks of cancer and recent evidence indicates that tumors induce profound alterations in platelet transcriptomes. However, underlying mechanisms that contribute to the transfer of tumor cell RNA to tumor educated platelets are incompletely understood. We hypothesized that hypercoagulability contributes to platelet transcriptome changes in tumor bearing mice.

Aims: We determined tumor-induced platelet transcriptome alterations in WT and hyperthrombotic, thrombomodulin mutated TM^{Pro} mice. We also deleted PAR4 to assess the role of thrombin signaling in tumor education of platelets.

Methods: We characterized in transplanted tumor models tumor cell expressed genes, composition of the tumor microenvironment, and platelet transcriptomes by next generation sequencing and quantitative real time PCR.

Results: Tumor growth rates were similar in all mouse strains with no apparent differences in immune cell infiltration of the tumor microenvironment. Spontaneous lung metastasis was increased in hyperthrombotic TM^{Pro} mice, but metastasis was low and similar to wild-type mice in $TM^{Pro}/PAR4^{-/-}$ with abolished platelet thrombin signaling. Platelet counts were lower in tumor bearing compared to tumor free animals. In contrast to markedly increased platelet consumption in hyperthrombotic TM^{Pro} mice, platelet counts of $TM^{Pro}/PAR4^{-/-}$ did not change upon tumor implantation. We detected 200-300 transcript changes in platelets from tumor-bearing TM^{Pro} mice relative to tumor free animals as well as in comparisons of tumor bearing TM^{Pro} or $TM^{Pro}/PAR4^{-/-}$ mice with wild-type. Approximately 70% of these transcripts were also found in isolated tumor cells or primary tumors, indicating that the RNA species are tumor derived.

Conclusions: Hyperthrombotic mice have alterations in platelet transcriptomes in comparison to tumor bearing wild-type mice. The majority of these platelet transcriptome changes can be traced to tumor cell transcripts and appear to occur independent of platelet hyperreactivity in tumor bearing hosts.

PB1801 | Generation of 12-HETE in Platelet-Cancer Cell Crosstalk

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Background: Platelets can influence the phenotype of cancer cells through the release of eicosanoids. Among them, the 12-hydroxyeicosatetraenoic acid (12-HETE), a product of platelet-type 12-lipoxygenase (p12-LOX), is considered a key modulator in cancer metastasis.

Aims: We aimed to investigate the production of 12-HETE (both released and intracellular) in human platelets and colon cancer cells (HT-29) cultured alone, and whether their exposure to platelets

influences 12-HETE generation and cancer cell phenotype via the p12-LOX pathway.

Methods: Human platelets ($2x10^8$) or HT-29 ($2x10^6$) cells were cultured alone or cocultured, and the levels of 12-HETE evaluated by liquid chromatography-mass spectrometry (LC-MS/MS), gene expression by qPCR and p12-LOX protein by Western blot.

Results: In HT-29 cells, 12-HETE and p12-LOX were undetectable. In platelets, expressing the enzyme, 12-HETE was detected mainly intracellularly (0.418 ± 0.138 nmol, mean±SD) versus the conditioned medium (0.018 ± 0.004 nmol). Cancer cells exposed to platelets acquired the capacity to generate 12-HETE (extracellular levels: 0.013 ± 0.004 nmol; intracellular levels: 0.024 ± 0.007 nmol). In HT29 cells cultured with platelets, p12-LOX mRNA was undetectable while the protein was found. CDC (30 μM), a selective inhibitor of p12-LOX, significantly reduced the levels of 12-HETE by 64%. In the coculture, platelet-derived microparticles (PMPs) were generated and expressed p12-LOX that was catalytically active. PMPs were internalized by HT29 cells, and this phenomenon was associated with the detection of p12-LOX in cancer cells and the capacity to synthesize 12-HETE. The crosstalk of platelets and cancer cells induced the expression of genes, such as ZEB1, vimentin, and fibronectin (involved in epithelial-mesenchymal transition), and RhoA (involved in cell migration). These effects were inhibited by the p12-LOX inhibitor CDC.

Conclusions: The interaction between platelets and HT29 cells caused the activation of platelets and the release of PMPs, which promoted the synthesis of 12-HETE via the transfer of p12-LOX to cancer cells. This phenomenon led to the induction of a more malignant phenotype of cancer cells, which was dampened by a p12-LOX inhibitor.

PB1802 | Platelet-derived Microparticles Regulate Migration, But Do Not Influence Proliferation of Breast Cancer Cells

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Background: Cancer cell-induced platelet activation was shown to favour metastasis through various mechanisms. Platelet-derived microparticles (PMPs), released upon platelet activation, are gaining growing attention as potential players in cancer-host communication. Cancer cells induce the release of PMPs *in vitro* and, accordingly, cancer patients display increased levels of circulating PMPs. However, the functional interaction between PMPs and cancer cell is largely unexplored.

Aims: This study aims to investigate the impact of PMPs on the biology of breast cancer cells (BCCs).

Methods: Three metastatic (MDA-MB-231, SKBR3, MCF7) and one invasive ductal (BT474) lines of BCCs were co-cultured with PMPs released from thrombin-stimulated platelets. The interaction between PMPs and BCCs was investigated by confocal microscopy, flow cytometry and immunoblotting analysis. Ca^{2+} movements were measured on fura-2-loaded BCCs. Cell migration was evaluated by transwell assays. Cell proliferation and cell cycle progression were analysed by flow cytometry.

Results: MCF7 cells were unable to bind PMPs, that by contrast were efficiently bound and internalized by MDA-MB-231, SKBR3 and BT474. PMPs evoked rapid intracellular Ca^{2+} mobilization in MDA-MB-231, SKBR3 and BT474 cells, suggesting the activation of specific signalling responses. No relevant effects on important Ca^{2+}-dependent processes, such as proliferation and cell cycle progression, were detected in BCCs within 48 hours of co-culture. However, PMPs strongly potentiated migration of MDA-MB-231 cells, in association with the phosphorylation of key signalling proteins. No effects on migration or protein phosphorylation were detected in SKBR3 and BT474 cells.

Conclusions: PMPs can be internalized by BCCs and elicit functional responses, including intracellular Ca^{2+} movements, protein phosphorylation and increased migration. The functional effects of PMPs, however, strongly depend on the nature of recipient cells.

PB1806 | A Potential Role for the FcγRIIa in Vinorelbine Induced Thrombocytopenia and Neutropenia

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Background: Vinorelbine is a vinca alkaloid used to treat non-small-cell-lung carcinoma and metastatic breast cancer. Side effects include neutropenia and thrombocytopenia causing immunosuppression and a risk of bleeding events. A virtual-high throughput screen (vHTS) identified vinorelbine as a ligand for FcγRIIa.

Aims: As FcγRIIa is expressed on both platelets and neutrophils we investigated the possibility that vinorelbine-mediated thrombocytopenia and neutropenia may be FcγRIIa-mediated.

Methods: vHTS was performed using 3D pharmacophore matching *in silico* using Molecular Operating Environment (CCG, Montreal). Platelet adhesion assays, aggregometry, loss of single platelet, as well as ATP secretion assays were performed to characterise its effects on platelets. We further tested the effect on TNFα secretion in differentiated U937 cells stimulated with IgG.

Results: Vinorelbine inhibited platelet adhesion to IgG (IC_{50} of 11 μM), as well as heat-agglutinated IgG (HAIg)-induced platelet aggregation

(vinorelbine: 7% ± 6%; vehicle control 65% ±10%; $P < 0.05$). This inhibition was selective as no inhibition was seen for ADP-induced aggregation (ADP, 68% ± 9%; ADP + vinorelbine 71% ± 11%, NS). When added to PRP, vinorelbine induced platelet aggregation (29 ± 3.8%) associated with a 24% reduction in single platelets and an increase in ATP release (34% ± 7.8%; $P < 0.01$). Vinorelbine also inhibited IgG-induced TNFα secretion in U937 cells (90% ± 6% inhibition).

Conclusions: The ability of vinorelbine to inhibit both platelet adhesion to IgG and HAIgG-induced platelet aggregation confirms the vHTS result that vinorelbine is a ligand for FcγRIIa. Its ability to induce platelet activation suggests that it has agonist activity on FcγRIIa. This FcγRIIa agonist activity may explain the loss of both platelets and neutrophils in patients and suggests that the use of an FcγRIIa antagonist such as intravenous IgG may ameliorate this adverse effect.

PB1807 | Chemotherapy - Induced Thrombocytopenia in Pediatric Acute Lymphoblastic Leukemia: A Single-institution Report

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Background: Thrombocytopenia is one of the most common toxicities of chemotherapy. It can cause from mild petechiae up to debilitating bleeding, as well as lead to treatment delays and discontinuation, which subsequently affects the outcomes. The data on chemotherapy induced thrombocytopenia (CIT) is scarce, especially from the developing world and until now there is no consensus on the appropriate management of this complication.

Aims: The aim of this study was to evaluate CIT in pediatric patients with acute lymphoblastic leukemia (ALL).

Methods: Medical records of ALL patients, who were diagnosed and treated in 2019 at the Pediatric Cancer and Blood Disorders Center of Armenia, the only center treating pediatric hematologic and oncologic disorders in Armenia, were retrospectively reviewed.

Results: A total of 29 patients with ALL treated with ALL BFM 2009 protocol, who achieved remission, were included in the current study. 17 (58%) patients were males. Median age was 4.5y, range - [18 months - 17 years]. 5 patients had T-ALL and 24 patients B-ALL. During induction therapy 11 patients (38%) developed critical thrombocytopenia, platelet (PLT) counts ranging from 21 × 10^9/L to 46 × 10^9/L. During consolidation therapy 16 patients (55%) developed moderate thrombocytopenia, PLT counts ranged from 70 × 10^9/L to 86 × 10^9/L and 6 (21%) patients had critical thrombocytopenia, PLT counts ranged from 27 × 10^9/L to 36 × 10^9/L. During re-induction phase of therapy 21 (72%) patients developed critical CIT with platelet counts ranging from 13 × 10^9/L to 45 × 10^9/L.

Conclusions: During the course of therapy the vast majority of patients with ALL developed clinically significant thrombocytopenia. Although our study is small and involve the cases from one year, it includes all the ALL patients treated in Armenia. The study clearly shows that chemotherapy induced thrombocytopenia is a very common complication of ALL therapy and larger studies need to explore these findings.

PB1808 | The Assessment of Platelet Membrane Alteration in Patients with Chronic Myeloproliferative Neoplasms

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Background: Patients with *Chronic myeloproliferative neoplasms* (MPN) present at the onset or during evolution thrombotic complications.

Aims: We retrospectively analysed the platelet membrane parameter and correlation with treatment outcome, thrombosis events in order to identify characteristics of platelet function in patients with MPN especially in those with high thrombotic risk.

Methods: This retrospective study included 150 patients with MPN as well as 15 healthy volunteers. The diagnosis of MPN was made according to World Health Organization (WHO) classification of MPNs. We evaluated the flow cytometry markers of platelet adhesion (CD42a, CD42b), aggregation (CD41, CD61) and CD36. Production of reactive species (ROS) was examined using fluorescence method with DCFDA and was assessed by aria under curve (AUC) in serial measurements during 900 sec. The determination of platelet membrane potential was done by fluorescence method using 3,3′-diprop yl-2,2′-thiadicarbocyanine iodide (DiSC3-5)

Results: The ROS production is significantly higher in MPN group, p = 0.04. Patients in advanced phase of myeloid metaplasia (grade 5 Hackett of splenomegaly) has high level of ROS production compared with patients diagnosed in early stage (grade 1 or 2 Hackett of splenomegaly) median value: 13178014970 vs 12303298585, p = 0.001. There are no differences between groups that was splitted by type of treatment Ruxolitinib/Hydroxiureea or type of mutation diagnosed JAK2V617F/CALR/no mutation. Patients with MPN present higher resting membrane potential (RMP) compared with controls (median value - 63 mV vs - 57.5 mV, p = 0.05). The expression of CD36 receptor was higher in MPN group vs controlsp = 0.003. here are significant correlation between resting membrane potential and expression of CD42b (r -0.45, p = 0.04) and GlyA receptors (r -0.45, p = 0.04).

Conclusions: Patients with MPN especially those with have a higher level ono reports are regarding platelet membrane. It was proved that generation of intracellular ROS is associated with CD36 signaling and represent one start point for thrombosis.

PLATELETS AND INFECTION

PB1809 | High-throughput Mechano-Phenotyping of Single Platelet Activation Induced by Bacterial Proteins

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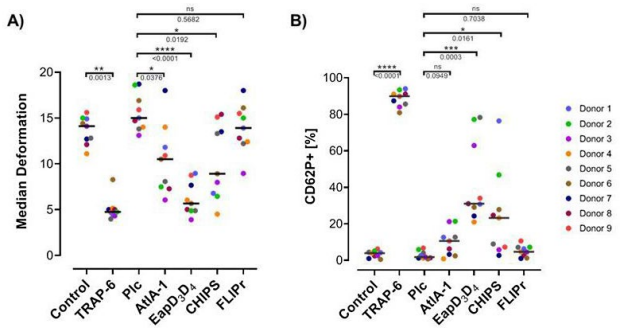
Background: Gram-positive *Staphylococcus aureus* is a leading cause of blood stream infection. Platelet activation and aggregation by *S. aureus* proteins plays an important role in the pathogenesis of infective endocarditis.

Aims: To quantify *S. aureus* protein induced changes in cytoskeleton dependent biomechanical properties and activation status of platelets.

Methods: High-throughput (>1000 single platelets/second) mechano-phenotyping of single platelets was performed in microfluidic real-time 1D-imaging fluorescence and deformability cytometry (RT-FDC). 50 µL of washed human platelets (300,000/µL) were incubated for 10 minutes with *S. aureus* proteins: phospholipase C (Plc, 4 µM), major autolysin (AtlA-1, 2 µM), extracellular adherence protein (EapD3D4 domain, 4 µM), chemotaxis inhibitory protein of *S. aureus* (CHIPS, 4 µM), and formyl peptide receptor-like 1 inhibitory protein (FLIPr, 4 µM). Thrombin receptor activator peptide 6 (TRAP6) was used as PAR1 agonist. Platelet deformation and activation (CD62P expression) were detected simultaneously in live-platelets by RT-FDC and F-actin by flow cytometry and confocal fluorescence microscopy.

Results: Mechano-phenotyping by RT-FDC revealed, except for FLIPr (median deformation = 0.145), that all tested *S.aureus* proteins lowered platelet deformability (i.e. increased stiffness) compared to the internal negative control Plc (median deformation 0.154) (Figure 1, A). While, $EapD_3D_4$ and CHIPS decreased deformation (median deformation 0.058 and 0.101) and increased CD62P expression (median fold increase 17.7 and 13.25) and F-actin content (median fold increase 1.43 and 2.54), intriguingly, AtlA-1 induced lower deformation (median fold increase 0.107) albeit without significant increase in CD62P expression but with increased F-actin content (median fold increase 1.65)
(Figure 1, A & B).

Conclusions: Changes in cytoskeleton dependent viscoelastic properties of platelets in the presence of *S. aureus* proteins reveal heterogeneity in platelet activation response in individual donors. Platelet deformation can be used as sensitive label-free activation marker for platelet activation even in the absence of CD62P expression.



Platelet deformation and activation status. A) Platelet deformation induced by *S. aureus* proteins in human platelets. Data shows median deformation of n=9 donors based on CD41⁺ gated events from RT-FDC. B) Percentage of CD62P positive events from RT-FDC showing activation in platelets gated for CD41⁺ events in the presence of *S. aureus* proteins. Data representative of n=9 donors. Statistical analysis: Friedman test followed by Dunn's multiple comparison test.

FIGURE 1 S. aureus proteins induce platelet deformation and activation

PB1810 | Risk Factors, Outcomes, and Score Models of Serious Pulmonary Infection in Non-Splenectomized Immune Thrombocytopenia Adults

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Background: Infection is one of the primary causes of death from immune thrombocytopenia (ITP). The lung is the most common location. However, little is known about the factors associated with serious pulmonary infection and prognosis.

Aims: To identify the factors associated with serious pulmonary infection in non-splenectomized ITP adults and prognosis, and establish two score models to predict the incidence of serious pulmonary infection and prognosis, respectively.

Methods: In all, 73 non-splenectomized ITP adults with serious pulmonary infection were included. Each patient was randomly matched with 3 controls based on the diagnosis time of ITP (±6 months) and

TABLE 1 Multivariate cox regression model for all-cause death in ITP patients with serious pulmonary infection

	Univariate analysis	Multivariate analysis		
Risk factor	**P Value**	**HR**	**95% CI**	**P Value**
Microscopic urinary bleeding	0.001	3.005	(1.266, 7.134)	0.013
ALC at admission	< 0.001	0.222	(0.057, 0.858)	0.029

TABLE 2 Risk factors of Pulmonary infections in ITP patients by univariate analysis and multivariate analysis

	Univariate analysis	Multivariate analysis		
Risk factor	**P Value**	**HR**	**95%CI**	**P Value**
Age at diagnosis of ITP	< 0.001	5.759	(2.984,11.116)	< 0.001
Smoke ever	0.050	1.907	(1.084, 3.354)	0.025
Charlson score	< 0.001	1.421	(1.208, 1.672)	< 0.001
Respiratory tract bleeding	< 0.001	2.925	(1.485, 5.761)	0.002
Glucocorticoids before (within 1 month)	< 0.001	3.017	(1.734, 5.248)	< 0.001
Danazol before (within 1 month)	0.005	3.004	(1.036, 5.581)	0.041
IVIg before (within 1 month)	0.049	3.206	(1.421, 7.235)	0.005

duration of ITP. Point values were assigned to the risk factors on the basis of regression coefficients in Cox regression analysis to establish a risk score model. Additionally, a simple scoring system was developed to predict the mortality of patients with serious pulmonary infections.

Results: Risk factors of serious pulmonary infections were shown in Table 1. Point values ranging from 0 to 6 were assigned to these factors. Receiver operating characteristic (ROC) analysis indicated that the risk score model performed well in discrimination of different groups (AuROC = 0.775; 95% CI, 0.709-0.840). Factors associated with the prognosis were listed in Table 2. We found the cut off value of $0.67*10^9$/L of ALC at admission through ROC curve. A simple scoring system was established that gave a score of 1 to patients who had an absolute lymphocyte count (ALC) at admission of < $0.67*10^9$/L or history of microscopic urinary bleeding. ROC analysis indicated that the discriminatory performance in the prognosis of the scoring system was great, with an AuROC of 0.819.

Conclusions: Risk factors for serious pulmonary infections and death were identified, and then two models were established to predict the occurrence of serious pulmonary infection and prognosis. They might help recognize high-risk patients and provide timely treatment.

PB1813 | Platelet Activation in Community-acquired Pneumonia Remains Increased Until 30 Days Later

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Background: Platelets have been shown to have a thrombotic and immune role in infections. However, its potential activation in community-acquired pneumonia (CAP) remains poorly studied.

Aims: We aimed to evaluate platelet activation by flow cytometry in hospitalized CAP patients at day 1, 4/5 and at 30 days.

Methods: We conducted a prospective study in hospitalized CAP. Peripheral blood samples were obtained from CAP patients (day 1, 4/5 and 30) and in healthy controls (day 1). Activation was evaluated through flow cytometry measuring heterotypic aggregates (leukocyte-platelet [CD45/CD61$^+$], and monocyte-platelet aggregates [CD14/CD61$^+$]) and the expression of P-selectin (CD61/CD62$^+$) on the surface of platelets. Patients were followed-up for 30 days.

Results: We included 126 patients and 20 controls. CAP patients showed a significant higher percentage of leukocytes (Figure 1A) and monocytes (B) with platelets aggregated and a significant higher percentage of platelets expressing P-selectin (C) at day 1, day 4/5 and day 30 compared to healthy controls. A direct correlation was found between P-selectin, leukocyte-platelet, and monocyte-platelet day 1 (rho: 0.489, $p < 0.01$; rho: 0.289, $p < 0.01$) in patients.

Conclusions: Greater platelet activation is established in hospitalized CAP patients during hospitalization and it remains until 30 days later. Platelet activation and its association with early and long-term clinical outcomes should be considered.

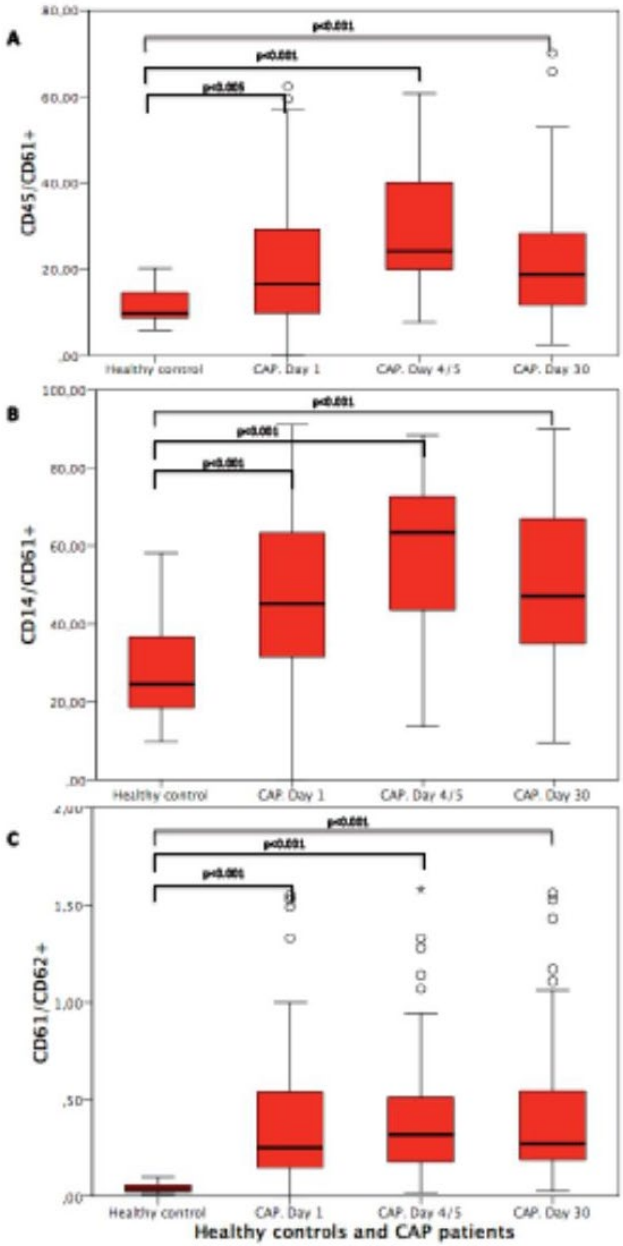

FIGURE 1

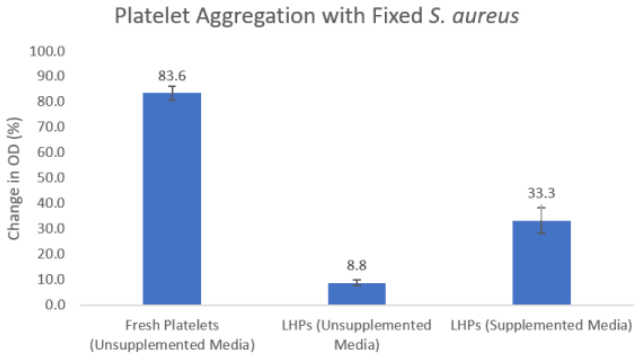

FIGURE 1 Platelet aggregation with fixed S. aureus

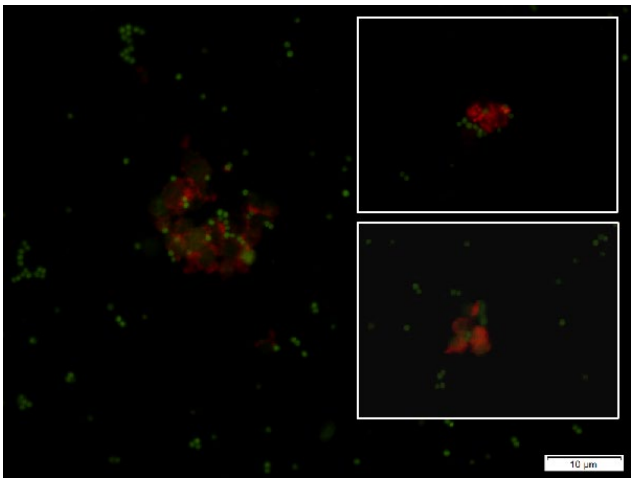

FIGURE 2 Fluorescence microscopy of LHP (red) association with fixed S. aureus (green)

PB1816 | **Lyophilized Human Platelets Exhibit Adhesive Interactions with** *Staphylococcus aureus*

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Background: Physical interactions between platelets and many bacteria is a key feature of innate immunity. Platelets are known to bind strains of *Staphylococcus aureus* via fibrinogen bridging with bacterial clumping factors. Platelet binding to bacteria assists in recruitment of additional immune factors via physical immobilization, chemokine signaling, and leukocyte recruitment. Such binding frequently results in platelet activation and aggregation, and thrombocytopenia is common with sepsis.

Aims: To determine whether lyophilized human platelets (LHPs), a platelet derived hemostatic agent currently under clinical development (Thrombosomes®), are capable of physical interactions with bacteria in a manner like fresh platelets.

Methods: LHPs or fresh drawn platelets were incubated with chemically fixed *S. aureus* (Cowan I strain) with or without supplementation of 1 mg/mL fibrinogen, 1 mM Ca^{2+} and Mg^{2+} and assessed by light transmission aggregometry. Platelets were biotinylated and streptavidin stained and *S. aureus*was labeled with BODIPY-FL to facilitate detection by flow cytometry and fluorescence microscopy.

Results: Fresh platelets activated and aggregated with chemically fixed *S. aureus* with a 5-minute lag time. LHPs agglutinated with *S. aureus* to a lesser magnitude than did fresh platelets (Figure 1). The interaction of *S. aureus* with LHPs was greater in the presence of fibrinogen and cations. Physical association of LHPs with *S. aureus* was confirmed by flow cytometry and fluorescence microscopy using orthogonal fluorescence staining methods (Figure 2).

Conclusions: Lyophilized human platelets participate in physical interactions with the Cowan I strain of *S. aureus in vitro* in a fibrinogen-dependent manner, though to a lesser extent than fresh platelets. Additional investigation is necessary to determine implications for *in*

vivo immune responses and interactions with other pathogenic species upon LHP transfusion, and to determine whether LHP transfusion is suitable in sepsis treatments.

PB1817 | Effects of the Bacterial Toxin Cytotoxic Necrotising Factor 1 on Platelet Function

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Background: Besides their roles in hemostasis and as components of the circulating immune system, platelets also constitute a potential target for bacteria that enter the bloodstream. Bacteria produce diverse toxins capable of modulating platelet function. The cytotoxic necrotizing factor 1 (CNF1) is the major virulence factor of extraintestinal pathogenic *E. coli* (ExPEC), which is the leading cause of bacteremia world-wide and a frequent cause of sepsis. CNF1 is internalized upon binding to specific plasma membrane receptors and processed to release the enzymatic domain into the cytoplasm. This domain catalyzes deamidation of a specific glutamine residue of Rho GTPases rendering them constitutively active and causing profound morpho-functional alterations in the target cell.
Aims: While the molecular and functional effects of CNF1 have been studied to some extent in epithelial cells, fibroblasts and leukocytes, no studies have addressed the impact of this toxin on platelet function.
Methods: We used platelets isolated from volunteer donors. Platelets were incubated with CNF1 at 37°C for up to 4 hours. Secretion and integrin activation were assayed by flow cytometry. Spreading was assayed by immunocytochemistry.
Results: Platelets express both laminin receptors, p37/67 and Lu/BCAM, involved in binding of CNF1. CNF1 becomes internalized in platelets and causes a shift in the electrophoretic mobility of RhoA, indicating that it becomes deamidated. CNF1 causes an increase in the levels of basal F-actin and impairs secretion of alpha and dense granules as well as integrin αIIbβ3 activation. The toxin does not impair adhesion to collagen or fibrinogen, however one third of the platelets adopt a strikingly spiky morphology. We are investigating the molecular mechanisms underlying these alterations and how they relate to Rho GTPase function.
Conclusions: By affecting platelet function CNF1 may contribute to the pathogenesis of sepsis by *E. coli*.

PB1818 | From the Antiplatelet Drug Ticagrelor to New Antibiotics

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Background: Ticagrelor is a widely administered orally active antiplatelet drug belonging to 1,2,3-triazolo[4,5-*d*]pyrimidines, which acts by reversibly inhibiting the platelet P2Y12 receptor. Importantly, we recently discovered that ticagrelor and AR-C124910, its main metabolite, exert bactericidal and anti-biofilm activity against gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA).
Aims: The present work aimed at establishing structure-activity relationships of ticagrelor molecule derivatives represented by the general formula (I) in order to identify structural determinants of antiplatelet and antibacterial activity and to assess the possibility of dissociating the two properties.

Methods: A series of pyrimidine derivatives, comprising 22 triazolopyrimidines (X_1 and X_2=N), including five ticagrelor metabolites, 23 purines (X_1=C and X_2=N) and 6 pyrazolopyrimidines (X_1=N and X_2=C) were synthetized. Molecule purity was assessed by NMR spectroscopy and elemental analysis. The antiplatelet activity of the test molecules was analysed by light transmission aggregometry upon platelet stimulation with ADP in citrated human platelet-rich-plasma using doses equal to ticagrelor IC_{50}. The antibacterial activity

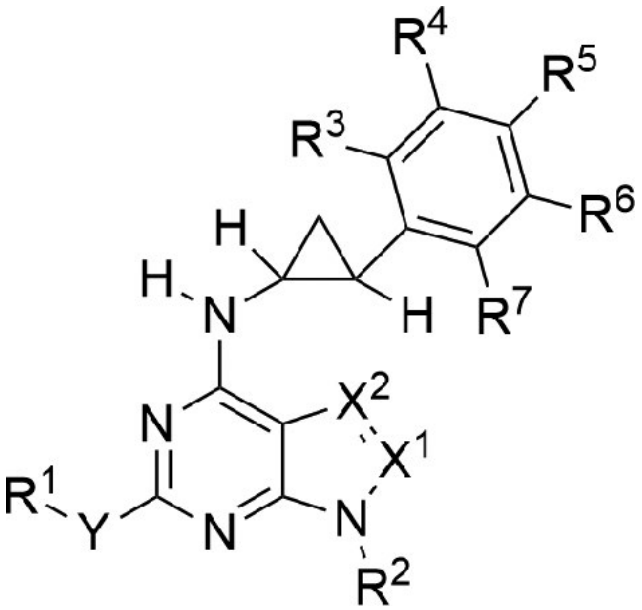

FIGURE 1 General formula for pyrimidine derivatives

against MRSA (ATCC BAA-1556) was determined by the broth microdilution method as recommended by EUCAST guidelines, as well as in citrated whole blood.

Results: Among triazolopyrimidines, several molecules showed antiplatelet activity, but only ticagrelor and its metabolite AR-C124910 had both antiplatelet and antibacterial properties. In whole blood, antibacterial dosage of ticagrelor (20 µg/mL) significantly reduced bacteria survival by about 35% while antiplatelet-only molecules were inactive against bacteria. Importantly, we discovered new pyrazolopyrimidine and purine molecules that were able to kill bacteria as potently as ticagrelor, while they lacked antiplatelet effect, e.g., N-((1R,2S)-2-(3,4-difluorophenyl) cyclopropyl)-6-(ethylthio)-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine hydrochloride and N-((1R,2S)-2-(3,4-difluorophenyl) cyclopropyl)-9-methyl-2-(propylthio)-9H-purin-6-amine.

Conclusions: Ticagrelor exerts direct *in vitro* antibacterial activity in whole blood. Antibacterial and antiplatelet activities could be dissociated by chemical structure modifications, which may lead to the discovery of a new class of antibiotics.

PLATELETS AND INFLAMMATION

PB1819 | Effect of cART on Platelet:Lymphocyte Ratio in HIV Patients Initiating Treatment

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Background: HIV infection is associated with varying degrees of inflammation and as the disease progresses, inflammation becomes severe and is associated with many of the complications seen in HIV infection. A novel marker for inflammation - Platelet-Lymphocyte ratio (PLR) is known to correlate with inflammation, atherosclerosis and platelet activation. We prospectively assessed the effect of cART on the PLR in HIV+ patients presenting to commence cART in Rivers State University Teaching Hospital.

Aims: This study aimed to evaluate the effect of Anti-retroviral Therapy on PLR, determine any significant relationship between the HIV Viral Load and PLR and acertain if the effect is sustained for up to 6 months into cART.

Methods: Consenting adults were recruited, 10mls of EDTA samples were collected at entry into the study, after 3 months and 6 months on ART respectively for Full Blood Count using a 3-part Sysmex XP300 and HIV Viral Load with RT-PCR using Cobas TaqMan version 1.5 (Roche Molecular Systems).

TABLE 1 Effect of cART ON Haematocrit, HIV Viral load, Platelet:Lymphocyte ratio

VARIABLE	MEAN+SD	F- VALUE	P-VALUE
HCT Month 0	31.650 ± 7.303	20.920	<0.001
HCT Month 3	36.435 ± 4.291		
HCT Month 6	38.960 ± 2.707		
VL Month 0	215767.850 ± 360338.04	14..293	<0.001
VL Month 3	705.650 ± 684.220		
VL Month 6	29.330 ± 17.869		
PLR Month 0	7.823 ± 2.90	26.50	<0.001
PLR Month 3	5.648 ± 1.439		
PLR Month 6	4.721 ± 1.053		

Results: A total of 40 clients were recruited, with a mean age of 36.20 years, 14 (35%) of them were males. Mean PCV, Platelet: Lymphocyte ratio and HIV VL at Month 0 were 31.65 ± 7.30%, 7.82 ± 2.90 and 215767.85 ± 360338.04cp/ml respectively. There was a statistically significant increase ($p < 0.001$) in the haematocrit by the 6th month on cART, the reduction in Platelet: Lymphocyte count and of HIV VL by the 6th month was also significant ($P < 0.001$). Interestingly, PLR positively correlated with the VL at baseline (0.3676), however, there was a negative correlation at 3 months (-0125) and 6 months (-0.028).

Conclusions: The findings from this study support a growing body of evidence that cART significantly reduces the viral load and inflammation in HIV+ patients, however, it also shows that a low-level inflammation persists which may lead to a chronic inflammatory state, morbidity and mortality in this group of patients.

PB1820 | Acute Exacerbations of COPD Induce a Prothrombotic State through Platelet-Monocyte Complexes, Endothelial Activation and Increased Thrombin Generation

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Background: Patients with chronic obstructive pulmonary disease (COPD) are at increased risk for cardiovascular events, particularly following an acute exacerbation (AE-COPD). Exacerbations are

associated with increased systemic inflammation, which may drive coagulation.

Aims: This prospective cohort study aimed to determine the effects of an AE-COPD on platelet activation, the endothelium and plasmatic coagulation, and its association with systemic inflammation.

Methods: This study was approved by the Maastricht University Medical Centre ethics committee. Fifty-two patients with an AE-COPD were included. After informed consent, blood samples were taken during AE-COPD, at day 3 of treatment and at convalescence (for 32 patients). Platelet-monocyte complex (PMC) formation, monocyte activation (Mac-1 expression) and platelet (re)activity (P-selectin expression, GPIIbIIIa activation) were measured by flow cytometry. Von Willebrand Factor (VWF) and thrombin generation (TG) were determined as measures of endothelial activation and plasmatic coagulation, respectively.

Results: Acute exacerbations of COPD were associated with increased PMCs compared to convalescence (MFI 31.3 vs 23.8, p = 0.004). Increased PMC formation was not accompanied by changes in platelet function. Secondly, VWF antigen, activity and the active VWF fraction were significantly higher during AE-COPD (medians 218%, 157%, 151%, respectively) compared to convalescence (182%, 135%, 125%, respectively). Thirdly, thrombin generation peak, ETP and velocity index were significantly elevated during AE-COPD compared to convalescence. PMC formation, VWF and thrombin generation were positively associated with the inflammatory marker CRP. Moreover, platelet hyperreactivity on admission was associated with an increased risk for exacerbation relapse.

Conclusions: In conclusion, acute exacerbations induce an inflammation-associated prothrombotic state, characterized by increased PMCs, endothelial activation and plasmatic coagulation. These findings provide direction for future studies on markers that predict the risk of cardiovascular events and guide thromboprophylaxis.

PB1821 | Role of Formyl Peptide Receptor 2 (FPR2/ALX) and its Anti-Inflammatory Ligand, Lipoxin A4 Analogue (BML-111) in the Modulation of Platelet Function

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Background: Formyl peptide receptor 2/ALX (FPR2/ALX), a member of seven transmembrane G protein-coupled receptors superfamily, exhibits dual effects of pro- and anti-inflammatory responses based on the nature of stimulating ligand type. Recently, FPRs have been shown to be present in platelets. However, FPR2/ALX and the effect of its anti-inflammatory ligands on the regulation of platelet function has not been addressed yet. Platelets play significant roles in the regulation of inflammation and host defence in addition to haemostasis and thrombosis. They act as sentinels through regulating inflammatory responses due to their large number in circulation and their ability to rapidly release different kinds of immunomodulatory cytokines, and other inflammatory mediators.

Aims: Given the anti-inflammatory effects of FPR2/ALX ligands, we sought to study the role of its anti-inflammatory ligand, Lipoxin A4 analogue, BML-111 in regulating platelet activation and thrombus formation.

Methods: The expression of FPR2/ALX in human and murine platelets as well as megakaryocytes was confirmed using immunoblot analysis. The subcellular distribution of FPR2/ALX in platelets was demonstrated by immunofluorescence.

Results: BML-111 inhibited a range of platelet function such as aggregation, fibrinogen binding to integrin $\alpha_{II}b\beta_3$, α-granule secretion, dense granule secretion, platelet spreading, and clot retraction suggesting that it may act as a modulator of platelet function.

Conclusions: This study demonstrates the significance of FPR2/ALX and its anti-inflammatory ligand, BML-111 in the modulation of platelet function. This suggests that FPR2/ALX may act as novel target to control thrombo-inflammatory conditions.

PB1822 | Prognostic Value of Platelet-Derived Parameters in Septic Patients: A Prospective Study

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Background: Septic shock is a life-threatening disease associated with high mortality.

Aims: To identify the prognostic value of the different platelet-derived parameters.

Methods: The study was conducted in a 28-beded intensive care unit (ICU) of a university hospital. All Adult patients admitted with sepsis or septic shock were included. Two citrated blood samples were collected on admission from consented patients and processed immediately using Sinnowa PL-12. Two platelets parameters were measured [Platelet count (PLT) and Mean Platelet Volume (MPV)] before and after the addition of four agonists [Arachidonic acid (AA), Adenosine diphosphate (ADP), Collagen and Epinephrine]. Maximum aggregation rate (MAR)% calculated as following: [1-(lowest platelet count/initial platelet count)] x 100. The variation of MPV after exposure to each agonist (delta MPV) was also calculated. Demographic data were also recorded. Two groups were compared: survivors and dead at discharge from ICU.

Results: Forty-seven cases were included. Median age was 57[39-74] years. Median SOFA score was 6[2-9]. Median platelets count on admission was 258[151-346] G/L. Median MPV was 8[8-9.6]. Ten patients (21.3%) experienced disseminated intravascular coagulation. Twelve patients died (ICU mortality = 25.5%). (MAR)% was similar between dead and survivors after exposure to AA (26.4[17.6-31.8] versus 42.4[22.9-57.6]; p = 0.056), after exposure to ADP (22.9[17.6-30.7] versus 35[21.6-49.7]; p = 0,127) and after exposure to epinephrine (26.1[16.3-34.5] versus 37.5[23.5-45.4]; p = 0,151). Delta

MPV was significantly higher after exposure to AA (0.4[0.1-0,54] versus 0,8[0.3-1.3]; p = 0.03) but not after exposure to ADP (0,2[0,1-0,67] versus 0.5[0.1-0.9]; p = 0.166) nor epinephrine (0.4[0.1-0.7] versus 0.7[0.1-0.9]; p = 0.577). ROC curve analysis shows that Delta MPV post-AA > 0.54 predicts mortality with a sensitivity of 62% and a specificity of 63%.

Conclusions: MPV variation after AA exposure is a useful marker for predicting mortality in septic patients.

PB1823 | Platelet and Extracellular Vesicle Mediated Inflammasome Activation Impairs Embryonic Survival by Reducing Placental Thrombomodulin Expression

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Background: Thrombomodulin (TM) deficiency causes platelet-mediated embryonic lethality in mice. Procoagulant extracellular vesicles (EVs) activate maternal platelets resulting in inflammasome activation in the trophoblast and preeclampsia (PE). We speculated that loss of trophoblast TM-expression unleashes platelet-dependent placental inflammasome activation, promoting embryonic lethality.

Aims: We investigated the interaction and functional relevance of EV-mediated thrombo-inflammation in placenta and placental thrombomodulin expression.

Methods: Inflammasome inhibition was conducted in TM-null placenta. Procoagulant EVs were injected into C57/Bl6 pregnant mice. Placental-TM expression and trophoblast proliferation was studied using immunoblotting and Ki-67 staining respectively. Differentiated mouse trophoblast stem cells were treated with EVs or IL-1β to study inflammasome associated loss of TM and proliferation. TM was restored in EV-injected pregnant mice using soluble TM (solulin) or transgenic TM expression to establish causality. Analysis of human PE placentae and trophoblast cells was conducted to scrutinize translational relevance.

Results: Genetic (*NLRP3*$^{-/-}$) or pharmaceutical (anakinra) inflammasome inhibition did not rescue the TM-null embryos from lethality, establishing that the inflammasome activation does not cause lethality of TM-null embryos. However, treatment of mouse or human trophoblast cells with exogenous IL-1β reduced TM expression indicating that loss of TM is a consequence of inflammasome activation. Furthermore, EV treatment reduced TM expression and trophoblast proliferation *in-vivo* and *in-vitro*. Importantly, anakinra restored these effects. In human PE placentae, TM expression was negatively correlated with IL-1β expression and positively correlated with platelet counts and proliferation. Solulin treatment or transgenic expression of TM in placenta ameliorated the PE-like phenotype in mice, prevented thrombo-inflammation and restored proliferation.

Conclusions: These results establish a unidirectional relation of inflammasome activation and reduced trophoblast TM-expression, which contributes to the placental defect and embryonic demise in PE. Restoring TM levels may be a safe therapy in thrombo-inflammatory placental defects associated with excess platelet activation and placental inflammasome activation, as observed in PE.

PB1824 | Is Platelet Hyper-Reactivity a Modifiable Risk Factor in Inflammatory Arthritis?

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Background: Patients with inflammatory arthritis die prematurely of cardiovascular disease. Inflammation activates platelets.

Aims: To assess changes in dynamic platelet function in patients after treatment and improvement in inflammatory markers.

Methods: 20 consecutive patients with acute inflammatory episodes were recruited from a Rheumatology clinic. Following informed consent and clinical review, patients were treated per local protocol. Dynamic platelet function was measured at baseline and again after a 3-month interval once markers of inflammation had improved. The assay utilises video microscopy to measure dynamic platelet behaviour in microliters of blood perfused over VWF at arterial shear rates (1500 s^{-1}). Tracking multiple individual platelets from frame to frame with motion-analysis software, the assay measures the total number of platelets 1) interacting with VWF, 2) translocating across VWF (GPIb dependent), and 3) stably adhering to the surface ($\alpha_{IIb}\beta_3$ dependent), and 4) the speed and distance platelets travel across VWF. Platelet parameters pre and post treatment were compared using a paired student t-test and post treatment changes
in platelet function were correlated to inflammatory disease markers by Pearson Correlation.

Results: 18 of 20 patients completed the study. Of the 18, 3 had a confirmed diagnosis of ankylosing spondylitis,
6 had psoriatic arthritis and the remaining 9 presented with sero +ve rheumatoid arthritis. Platelet adhesion decreased (p ≤ 0.0001)

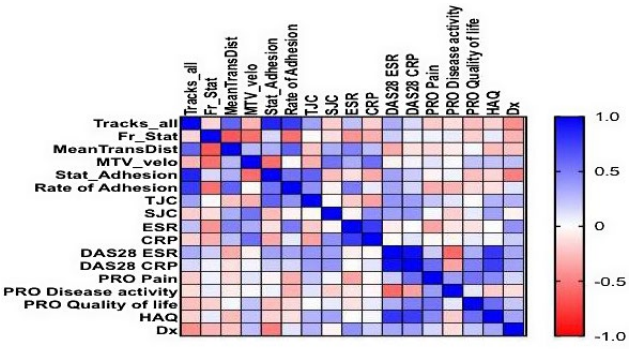

Figure 1: Pearson correlation matrix outlining links between dynamic platelet function and markers of disease activity in inflammatory arthritis

FIGURE 1 Pearson correlation matrix of dynamic platelet function and markers of disease activity

with a corresponding increase in platelet motion ($p \leq 0.005$) following treatment. Tender joint count correlated with platelet adhesion (Pearson $r = 0.616$, $p \leq 0.01$) while CRP correlated with velocity of platelet movement (Pearson $r = 0.563$, $p \leq 0.01$) indicating that changes in clinical markers of inflammation were linked to changes in platelet function (figure 1).

Conclusions: Improvement in clinical markers of inflammation are associated with a corresponding change in platelet function.

PB1825 | Role of Platelets in Processes of Myocardial Healing After Myocardial Ischemia and Reperfusion in Mice

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Background: Platelets play a crucial role in ischemic diseases, which can lead to myocardial infarction (MI), the most leading cause of death in Western countries. In the acute phase of myocardial infarction (AMI) in patients antiplatelet intervention inhibits inflammatory cell recruitment into the infarcted myocardium. In mice knock down or inhibition of specific platelets proteins like PAR-4, GPVI or P-selectin leads to reduced infarct size and reduced inflammation in the acute phase after I/R injury.

Aims: Aim of this project is to analyze the influence of platelets in processes of inflammation and remodeling after I/R in mice besides of their thrombotic potential.

Methods: *In vitro* and *in vivo* analysis of thrombocytopenic mice after I/R injury.

Results: In the acute phase after I/R injury platelets showed enhanced integrin activation and degranulation. Thrombocytopenia in mice leads to reduced infarct size and an improved left ventricular function 24 hours and 21 days after myocardial infarction. Improved heart function was accompanied by reduced inflammation, including decreased cell-migration into the infarcted myocardium and improved cell survival in the infarct border zone as detected by reduced expression of pro-apoptotic proteins in the acute phase and reduced active caspase3 5 days after I/R.

Conclusions: Our results show that platelets modulate inflammation and cell-survival after myocardial infarction indicating platelet interaction with inflammatory and endothelial cells and migration into the infarcted border zone to induce cell apoptosis of cardiomyocytes and / or inflammatory leukocyte.

PB1827 | Toll-Like Receptor 2 Ligands Promote and Enhance Platelet Activation

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Background: In addition to their hemostatic role, platelets regulate the immune and inflammatory responses. Among other immune receptors, platelets express Toll-like receptors (TLRs), which are conserved sensors of pathogen and damage associated patterns. One of the most relevant TLRs is TLR2, which mediates its signaling via binding to either TLR1 or TLR6 as coreceptors. It has been shown that the exposure of platelets to Pam3CSK4, a bacterial surrogate and specific TLR2/TLR1 ligand, promotes platelet hyperactivation, which directly links inflammation with thrombosis. However, the mechanisms and functionality of platelet TLR2/TLR6 are poorly understood.

Aims: To determine whether the bacterial motif and specific TLR2/TLR6 activator Pam2CSK4 (Pam2) can activate human platelets.

Methods: Human washed platelets were isolated from healthy volunteers and exposed to Pam2. Platelet activation and functionality were assessed by Western Blot, static adhesion assays, flow cytometry and secretion assays.

Results: Pam2 induced the activation of a series of signaling cascades in human platelets, including the phosphorylation of the subunit p65 of the nuclear factor (NF)-kB, a master regulator of inflammatory responses. Moreover, Pam2 increased platelet adhesion to collagen-coated surfaces. Flow cytometry analysis demonstrated that Pam2 induces P-selectin exposure in the membrane, a marker of α-granule secretion, as well as integrin $\alpha_{IIb}\beta_3$ activation. Dense granule secretion was also induced by Pam2, as shown by ATP-induced luciferin-luciferase luminescence. Mechanistically, apyrase pretreatment showed that an ADP positive feedback loop is involved in Pam2-induced responses. Finally, Pam2 showed cooperation with the physiologic platelet agonist collagen-related peptide on the induction of P-selectin expression, integrin activation, and ATP release.

Conclusions: TLR2/6 engagement by bacterial motifs induces human platelet activation, which is further enhanced in the presence of physiologic agonists. This work may help identify novel platelet inflammatory-specific targets in the context of thrombosis.

PB1828 | Platelets Express a Stable and Functional Proteasome System and All Components of the Antigen Processing and Presentation Pathway (APP) Machinery

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Background: The proteasome and especially the immunoproteasome with its increased catalytic activity are major proteolytic components contributing to immune cell functions by generating the peptides essential for MHC class I presentation. Platelets express active proteasome complexes that are responsible for protein degradation and regulation of signal transduction processes. However the actual role of the proteasome in platelets remains unclear.

Aims: Characterization of the proteasome and antigen processing and presentation pathway (APP) machinery of platelets

Methods: We identified the catalytically active standard and immunoproteasome subunits as well as APP components in freshly isolated platelets as well as in stored platelets by immunoblot. Proteasome components were purified from washed platelets and their proteasomal activity was determined by fluorogenic peptide substrates. Protein synthesis in stored platelets was inhibited by puromycin treatment.

Results: We identified both, the catalytically active standard proteasome subunits β1/delta, β2/Z and β5/MB1 and immunoproteasome subunits β1i/LMP2, β2i/MECL-1 and β5i/LMP7 in platelets of > 10 healthy donors (Fig 1A, B). For these subunits we observed chymotrypsin-like, caspase-like and trypsin-like proteasome activity, respectively in freshly prepared platelets, which was maintained during 7 days of storage, even when protein synthesis was inhibited. We identified the expression of all APP components required for successful MHC class I presentation (Fig. 1C, D).

Conclusions: The standard proteasome and immunoproteasome are fully functional in platelets. In contrast to other immune cells, the platelet proteasome has a 20 times longer halflife. The high stability of the platelet proteasome might foster platelet functions in hemostasis and immune response by modulation of proteins involved in signaling or MHC class I antigen presentation.

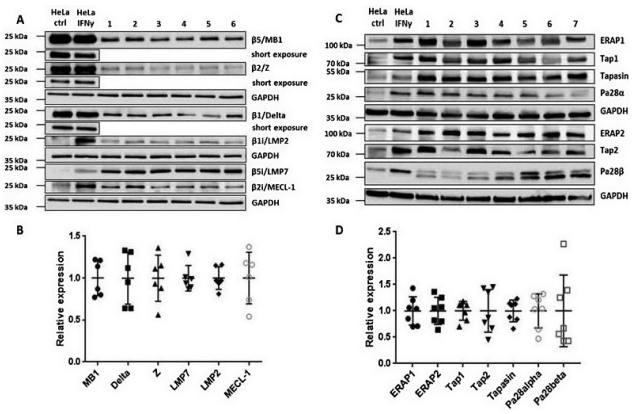

FIGURE 1 Human platelets were analyzed for their proteasome expression (A, B) and antigen processing and presenting pathway (APP) components (C, D)

PB1830 | Sirolimus Is Effective in Autoimmune Lymphoproliferative Syndrome (ALPS)/ALPS-Like Syndrome: Results of a Single Centre Experience

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Background: Autoimmune lymphoproliferative syndrome (ALPS) is a rare condition characterized by a lymphoproliferative disease with clinical manifestations, including lymphadenopathy, hepatomegaly, splenomegaly, autoimmune disease, and secondary malignancies. IAn increased number of a characteristic T-cell population termed "double negative T cells" (DNTs).

Aims: Summary the detailed genetic results of completely relieved ALPS and ALPS-like patients with the application of rapamycin in our center .Analysis the DNT/Treg axis imbalance of rapamycin-benefit patient before taking the drug.

Methods: Patient enrollment:

1. Diagnosed as ALPS and ALPS-like
2. Undergone genetic testing
3. Treatment with rapamycin and relieved.

Flow cytometry: DNT: CD3 + CD4-CD8-TCRab+;

Treg: CD3 + CD4 + CD25 + Foxp3 +

Results: 12 patients were enrolled.

ALPS-sFAS: c.234T>G, p.D78E and c.857G>A, p.G286E

ALPS-caspase10: c.683C>T, p.P228L

ALPS-U: PIK3CD (c.3061G>A, p.E1021K), CTLA4 (c.523dupT, p.Y117Lfs*2), LRBA (c.5003dupc, p.S1669Vfs*18 & c.928C>T, p.R310X), KRAS (c.38G>A, p.G13D), NRAS (c.38G>A, p.G13D), STAT3 (c.833G>A, p.R278H) and other 3 patients with no identifiable mutation.

The DNT/CD3 + : 2.735% (2.38%-3.60%)

The DNT/Lymphocyte: 2.295 (1.42%-3.93%)

The Treg/CD4 + proportion: 4.5% (1.9%-4.8%).

Conclusions: Early clinical identification of ALPS/ALPS-like disease may be facilitated by genetic testing and DNT/Treg detection

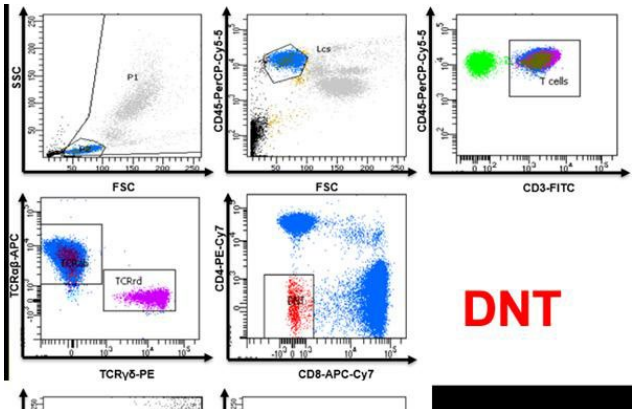

FIGURE 1 Flowcytometry of DNT and Treg

TABLE 1 Results-Detailed genetic results

Patients	Genetic	cDNA mutation	Protein level mutation	PolyPhen2 T	SIFT	EXAC-EAST Asian	DNT/ CD3+	DNT/Lym	Treg/ CD4+
1	FAS	c.234T>G	p.D78E	0.546	0.144	-	3.60	2.29	3.76
2	FAS	c.857G>A	p.G286E	0.822	0.016	-	4.95	4.67	-
3	CASP10	c.683C>T	p.P228L	0.419	0.022	0.0006	-	-	6.5
4	PIK3CD	c.3061G>A	p.E1021K	0.984	0.002	-	-	-	1.9
5	CTLA4	c.523dupT	p.Y117Lfs*2	-	-	-	2.61	2.30	2.53
6	LRBA	c.928C>T,c.5003dupc	p.S1669Vfs*18 p.R310X	-	-	-	4.42	3.93	1.81
7	KRAS	c.38G>A	p.G13D	0.506	0.032	-	1.44	1.31	7.5
8	NRAS	c.38G>A	p.G13D	0.383	0.029	0.00001	2.38	1.42	10.47
9	STAT3	c.833G>A	p.R278H	0.989	0.056	-	6.80	4.11	5.47

IITargeting imbalanced DNT/Treg Axis, Sirolimus is Operative in Autoimmune Lymphoproliferative Syndrome (ALPS)/ALPS-Like Syndrome.

PB1832 | Cold Storage of Apheresis Platelets Promotes IL-8 Release in Platelet: Leukocyte Co-cultures

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Background: Platelet products (PLT) are transfused both prophylactically and therapeutically to prevent and stop bleeding, respectively. United States (US) regulatory guidelines dictate PLT be stored at room temperature (20-24°C, "RTPLT") for up to 5 days. However, cold storage of PLT (2-6°C, "CSPLT") out to 21 days has been shown to improve PLT hemostatic function, prolong shelf life, and decrease contamination risks. CSPLT are now used in US Military combat hospitals and at one civilian institution via Food and Drug Administration (FDA) variance. While platelets are typically linked mechanistically to hemostasis, platelets are immunologically active and modulate leukocyte function. The immunological effects of CSPLT are poorly understood, and as such, need to be more clearly defined if CSPLT are to be transfused into patients with altered immune states (eg. immunocompromised or septic patients).

Aims: To use PLT:whole blood (WB) co-cultures to determine if CSPLT potentiate or dampen leukocyte function.

Methods: PLT (type O+) were stored in plasma with agitation at either 22°C (0, 5 days) or 4°C (0, 5, 10, 21 days) and then mixed with fresh donor WB (type O+). Co-cultures were assessed by flow cytometry for myeloid respiratory burst (DHR+) and degranulation (CD107a+), and by Luminex for cytokine secretion in response to lipopolysaccharide (LPS) stimulation.

Results: Upon PLT:WB co-culture, there was reduced monocyte respiratory burst with early-stored (D0, D5) CSPLT and RTPLT. However, as storage time increased (> 10 days), CSPLT lost the ability to suppress monocyte respiratory burst. Cold storage out to D21 increased PLT-mediated neutrophil degranulation. There were minimal changes in myeloid degranulation. The longer CSPLT were stored, the more they potentiated LPS-induced IL6 and IL8 production in PLT:WB co-cultures. IL8 induction was specific to monocytes and required intact platelets.

Conclusions: CSPLT promote proinflammatory leukocyte responses (increased IL8 secretion and cellular degranulation) upon co-culture with WB from healthy volunteers.

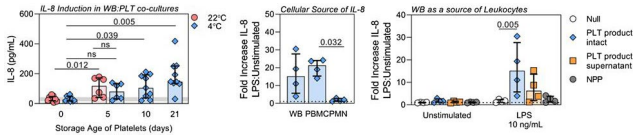

FIGURE 1 Cold-stored platelets potentiate monocyte-specific IL8 secretion in LPS-stimulated PLT:whole blood cultures

PB1833 | Platelets Are Active Players as Inflammatory Cells in Nonalcoholic Fatty Liver Disease

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Background: Non-alcoholic fatty liver disease (NAFLD) is the main cause of chronic liver disease and ranges from simple steatosis to non-alcoholic steatohepatitis (NASH). Recently, a platelet role in NAFLD pathogenesis and progression has been reported in mouse models and in patients.

Aims: Aim of this study was to investigate the role of platelets in NAFLD as active players in liver disease and systemic inflammation.
Methods: We recruited 24 consecutive patients with biopsy-proven diagnosis of NAFLD. In these patients we investigated gene expression profile of the pro-inflammatory cytokines TLR2, TLR4, IL6, IL1R1, MPO, CXCR1, ICAM1,TNF and of hemostatic effectors GPVI, GPIB, F2R (Thrombin receptor PAR1), P2Y1, P2Y12 and thromboxane A2 receptor (TBXA2R) of circulating platelets. We measured plasma levels of leptin, IL-6, TNF-alpha, Il-8 and lipopolysaccharides (LPS) by ELISA commercial Kits. We measured platelet accumulation in liver sections using CD41 antibody, neutrophil extracellular traps (NET) deposition in liver sections using anti-H3 citrullinated histones antibodies.
Results: NAFLD patients had increased plasma levels of systemic inflammation markers and lipolysaccharides. We found an increased inflammatory gene expression in circulating platelets and not in leukocytes of NAFLD subjects compared with healthy controls. Expression of hemostatic genes was unchanged in NAFLD subjects compared with healthy controls. We demonstrated increased intrahepatic platelet accumulation that correlated with NAS score and intrahepatic neutrophil extracellular traps (NET) formation in liver biopsies of NAFLD patients. NET formation was higher in livers with higher NAS and inflammation scores.
Conclusions: The mutual relationship between liver platelet adhesion and NET formation, together with low-grade endotoxemia, low-grade systemic inflammation and pro-inflammatory changes of circulating platelets, suggests that platelets participate on systemic and liver inflammatory changes associated with NAFLD. Altogether, these results indicate that platelets may act primarily to protect the liver from invading microorganisms but contribute to the pathogenesis of liver damage and NAFLD progression.

PB1834 | Sarcoidosis and Autoimmune Thrombocytopenic Purpura: Random Association or Hematologic Manifestation of Sarcoidosis?

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Background: Thrombocytopenia in sarcoidosis is a rare manifestation essentially secondary to hypersplenism or bone marrow involvement.
Aims: Autoimmune thrombocytopenic purpura (ATP) has been rarely described associated with sarcoidosis.
Methods: We present a new case report.
Results: Mrs. FR, aged 50, with a history of pulmonary tuberculosis 8 years ago, has been followed up for mediastino-pulmonary sarcoidosis with sinusal involvement for 3 years.
The diagnosis was based on cutaneous lesions such as lupus pernio and erythema nodosum of lower extremities, nasal obstruction, mediastinal and intraperitoneal lymphadenopathy and bilateral pulmonary infiltrates, increased angiotensin-converting enzyme (ACE) level and the presence of an epithelioid granuloma at salivary gland biopsy and nasal mucosa. She was treated with oral corticosteroid and the evolution was, initially, favorable.
While the patient was taking 10 mg of Prednisone per day, she presented a great abundance epistaxis resisting to local treatment. Laboratory testing revealed thrombocytopenia at 3000el/mm3 and anemia at 5 g / dl of hemoglobin. Myelogram, liver function test, LDH and complement were normal. Anti-nuclear antibodies, anticardiolipin and anti-β2 GP1 were negative. There were no signs of infection. Abdominal ultrasound was normal. The diagnosis of ATP associated with a Sarcoidosis was set.
The patient was treated with tranexamic acid and red blood cells transfusions. Prednisone was increased to 1 mg / kg / day. The evolution was marked by a rise in platelet count within 72 hours and nasal bleeding stopped after 24 hours.
Conclusions: Thrombocytopenia in sarcoidosis is explained by three mechanisms: hypersplenism, medullary invasion by granulomas or more rarely an immunological mechanism. ATP occurs during sarcoidosis in less than 2% of cases and is particularly severe, often complicated by hemorrhage and associated with a high mortality rate of up to 15% of patients. Sarcoidosis associated with ATP is characterized by severe systemic involvement and frequent relapses needing prolonged systemic treatment.

PB1835 | Immature Platelet Fraction and Platelet Indices in Acute Coronary Syndrome

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Background: Acute coronary syndrome (ACS) comprises of ST elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NSTEMI) and unstable angina (UA). Platelet plays important roles in ACS pathogenesis. Immature platelet fraction (IPF) and platelet indices can reflect platelet activations. Platelet indices consist of platelet count, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT).
Aims: This study aimed to analyze the differences in number of IPF and platelet indices among ACS patients.
Methods: This study was an observational analytical cross sectional study conducted at the Dr. Soetomo Hospital, Surabaya, Indonesia, during May to September 2019. The EDTA-samples were performed for IPF and platelet indices using Sysmex XN-1000. This study was approved by ethical clearance committee of Dr. Soetomo Hospital. Informed consents were taken from all study subjects. The differences of IPF and platelet indices among STEMI, NSTEMI, and UA patients were analyzed using Kruskall-Wallis and Mann-Whitney test.

Results: The subjects were consisted of 30 STEMI, 25 NSTEMI and 24 UA patients. The IPF values showed significantly difference between groups ($p < 0.001$), with the highest median value is STEMI, and be followed by NSTEMI and UA groups.The value of MPV, PDW, and P-LCR showed significantly higher in STEMI towards UA ($p < 0.001$ for each parameter), and NSTEMI towards UA ($p = 0.002$; $p < 0.001$; $p < 0.001$ respectively for each parameter).

Conclusions: The IPF values can be considered as an alternative indicator to differentiate the STEMI, NSTEMI and UA. The MPV, PDW, and P-LCR can be considerd as an alternative indicator to differentiate STEMI and UA, and also NSTEMI and UA.

PB1837 | Platelet β1 and β3 Integrins Cooperate to Stop Cutaneous and Cerebral Bleeding under Inflammatory Conditions in Mice

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Background: Platelets ensure the arrest of bleeding under inflammatory conditions through a mechanism relying primarily on GPVI and to a lesser extent on CLEC-2. However, this role of platelets appears independent of the receptors classically involved in the arrest of bleeding i.e. the GPIb-V-IX complex and integrin αIIbβ3 in the reverse passive Arthus (rpA) model. This questions which platelet receptors support stable platelet adhesion at inflammation site, a role played notably by integrins during a trauma.

Aims: To evaluate the role of platelet β1 and β3 integrins in the arrest of bleeding under inflammatory conditions.

Methods: We used an rpA model based on anti-bovine serum albumin (BSA) antibody intradermal injection and BSA intravenous injection to induce cutaneous inflammation. The intranasal lipopolysaccharide and transient middle cerebral artery models were used to induce lung and cerebral inflammation, respectively.

Results: Contrary to mice totally deficient for β3 integrins, those deficient for β3 integrins in the platelet lineage (PF4Cre-$\beta3^{-/-}$) presented localized petechial bleedings in the cutaneous rpA model, which was confirmed using αIIb-deficient mice. These results identify a role for platelet αIIbβ3 integrin in inflammatory bleeding. Mice deficient for platelet β1 integrins did not present bleeding in this model, however, mice deficient for both platelet β1 and β3 integrins (PF4Cre-$\beta1^{-/-}/\beta3^{-/-}$) presented more severe cutaneous bleeding compared to PF4Cre-$\beta3^{-/-}$ indicating a secondary role for platelet β1 integrins and a cooperation between both integrin families in the arrest of cutaneous bleeding under inflammatory conditions. Similar results were obtained under cerebral inflammatory conditions since PF4Cre-$\beta1^{-/-}/\beta3^{-/-}$ mice developed cerebral bleeding. On the contrary, platelet integrins do not appear to play a major role in the arrest of pulmonary bleeding under inflammatory conditions.

Conclusions: This study identifies a cooperation between platelet β1 and β3 integrins in the arrest of cutaneous and cerebral bleeding under inflammatory conditions.

PB1838 | Platelets in Resolution of Liver Inflammation and Tissue Regeneration

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Background: Mechanisms of hepatic ischemia-reperfusion injury (IRI), caused by liver surgery, transplantation and trauma, remain unclear and therapy is limited. IRI is characterized by inflammatory cell infiltration and parenchymal cell injury. The tissue damage can be limited by prompt removal of accumulated leukocytes restoring homeostasis by properly completed resolution of inflammation. This phase of inflammation is a biochemical and metabolic process orchestrating the function and migration of immune cells resulting in correct host defense. Impaired resolution disturbs tissue integrity leading to organ failure. Platelets play an immunomodulatory role during onset of liver IRI by interacting with hepatic sinusoidal endothelium and promoting the recruitment of proinflammatory cells. Its role in resolving processes has not been studied yet.

Aims: We aimed to unravel the properties of platelets in hepatic IRI in the resolution phase of inflammation and restoration of homeostasis.

Methods: Human PBMC were stimulated with GM-CSF and challenged with platelets and TNF-α. M1/M2 markers were analyzed by qRT-PCR.

The hepatic IRI C57BL/6 mice were treated with platelet-depleting anti-GPIbα antibody or IgG control. Leukocytes were assessed by flow cytometry; serum was analyzed for liver injury markers; proresolving lipid mediators and its derivates were measured by LC-MS/MS.

Results: We showed that platelets control the phenotypic polarization of macrophages by shifting them from proinflammatory M1 to the anti-inflammatory M2 phenotype depending on stimuli.

We demonstrated sustained proinflammatory state in platelet depleted hepatic IRI mouse model with augmented infiltration of PMN with decreased clearance of apoptotic cells.

Conclusions: Our results reveal dual role of platelets orchestrating leukocyte infiltration in early phases and supporting resolution at later stages of liver inflammation. Further studies will elucidate platelets' impact on tissue regeneration, its signaling events and link to specialized lipid mediators. Thus, platelets and its metabolites are

potential therapeutic targets modulating the outcome of liver IRI and tissue regeneration.

PB1839 | Effect of Human Myeloperoxidase on Ca^{2+}-response, Membrane Conductivity and Phosphatidylserine Exposure on Platelet Surface

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Background: Activation of the immune system strongly influences blood coagulation and pathological thrombus formation. Antimicrobial agents that are released during leukocyte degranulation can directly activate platelets and induce the coagulation cascade. Myeloperoxidase (MPO) is an enzyme that is contained in azurophilic granules of neutrophils. Recently MPO was shown to potentiate platelet aggregation, so it may contribute to pathological thrombosis during inflammation, but the mechanism is poorly understood.

Aims: To investigate MPO binding to platelet membrane and to assess MPO effect on primary platelet signaling (cytosolic Ca^{2+} concentration, membrane potential and ionic currents, phosphatidylserine (PS) exposure on platelet surface).

Methods: Platelets were isolated from citrate-stabilized blood from healthy volunteers. Single channel ionic currents were registered using patch-clamp technique in a voltage-clamp mode. Platelet calcium responses were measured by TIRF miscroscopy using CalBryte 590. PS exposure was measured by flow cytometry using annexin V-Alexa Fluor 647.

Results: MPO (100 nM) increased frequency (2-fold) and duration of calcium oscillations compared to the basal level. This effect was accompanied by plasma membrane hyperpolarization by 10-15 mV. To inactivate voltage-gated potassium channels patch was hyperpolarized and registration of single channel events was performed. It revealed MPO-induced currents from 7 to 20 nSm (outward Cl^- or inward K^+ movement) in Ca^{2+}-containing KCl and K-aspartate solutions, indicating the involvement of Ca^{2+}-dependent chloride and potassium channels. Since platelet procoagulant activity closely relates to its calcium response, we next examined the effect of MPO on PS exposure on platelet surface. At MPO concentrations higher than 100 nM there was a significant increase in number of PS-positive platelets.

Conclusions: Obtained results indicate that MPO binding to platelets increases the rate of calcium oscillations as well as Ca^{2+}-dependent ionic permeability that may lead to exposure of a procoagulant platelet surface. This suggests possible targets for regulation of platelet response at sites of inflammation.

PB1840 | Influence of HOCl-modified Myeloperoxidase on Platelet and Neutrophil Activity

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Background: Platelets and neutrophils interact during an infection, inflammation and thrombosis and regulate functions of each other. These interactions are mostly initiated by soluble mediators. One of the principal enzymes of neutrophils is myeloperoxidase (MPO). It is a heme protein that not only generates cytotoxic oxidants but also modulates cellular signal cascades. Hypochlorous acid (HOCl) produced by MPO is the main bactericidal agent that can also damage host biomolecules, and MPO can be one of the primary targets.

Aims: To investigate the ability of HOCl-modified MPO (MPO-HOCl) to affect platelet's and neutrophil's functional responses.

Methods: Purified MPO was pre-treated with HOCl at room temperature for 2 h before analysis. The *Western blotting*, fluorescence and surface-enhanced *Raman scattering* techniques were *used* to *analyze* MPO structure *after* treatment with *HOCl*. Cell activation was investigated via aggregometry, flow cytometry, fluorescence techniques.

Results: Flow cytometry analysis and confocal microscopy revealed efficient binding of MPO (25-200 nM) to human neutrophils and platelets while HOCl-modified MPO displayed *significantly reduced* binding to cellular surface. Though MPO-HOCl induced calcium signaling in neutrophils, this effect was less than the effect of native MPO and decreased with increasing protein modification by HOCl. However, MPO-HOCl failed to induce exocytosis of lactoferrin, marker of specific granule, and elastase, marker of azurophilic granule in neutrophils. MPO-HOCl in contrast to native MPO did not enhance ADP- and thrombin-induced platelet aggregation and wasn't able to induce formation of platelet-leukocyte aggregates.

Conclusions: Elevated level of MPO in circulation is associated with inflammation and increased oxidative stress. Our data suggests that MPO can have dual effect:

(i) regulating of platelet and neutrophil activity in order to induce pro-inflammatory responses

(ii) under oxidative stress HOCl-induced modification of MPO downregulates chronic inflammatory processes in vessels.

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PB1841 | Specific Inhibition of MRP4 Affects Several Signaling Pathways and Thrombus Formation in Human Platelets

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Background: The multidrug resistance protein 4 (MRP4) is a versatile efflux transporter highly expressed in platelets and transports cyclic nucleotides as well as certain lipid mediators including sphingosine-1-phosphate (S1P).

Aims: Aim of the present study was to comprehensively characterize the effects of the selective MRP4 inhibitor Ceefourin-1 on different signaling pathways in platelets.

Methods: Platelet activation was determined by light transmission aggregometry and flow cytometry. Thrombus formation in whole blood was analyzed in collagen-coated microchannels under high arterial shear conditions. Phosphorylation of vasodilator-stimulated phosphoprotein (VASP) was quantified by flow cytometry; calcium influx in Fura-2 loaded platelets. Transport of 3H-cGMP, fluorescein (FITC)-labelled S1P and thromboxane B2 (TXB2) was studied in membrane vesicles from MRP4-overexpressing SF9 cells and human platelets.

Results: Ceefourin-1 concentration-dependently inhibited the MRP4-mediated membrane transport of cyclic nucleotides, TXB2 and S1P. In ex-vivo aggregometry studies in human platelets, Ceefourin-1 significantly inhibited platelet aggregation by about 30 to 50% when ADP or collagen were used as activating agents, respectively. Furthermore, Ceefourin-1 significantly lowered the ADP-induced activation of integrin αIIbβ3, indicated by binding of FITC-fibrinogen (about 50% reduction at 50 μM Ceefourin-1). Platelets incubated with Ceefourin-1 (50 μM) showed a reduced Calcium influx and a 1.5-fold and 1.7-fold increase in PGE1- and Cinaciguat-induced VASP phosphorylation, indicating increased cytosolic concentrations of cAMP and cGMP, respectively. The release of TXB2 and S1P from activated human platelets was also attenuated. Moreover, selective MRP4 inhibition significantly reduced both the total area covered by thrombi and the average size of thrombi by about 40% in the flow chamber model.

Conclusions: Selective MRP4 inhibition causes reduced platelet adhesion and thrombus formation under flow conditions. This is reflected by an inhibition of integrin αIIbβ3 activation, elevated VASP phosphorylation and reduced calcium influx. Beside inhibition of cyclic nucleotide transport, release of pro-inflammatory mediators such as S1P is also affected.

PB1843 | Critical Role of Platelets during Pulmonary Fibrosis

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Background: Idiopathic Pulmonary Fibrosis (IPF) is a chronic, progressive and irreversible disease leading to death between 2 to 5 years after diagnosis. Despite the significant morbidity and mortality associated with IPF, its pathogenesis remains poorly understood and there is no curative treatment. Recent evidence showed that platelets are in a preactivated state in blood of IPF patients. However, the role of platelets and activation mechanisms during the development of IPF have not been established yet.

Aims: To decipher the role of platelets during pulmonary fibrosis:

Methods: We used intratracheal instillation of bleomycin to induce pulmonary fibrosis in mice and collected their bronchoalveolar lavages (BAL) fluids, blood and lungs. Mice treated with intratracheal saline were used as controls.

Results: Wild-Type (WT) mice display a significative higher mortality rate 14 days after bleomycin instillation compared to the control group. Platelets and white blood cells counts increase in BAL of bleomycin-treated mice. Interestingly, thrombocytopenic (TP) mice challenged with bleomycin died prematurely after instillation. To further identify platelet mechanisms involved, mice lacking the GPIbα subunit of the von Willebrand factor receptor (hIL4R/GPIbα) died prematurely 6 days after instillation. Remarkably, mice deficient for the collagen receptor, GPVI, are protected from mortality after bleomycin instillation. Hemoglobin level, in the BAL, was significantly higher in bleomycin-induced TP and hIL4R/GPIbα mice compared to bleomycin-induced GPVI deficient and WT mice. Moreover, platelet factor 4 level in BAL from GPVI deficient mice is significantly decreased compared to WT mice.

Conclusions: Here we showed that platelets play a protective role during pulmonary fibrosis. We uncovered that GPVI plays a deleterious role in pulmonary fibrosis and GPIbα is required for the maintenance of vascular integrity in bleomycin-treated mice. Our studies require further experiments to explore other platelet signaling molecules and highlight a new potential therapeutic target in IPF.

PB1844 | Critical Role for Platelet Ral GTPases in the Regulation of Venous Thrombosis

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Background: The ubiquitously expressed small GTPases RalA and RalB are involved in cell proliferation and tumour metastasis. We

reported that RalA/B conditional double knockout (RalAB DKO) in mouse platelets resulted in near complete defect in P-selectin externalisation on platelets upon activation, with no other significant secretion defect.

Aims: To address whether platelet-expressed Ral GTPases, through regulation of platelet surface expression of P selectin, control venous thrombosis *in vivo* in mice.

Methods: We used an *in vivo* inferior vena cava ligation model of venous thrombosis in mice, and analyses of neutrophil NET formation.

Results: RalAB DKO led to ablation of venous thrombosis in mice (thrombus weight in DKO: 0.4 ± 0.2 mg vs 15.35 ± 3.20 mg in Wt control, n = 8 in DKO and n = 10 in WT, $p < 0.001$). Immunohistochemical staining of thrombus sections demonstrated that in DKO mice there was no NET formation, and less neutrophil infiltration than in WT mice, where strong NETosis was detected. Platelet-specific conditional knockout of SNAP23 however did form (diminished) venous thrombi, even though absence of SNAP23 resulted in complete ablation of secretion. Histological examination of SNAP23$^{-/-}$ thrombi showed similar structures to WT, although diminished in size, and NETosis formation was normal, suggesting that secretion defects, particularly P-selectin externalisation, are only partially responsible for the ablated venous thrombosis in RalAB DKO mice. In addition to the defect in NETosis, we also found that P-selectin and HMGB1 did not colocalize in thrombus sections of RalAB DKO as they did in WT control mice.

Conclusions: Platelets play a critical role in venous thrombosis in a ligation model in mouse. Platelet-derived P-selectin and HMGB1 recruit and activate neutrophils and NET formation, coordinated by platelet-expressed RalA and RalB. As Rals are targets for drug development, future work is required to determine whether platelet Rals represent good novel targets in venous thrombosis management.

PB1845 | Fat Emulsion for Parental Nutrition as Well as Lipid Emulsion of the Drug Propofol Strongly Enhance Platelet Activation Induced by Human Albumin, Misfolded by Structural Modification with the Granulocyte Defence Weapon HOCl

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Background: Misfolding of extracellular proteins, induced by environmental factors as mechanical stress, glycation, oxidative stress, proteolysis and electrophilic stress leads to activation of platelets. Lipid rafts, ganglioside- and cholesterol enriched membrane microdomains directly promote aggregate formation of misfolded proteins.

Aims: As the general anaesthetic propofol is formulated in a lipid emulsion and as fat emulsions are used for parenteral nutrition in intensive care patients, we studied the effect of such formulations on the activation of platelets induced by HOCl modified proteins.

Methods: Platelet function was analysed using flow cytometry, adhesion assays, aggregation, procoagulant activity and confocal laser scanning microscopy.

Results: We observed a strong enhancement of platelet activation induced by HOCl modified albumin in all used assays with lipid formulated propofol and clinically relevant concentrations. Pure propofol without lipids had no effect. In addition, Lipofundin MCT 20 showed the same enhancing effects as lipid formulated propofol.

Conclusions: It is likely that the fat emulsions induced stronger platelet activation by enhancing the aggregate formation of the misfolded albumin, leading to enhanced crosslinking of platelet receptors and hence stronger activation. Infusion of propofol and any other lipid-based drug or parenteral nutrition might have an influence on prothrombotic tendency under inflammatory conditions.

PB1846 | Platelet Depletion Limits the Severity But Does Not Prevent the Occurrence of Experimental Transfusion-related Acute Lung Injury

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Background: Transfusion-related acute lung injury (TRALI) is a severe pulmonary reaction due to blood transfusions. The pathophysiology of this complication is still not widely elucidated by the scientific community, especially regarding the direct role of blood platelets within the cellular mechanism responsible for the development of TRALI.

Aims: Beside the recently described proinflammatory functions of platelet, the conflicting reports of platelets involvement in TRALI have led us to investigate their role in its onset. In order to address this question, we developed a two-hit mouse model of TRALI induced by injections of lipopolysaccharide (LPS) and anti-MHC I antibody, which is the equivalent of HLA Class I in Human.

Methods: In this study, a mouse model was used to induce the development of antibody-mediated acute lung injury (ALI) through injections of lipopolysaccharide (LPS) and an anti-MHC class I antibody. BALB/c mice were pretreated with an anti-GPIbα antibody, which induces platelet depletion, or ML354, a PAR-4 receptor pathway inhibitor, 30 minutes prior to TRALI induction.

Results: Depletion of platelets prior to TRALI induction appeared to reduce the severity of TRALI without completely inhibiting its development. Also, inhibition of platelet activation by ML354 did not prevent the onset of TRALI. Finally, the stimuli used of TRALI induction also triggered specific platelet activation upon ex vivo stimulation.

Conclusions: This study suggests that blood platelets are not critically required for TRALI induction although they are to some extent involved in its pathophysiology.

PB1847 | P-selectin (CD62P) Deficiency Is Associated with Increased Coxsackievirus B3 Myocarditis But Not Influenza A Virus Infection in Mice

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Background: Platelets have been shown to play a critical role in host survival after Coxsackievirus B3 (CVB3) infection. Recently, we showed that protease-activated receptor 4 (PAR4), the major thrombin receptor on mouse platelets, contributes to anti-viral responses in mice infected with CVB3 or influenza A virus (IAV). Thrombin stimulation of platelets leads to P-selectin exposure and platelet-leukocyte interaction that participates in innate immune responses.
Aims: To investigate the effect of P-selectin (CD62P) deficiency on CVB3 myocarditis and IAV infection in mice.
Methods: CD62P$^{-/-}$ and wild-type (WT) mice (6-8 weeks old) were infected with 10^5 pfu of CVB3 (Nancy strain). In addition, mice (8-12 weeks old) were infected with 0.02 HAU influenza A/Puerto Rico/8/1934 (PR/8) virus. Progression of CVB3 myocarditis (day 8) and IAV infection (day 3, 5 and 7) were analyzed.
Results: CD62P deficiency resulted in significant increased CVB3 virus load in hearts of mice at day 8 compared to WT mice. This was associated with increased systemic inflammation measured by elevated white blood cell (WBC) and neutrophil numbers in the blood of CD62P$^{-/-}$ mice 8 days after CVB3 infection compared to WT mice. The more severe myocarditis in CD62P$^{-/-}$ mice resulted in increased mortality compared to WT mice. However, a lack of CD62P did not significantly influence the immune cell numbers (total WBC, neutrophils, lymphocytes, monocytes) in the air space of CD62P$^{-/-}$ mouse lungs 3, 5 or 7 days after IAV infection compared to WT mice. Furthermore, CD62P deficiency had no effect on CXCL1, CXCL2, CCL2 or CCL5 levels in the lung 3, 5 or 7 days compared to WT mice after IAV infection.
Conclusions: CD62P contributes to protection in systemic viral infection, such as CVB3 myocarditis, but is not needed for immune responses in localized IAV infection of the lung.

PB1848 | Defining the Effects of Storage Lesion on Platelet Autophagic Activity

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Background: Numerous autophagy proteinic components such as Beclin1, ATG7, ATG12-5 and LC3a/b are not only present but also modulated in platelets following stress, activation or starvation. Autophagy mechanisms are essential to the integrity of platelet functions. Platelet storage lesions (collection, processing, and storage) are considered as stress signals.
Aims: Our objective was to assess the impact that the Pathogen Inactivation (PI) procedure for platelet concentrates (PC) has on storage lesions and more specifically on autophagy and platelet functionality.
Methods: In order to do so, we evaluated both the importance and the evolution of autophagy biomarkers on PC. Autophagy was indirectly measured by the levels of LC3a/b, ATG5, SQSTM1, analysed by Western Blot i) on platelet-rich plasma (PRP) lysates with or without thrombin receptor-activating peptide (TRAP) stimulation and ii) on platelet concentrates lysates before and after Pathogen inactivation process.
Results: After PI, we observed modulations in the levels of typical markers of autophagy. Indeed, LC3a was increased in platelets when compared to LC3b. ATG5 was also increased suggesting a modulation of autophagy process following PI. The differential levels of ATG5 and LC3a/b were reminiscent of those we observed after TRAP activation of platelet rich plasma. Surprisingly, we could not detect LAMP-2 after TRAP activation, a result that remains to be investigated.
Conclusions: These preliminary results suggest that PI process impacts platelet autophagy which we suggest could contribute to the storage lesions of PC. Despite many connections between autophagy and apoptosis a strong causal relationship wherein one process controls the other, has not been adequately demonstrated in the context of transfusion. Contemporary efforts in elucidating platelet autophagy pathways now allows to focus on the transfusion thematic and help us to understand how autophagy can modulate transfusion yields.

PB1849 | Platelets Induce Rapid Subset and Phenotypic Switching in Monocytes in Whole Blood

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Background: The majority of circulating monocytes exist in a classical phenotype ($CD14^+$, $CD16^{low}$). Intermediate phenotype monocytes ($CD14^+$, $CD16^{high}$) are normally rare but greatly expand in a range of disease conditions including sepsis, coronary artery disease and autoimmune disease, where their presence is associated with poor outcome. Using *in vitro* culture models it has previously been suggested that platelets can induce changes in monocyte phenotype, however, whether these can occur in physiological conditions and timescales remain unknown.
Aims: To investigate whether acute stimulation of platelets can switch monocytes from classic to intermediate phenotype in whole blood *ex vivo*.
Methods: Whole blood was stimulated with collagen-related peptide (CRP-XL) and mixed at 37°C for 10-120 minutes. Flow cytometric analysis was performed to detect $CD14^+$ monocytes, their subtype (classical $CD16^{low}$; intermediate $CD16^{High}$) and the presence or absence of platelets (CD42b). Further cell markers were incorporated to detect altered expression profiles. Data are mean ±s.e.m from n = 7.
Results: CRP-XL stimulated platelets to bind to $CD14^+$ monocytes within 10 minutes (66 ± 4% vs 19 ± 3% vehicle). Concurrently, within 10 minutes, there was a proportional decrease in classical monocytes from 80 ± 1% to 38 ± 9% ($p < 0.05$) and the intermediate subset increased from 4 ± 1% to 42 ± 6% ($p < 0.05$). This response was maintained and unchanged for up to 2 hours. Of this intermediate subset, post CRP-XL stimulation, 91 ± 2% were platelet positive. Furthermore a significant increase (52 ± 11%) in pro-chemotactic CCR2 expression and a significant decrease (28 ± 7%) in antigen-presenting HLA-DR expression was observed only in platelet-induced intermediate monocyte subset and not platelet-bound classical monocytes.
Conclusions: Direct and selective activation of platelets in whole blood results in rapid emergence of a platelet-induced intermediate subset of monocytes with differential receptor expression.
These data strongly indicate a role for platelets as rapid regulators of monocyte phenotype and identify a potential means to control disease-associated monocyte phenotypes.

PB1850 | Evaluation of Analyser Flag 'Abnormal Platelet Distribution' in Routine Complete Blood Count (CBC)

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Background: Coronary artery disease (CAD) has become a global epidemic and has spawned multitudes of research in not only diagnosis and treatment, but also in preventive measures. As part of prevention, risk factors for CAD have been identified, such as smoking, diabetes mellitus, dyslipidaemia and hypertension. Inflammation has also been implicated in the aetiology of CAD. Thus, there were many studies done to investigate the value of cheap and easily available laboratory parameters such as neutrophil lymphocyte ratio and platelet volume indices in predicting the severity of the disease or the outcome of a surgical procedure.
Aims: The aim of our study is to assess the 'abnormal platelet distribution' instrument flag at our laboratory.
Methods: We reviewed all results of complete blood count with or without white cell differential, from our laboratory database in the month of October 2019. Data on erythrocytes, leukocytes and platelet parameters were collected and tabulated. We were using Sysmex XN550. We also recorded the patient's demographic data and clinical history, which was available on the request form.
Results: There were 1379 results of complete blood count (CBC), in which 75 (5.4%) tests flagged as 'abnormal platelet distribution'. Some patients have the tests repeated over the period of one month. 33 patients had 'abnormal platelet distribution' flags. 23 (70%) of these patients had coronary artery disease and/ or end stage renal failure. The means haemoglobin, white blood cell and platelet counts were 11.8 g/dL, 8.5 x 10^9/L and 187x 10^9/L, respectively. The platelet distribution width (PDW) and mean platelet volume (MPV) 11.2 fL and 15.7%, respectively. In about 50% of patients, the PDW and MPV were not available from the analyser.
Conclusions: In conclusion, abnormal platelet distribution may suggest presence of coronary artery disease but can also be a transient finding in some patients.

PROTEASES AND CANCER

PB1851 | Hypochlorous Acid Modification to Antithrombin Results in Generation of N*eo*-epitopes, and a Shift towards Prothrombotic and Proangiogenic State in Cancer Patients

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Background: Cancer patients are at high risk of developing venous thromboembolism leading to high rates of mortality. The reason

might be the elevation of procoagulant proteins and down regulation of natural anti-coagulants like antithrombin (AT). The AT modulates both coagulation and angiogenesis. During respiratory burst hypochlorous acid (HOCl) is generated *in vivo* which may cause alteration in various biomolecules like proteins and DNA. Structural alteration in proteins may lead to autoantibodies generation *in vivo*. Therefore, it is a thought of interest to investigate the role of HOCl in cancer.

Aims: To see the role of HOCl-modified AT in cancer.

Methods: The purified AT was modified with various concentrations of HOCl to asses structural and functional alterations by various physicochemical techniques and enzymatic assays. Sera of pre-treated cancer patients (N = 60) with mean age of 40 ± 25 were collected. Sera from healthy subjects (N = 30) served as negative control. Statistical study was done with the results obtained. This study had clearance from bioethical committee.

Results: HOCl induced secondary and tertiary structural changes in AT were observed by various techniques like absorbance and fluorescence spectroscopy, and Far-UV CD. While, the HOCl-modified native-AT showed fragmentation and polymerisation (further conformed by TEM), cleaved and latent conformers exhibited fragmentation only on PAGE. HOCl reduced the catalytic activity of AT as assessed by factor Xa and thrombin inhibition assays. Compared to the unmodified AT, the HOCl-modified counterparts were more specifically recognised by cancer autoantibodies as assessed by direct binding and competition ELISA, showing probable role of HOCl-modified AT in cancer.

Conclusions: We propose that *in vivo* generated HOCl, during respiratory burst, may cause structural alteration in AT and hence, generation of *neo*-epitopes on it. Also functional loss in AT points towards a shift in more prothrombotic and proangiogenic environment.

PB1853 | Hepsin Is Involved in the Tumorigenicity and in the Hipercoagulability of Colon and Gastric Cancer Cells

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Background: Venous thromboembolism is one of the most common complications in patients with digestive tumors and is associated with an increased morbidity and mortality. The bidirectional interaction between the hemostatic system and tumorigenic processes is based on pleiotropic molecules, whose complex effects are not

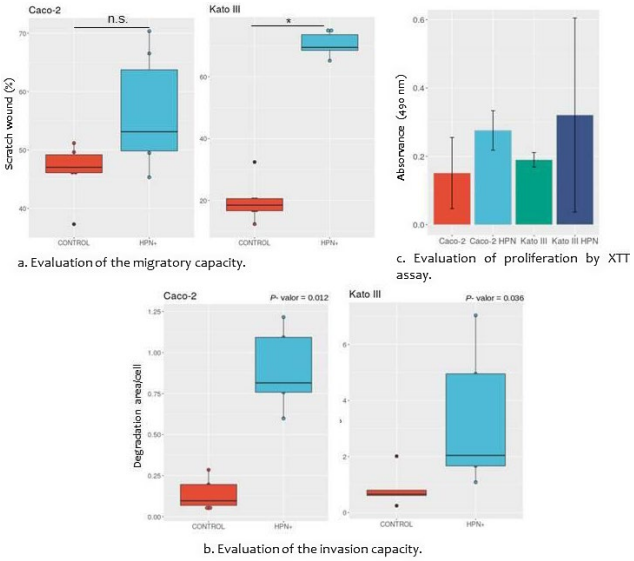

Figure 1. Hepsin effect on tumorigenicity of cancer cells. The hepsin overexpression increases the migration, invasion and proliferation capacity of both cell lines *in vitro*.

FIGURE 1 Hepsin effect on tumorigenicity of cancer cells

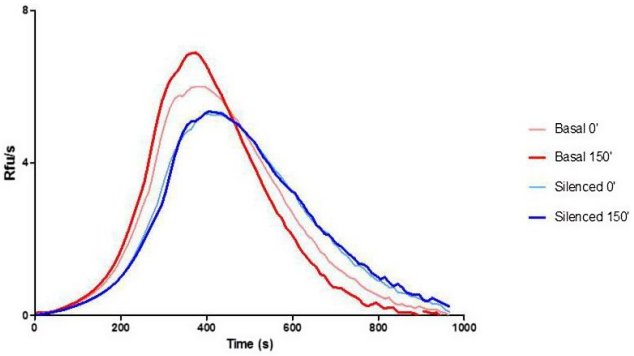

Figure 2. Fxa generation of tumor cells incubated with plasma. FXa generation was evaluated adding 5pM TF. Hepsin silencing prolonged the lag time (P=0.017), and decreased peak height (P=0.036) and AUC (P=0.017). At 150 minutes, the values obtained with KATO III incubated with plasma (basal vs silencing): Lag time (sec), 117 vs 143; peak (rfu/sec), 6.9 vs 5.3; AUC (rfu) 1781 vs 1697.

FIGURE 2 Fxa generation of tumor cells incubated with plasma

completely known. Hepsin, a serine protease involved in cancer progression and factor VII activation, is one interesting pleotropic molecule that could be considered as a new promising therapeutic target.

Aims: To study the influence of hepsin levels in tumorigenic processes and in plasma hypercoagulability.

Methods: Hepsin overexpression (HPN) was generated in two cancer cell lines: Kato III (gastric carcinoma) and Caco-2 (colorectal adenocarcinoma). Gene silencing was carried out with the ON-TARGETplus system. Hepsin expression levels were evaluated by western blot and qRT-PCR. Migration assays were performed by wound-healing. Invadopodia invasion was analyzed by fluorescence microscopy and the proliferation rate by XTT assay. The FXa generation was analyzed

by incubating plasma with the different cell lines. Continuous variables were compared using non-parametric tests.

Results: Kato III-HPN and Caco-2 HPN showed greater migration ($p = 0.008$ and $p = 0.120$, respectively) and invasiveness capacity ($p = 0.036$ and $p = 0.012$, respectively) than the basal cell lines (Fig. 1). Cell proliferation only showed a tendency to increase in HPN cells, while hepsin silencing decreased by 36% the procoagulant capacity of Caco-2 and KATOIII, HPN did not influence this procoagulant effect compared to control cell lines (Fig. 2).

Conclusions: Hepsin is involved in the capacity of migration and invasion of gastric and colorectal cancer cell lines. The silencing of the gene associates with a reduction in their procoagulant capacity, and, in FXa generation, prolonged time parameters and decreased peak height. The plasma FVIIa-AT concentration, likely reflecting FVIIa activation, might be coherently modulated. These data suggest that hepsin could be considered as a biomarker of tumor aggressiveness and thrombotic risk in these tumors.

PB1854 | Novel Urokinase-derived Peptides Inhibiting the Pro-tumoral Activity of Cancer-associated Fibroblasts

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Background: Progression of solid tumors not only depends on the complex events initiated by oncogenic lesions, but also on cancer cell ability to divert the function of stromal cells, which become supportive of cancer growth and dissemination.

Aims: In the framework of new anti-cancer strategies, our aim is to concurrently co-target neoplastic cells and stromal Cancer-Associated Fibroblasts (CAF).

Methods: Methodologies include peptide synthesis, cell culture migration and invasion assays, in vivo tumor dissemination experiments, organotypic assays with primary stromal CAFs.

Results: This work is focused on the biological and molecular effects of two novel deca-peptides, denoted as PEP1 and PEP2, which were rationally designed starting from previously disclosed anti-migratory peptides derived from human urokinase (uPA). In vivo, the cyclic derivative PEP2 markedly reduces the number and the size of lung metastases, originated from HT1080 fibrosarcoma cells injected in the mouse tail vein, showing a net anti-metastatic activity. In culture, both peptides counteract the migratory and invasive ability of HT1080 and MDA-MB-231 breast carcinoma cells in Boyden chambers and in 3D-organotypic assays. Noticeably, cell exposure to nanomolar concentrations of either peptides, human dermal fibroblasts (TIF) or primary CAF from breast carcinoma patients (intra- or peri-tumoral) exhibit a reduced pro-invasive ability in 3D-organotypic co-cultures, when tested in combination with either HT1080-GFP fibrosarcoma or MDA-MB-231 mammary carcinoma cells. These effects are accompanied by a reduced capacity to remodel collagen matrix and to secrete chemoattractants, together with a decreased α-SMA and increased Caveolin-1 protein levels, both well known CAF markers.

Conclusions: Overall, these data indicate that two novel decapeptides are efficacious co-targeting agents, counteracting tumor cell dissemination and modulating the pro-invasive activity of stromal fibroblasts.

THROMBOTIC MICROANGIOPATHIES

ADAMTS13 AND TTP

PB1856 | Oligosymptomatic Thrombosis in a Young Girl with Upshaw-Schulman Syndrome (USS) Not Receiving Prophylactic Plama Infusion

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Background: USS, or congenital TTP is a rare but potentially fatal thrombotic microangiopathy caused by a severe hereditary deficiency of ADAMTS13 activity. Because of the paucity of severe clinical signs during childhood prophylactic infusions of plasma to replace ADAMTS13 are often withheld.

Aims: We here report a retrospective evaluation highlighting the danger to omit regular plasma infusions in USS.

Methods: The early disease course up to the age of 5 yrs has been extensively documented (IJH 2012; 96:789 and Blood Advances 2019; 3:3191).

Results: The patient was now 12-year-old. A diagnosis of USS based on biallelic *ADAMTS13* mutations was made at the age of 4 yrs, but thereafter she had no appreciable clinical signs, and so her parents declined of prophylactic (non-sterilized) plasma infusions.

At the age of 12 yrs, the patient suddenly complained of right lower severe abdominal pain and was admitted to hospital. Abdominal CT scan revealed a large infarction of the right lower kidney and a small infarct of the left kidney (Fig. 1). She received fresh frozen plasma infusion. On the following day abdominal pain disappeared, and she was discharged in improved condition on the 6th hospital day. Because of frequent occurrence of ischemic cerebral infarctions in USS, a cerebral CT and MRI were performed which revealed multiple older ischemic infarcts (Fig. 1). Thus, prophylactic plasma infusions each

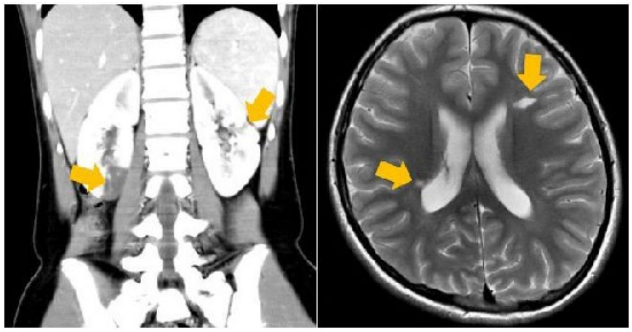

FIGURE 1 Abdominal CT and brain MRI at the time of onset

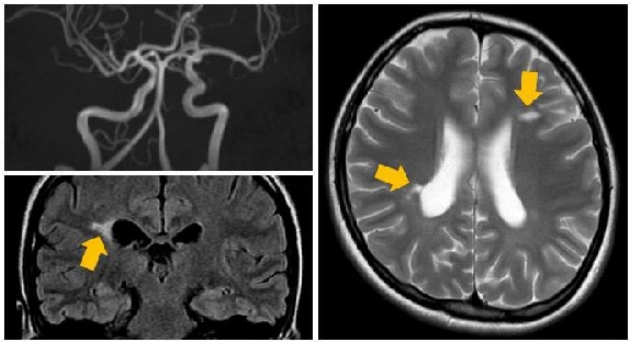

FIGURE 2 Reexamination of brain MRI after 3 years

every 2 weeks were started and so far 4 years passed without clinical symptoms and no new cerebral lesions in a control MRI (Fig. 2).

Conclusions: Whereas acute retroperitoneal pain led to the detection of kidney infarctions, the multiple cerebral ischemic infarctions had occurred largely without symptoms. This strongly suggests that regular prophylaxis to substitute the lacking ADAMTS13 should be considered.

PB1857 | Differential Diagnosis of Thrombotic Thrombocytopenic Purpura at Acute Phase by the Ratio of VWF Activity to VWF Antigen Based on Automatic Immunologic Assays

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Background: The absence of large von Willebrand factor (VWF) multimers in patients with thrombotic thrombocytopenic purpura (TTP) had been demonstrated in several studies, which can probably be explained by a sequestration of these large multimers within the microthrombi. This characteristic can be measured by electrophoresis of VWF multimers or some testing methods reflecting VWF activity, and thus might be useful for diagnosis and prognosis of TTP. However, many of these methods are time-consuming.

Aims: To evaluate the diagnostic value of the VWF activity / VWF antigen ratio for TTP at acute phase.

Methods: In a consecutive cohort of 71 patients with suspected TTP and admitting our hospital in 2019, we analysed the levels of VWF activity and VWF antigen at acute phase, the diagnosis of TTP was defined as ADAMTS13 activity < 10%. The VWF activity was measured by an automatic immunoturbidimetric assay, based on a specific anti-VWF monoclonal antibody directed against the platelet binding site of VWF (Glycoprotein Ibreceptor).

Results: All of these included patients manifested remarkable thrombocytopenia (< 50×10^9 platelets/L) and microangiopathic hemolytic anemia. Eventually, these patients were diagnosed as TTP (n = 22), hemolytic uremic syndrome (n = 9), disseminated intravascular coagulation (n = 18), megaloblastic anemia (n = 3), myelodysplastic syndrome (n = 1), and thrombotic microangiopathies secondary to-collagen disease (n = 8), malignancy (n = 7), organ transplantation (n = 2) and HELLP syndrome (n = 1).The ratio of VWF activity to VWF antigen was significantly lower in patients with TTP than in those without ($P < 0.001$), the median (interquartile range) were 0.69 (0.63-0.73) and 0.91 (0.78-1.04), respectively. The ratio of VWF activity to VWF antigen < 0.75 could yield a sensitivity of 90.9% and a specificity of 77.3% for TTP diagnosis.

Conclusions: The ratio of VWF activity to VWF antigen based on automatic immunologic assays might be useful for differential diagnosis of TTPat acute phase, especially when the ADAMTS13 assay is not available in time.

PB1858 | Prognostic Value of A Disintegrin and Metalloprotease with Thrombospondin Motif 13 in Patients with Severe Sepsis and Septic Shock

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Background: Despite a better knowledge of A Disintegrin and Metalloprotease with Thrombospondin Motif 13 (ADAMTS13), there is still a paucity of data regarding the prognostic value of ADAMTS13 in severe sepsis and septic shock.

Aims: ADAMTS13 level was evaluated as a predictor of mortality in patients with severe sepsis and septic shock, and compared with Acute Physiology and Chronic Health Evaluation II (APACHE II) scores.

Methods: This prospective observational study was conducted in the Medical and Surgical Intensive Care Units of King Saud University Medical City, King Saud University, Riyadh. A total of 84 patients aged 56.08 ± 18.18 years with severe sepsis and septic shock were recruited. Serial ADAMTS13 levels were determined at 24 intervals and APACHE II scores, hematological profiles, indices of organ hypo-perfusion, renal functions and coagulation profiles were recorded. Primary outcome was 30 days ICU mortality and secondary outcomes were its comparison with APACHE II score, length of ICU stay and use of vasopressor agents.

Results: The median ADAMTS13 levels were 336.65, 339.35 and 313.9, respectively. ROC analysis showed maximum area under

the curve for second ADAMTS13 (AUC = 0.760) compared with first (AUC = 0.660) and third samples (AUC = 0.707) and APACHE II scores (AUC = 0.662). Hypertension (53.6%) and diabetic mellitus (45.2%) were the commonest comorbidities. Patients were divided into low and high ADAMTS13 groups according to the best cut-off point. Mortality was high in the low ADAMTS13 level group [≤365 ng/ml, OR = 4.5] and was significantly associated with age, diastolic blood pressure, ADAMTS13, APACHE II score, DIC score and platelet count. ADAMTS13 (OR = 5.3), APACHE II (OR = 4.13) and DIC scores (OR = 7.32) were significant risk factors for mortality in multivariate stepwise logistic regression model.

Conclusions: Low ADAMTS13 levels are associated with increased mortality in patients with severe sepsis and septic shock and are comparable to APACHE II scores for predicting mortality.

PB1860 | Rituximab Prolongs the Time to Relapse in Patients with Immune Thrombotic Thrombocytopenic Purpura: Analysis of Off-Label Use in Japan

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Background: Immune thrombotic thrombocytopenic purpura (iTTP), a life-threatening disease caused by platelet thrombus in microvasculature, results from severe ADAMTS13 deficiency due to anti-ADAMTS13 autoantibodies. Rituximab (RTX) is an anti-CD20 monoclonal antibody often used to suppress anti-ADAMTS13 autoantibodies. In August 2019, RTX was approved for the treatment of refractory or relapsed iTTP by Japanese health insurance.

Aims: We evaluated the efficacy of RTX for off-label use in Japan.

Methods: Patients with confirmed severe ADAMTS13 deficiency (activity < 10%) and anti-ADAMTS13 inhibitors who experienced acute episodes of iTTP were enrolled from January 2006 to December 2018. Physicians assigned RTX treatment based on patients' resistance to conventional treatment. We compared the median time to relapse and relapse-free survival between RTX-treated and untreated groups. This study was approved by the ethics committee of Nara Medical University.

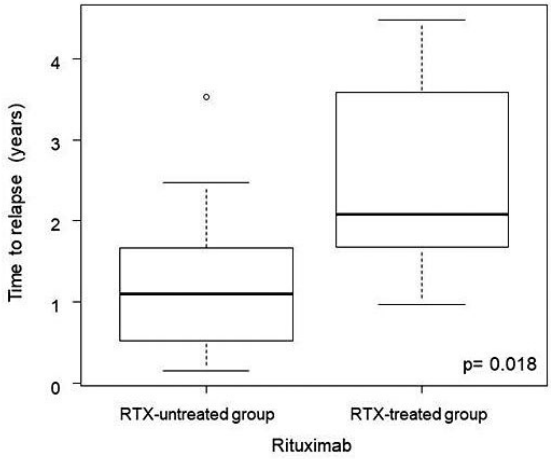

Figure 1. Median time to relapse in RTX-treated and RTX-untreated group. The median time to relapse was 2.1 years (range 1.0–4.5 years) in the RTX-treated group (n=7) and 1.1 years (range 0.2–3.5 years) in the RTX-untreated group (n=16). Mann-Whitney U test was used to compare the time to relapse between both groups.

FIGURE 1 Median time to relapse in RTX-treated and RTX-untreated group

Results: Among 158 patients with iTTP, 167 episodes were eligible. In the RTX-treated group, patients assessed as having resistance to treatment or relapse received RTX to treat 60 episodes (35.9%). In the RTX-untreated group (control), patients received conventional treatment to treat 107 episodes. RTX-treated patients had a significantly higher inhibitor titer compared with RTX-untreated patients (p = 0.002) (Table 1). Seven relapses (11.6%) in the RTX-treated group and 16 relapses (15.0%) in the RTX-untreated group were observed during a median follow-up period of 38.8 months (range 1.7-161 months). The median time to relapse was 2.1 years (range 1.0-4.5 years) in the RTX-treated group and 1.1 years (range 0.2-3.5 years) in the RTX-untreated group (Figure 1). Relapse-free survival at 2 years was significantly higher in the RTX-treated group compared with the RTX-untreated group (p = 0.04). The incidence of relapse at 5 years did not differ between groups (p = 0.35).

TABLE 1 Clinical characteristics and laboratory parameter of patients who received RTX and did not receive RTX

	RTX-treated group (n = 60)	RTX-untreated group (n = 107)	p-value
Age median (range)	50.5 (13-78)	59.0 (12-88)	0.10
Sex (Female : Male)	37:23	58:49	0.42
Type of Presentation (First-time : Relapse)	55:5	103:4	0.29
Hb, g/dl median (range)	7.7 (4.6-13.9)	7.9 (4.6-14.3)	0.36
Platelet, $\times 10^4$/μl median (range)	1.0 (0.4-6.3)	1.1 (0.1-7.4)	0.82
LDH, IU/L median (range)	1027 (209-4426)	980 (114-3024)	0.23
Cre, mg/dl median (range)	0.88 (0.47-12.9)	0.83 (0.35-11.4)	0.58
ADAMTS13 activity, % median (range)	<0.5 (<0.5-<0.5)	<0.5 (<0.5-3.2)	0.04
ADAMTS13 inhibitor, BU/mL median (range)	4.3 (0.8-17.2)	2.1 (0.5-20)	0.002

Conclusions: RTX appears to reduce the risk of relapse in patients with refractory or relapsed iTTP for 2 years.

PB1861 | Exploration of Coagulation Disorders, von Willebrand Factor and ADAMTS13 in a Cohort of 45 Patients with Hemophagocytic Lymphohistiocytosis

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare and life-threatening condition associated with multiorgan dysfunction. HLH is related to an inappropriate stimulation and proliferation of cytotoxic lymphocytes and macrophages inducing the secretion of cytokines leading to an uncontrolled hyperinflammatory response. Coagulation and fibrinolysis disorders are common in HLH patients. Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy (TMA) syndrome defined by a severe deficiency in ADAMTS13, the specific von Willebrand factor (VWF)-cleaving protease. Several cases of TMA syndrome associated to HLH have been reported.

Aims: To describe and correlate fibrinolysis disorders and VWF-ADAMTS13 system in patients with HLH.

Methods: Critically ill patients with a diagnosis of HLH were prospectively included in a monocentric study. An exhaustive analysis of clinical records was assessed. At diagnosis, fibrinolysis disorders were evaluated by the measurement of fibrinogen, D-dimers, fibrin monomers, plasminogen, t-PA and PAI-1. VWF-ADAMTS13 system was also investigated by the measurement of VWF antigen levels, VWF multimers analysis, ADAMTS13 activity, anti-ADAMTS13 IgG titer, ADAMTS13 antigen levels and ADAMTS13 conformation.

Results: Forty-five patients with HLH were included in the study: median age of 53 years, sex ratio of 1.6F/1M, clinical context of lymphoma in 32 patients. In most patients, hypofibrinogenemia, decreased plasminogen levels, increased D-dimers, fibrin monomers, t-PA and PAI-1 levels were consistent with hyperfibrinolysis. All patients exhibited increased VWF antigen levels and high molecular weight multimers. Surprisingly, a non-immune-mediated severe functional deficiency of ADAMTS13 (activity < 15%, negative anti-ADAMTS13 IgG) associated with a closed ADAMTS13 conformation (conformation index < 0.5) was reported in 16 patients who also exhibited thrombocytopenia, anemia, increased LDH levels. No correlation was found between hyperfibrinolysis and VWF-ADAMTS13 abnormalities.

Conclusions: We reported hyperfibrinolysis in most patients with HLH at diagnosis and a potential TTP-like syndrome in 1/3 of them. The study of degradation products of ADAMTS13 may be warranted to understand the loss of ADAMTS13 activity.

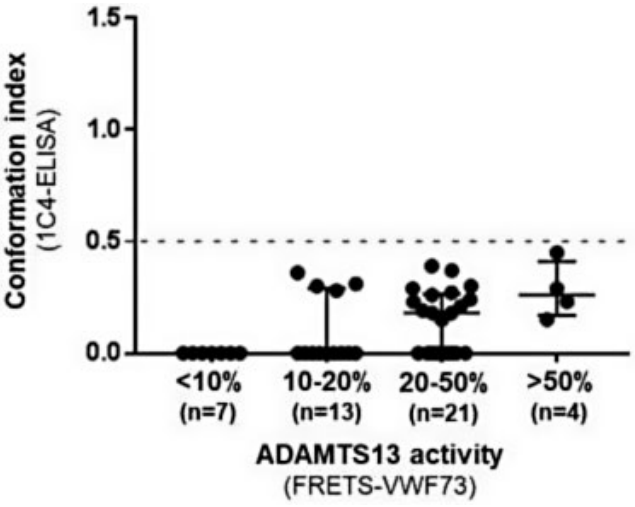

FIGURE 1 ADAMTS13 activity and conformation in 45 patients with hemophagocytic lymphohistiocytosis, at diagnosis

PB1862 | Automated Chemiluminescent ADAMTS13 Activity Immunoassay for Thrombotic Thrombocytopenic Purpura Diagnosis and Follow-up

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare, life-threatening thrombotic microangiopathy defined by a severe functional deficiency (activity < 10%) in ADAMTS13, the specific Von Willebrand factor cleaving protease. ADAMTS13 activity is a crucial assay: firstly, evidence of ADAMTS13 activity < 10% is mandatory to definitely diagnose acute TTP; secondly, monitoring of ADAMTS13 is likely to become essential to guide caplacizumab therapy in acute TTP; thirdly, monitoring of ADAMTS13 during follow-up in remission is useful to early detect TTP relapses requiring preemptive rituximab. Testing for ADAMTS13 activity remains currently performed in expert laboratories as it requires advanced technical skills and expertise. The prospect of a reliable automated, time-sparing technique could significantly improve the management of TTP and spare care resources.

Aims: We investigated whether a new fast, fully-automated chemiluminescent immunoassay could assess ADAMTS13 activity when compared to the FRETS-VWF73.

Methods: 270 citrated plasma samples were tested. ADAMTS13 activity had previously been assessed by FRETS-VWF73 (reference method) for all samples (range: < 10-150%). Analytic performances were extensively evaluated: detection limit, linearity, intra- and inter-assay precisions. Correlation with ADAMTS13 activity measured by FRETS-VWF73 was carried out, as was agreement between methods regarding ADAMTS13 activity < 10%, threshold for the diagnosis of TTP. Specific attention was given to the performances of this method in the 10-30% range, critical for both optimal monitoring of caplacizumab therapy and for preemptive strategies throughout follow-up.

Results: Analytic performances were highly satisfactory (limit of detection < 0.2%, intra- and inter-assay precisions < 5%). This technique showed good correlation with FRETS-VWF73 including within the 10-30% range (Fig. 1) and excellent agreement with FRETS-VWF73 for assessing ADAMTS13 activity < 10%, i.e. for the diagnosis of TTP (Table 1).

Conclusions: This new technique appears highly reliable and implementable for the diagnosis and monitoring of TTP. Real life experience data are now needed to further validate this method.

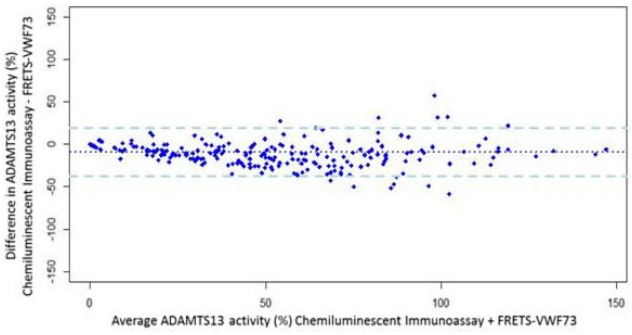

FIGURE 1 Bland-Altman plot of ADAMTS13 activity (%) comparing the Chemiluminescent Immunoassay and the FRETS-VWF73. Mean bias: -9.45%, LL: -37.76%, UL : 18.87

TABLE 1 Agreement between the Chemiluminescent Immunoassay and FRETS-VWF73 for assessing ADAMTS13 activity < 10% i.e. TTP diagnosis

	FRETS-VWF73 ADAMTS13 activity	
	< 10% (n = 64)	*> 10% (n = 206)*
Chemiluminescent Immunoassay ADAMTS13 activity		
< 10% (n = 62)	60	2
> 10% (n = 208)	4	204
Sensitivity	93.75%	
Specificity	99.03%	
Positive Predictive Value	96.77%	
Negative Predictive Value	98.07%	
Kappa Coefficient	0.938	

PB1863 | Caplacizumab in a Case of Acquired Thrombotic Thrombcytopenic Purpura (TTP) Poor Responsive to Plasma Exchange and Immunosuppressive Therapy

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Background: Acquired thrombotic thrombocytopenic purpura (TTP) is a rare and life-threatening condition. Until recently plasma-exchange (PE) and immunosuppressive therapy represented the only available therapeutic strategy, but not always are effective in preventing complications and mortality. Caplacizumab is an humanized immunoglobulin anti-von Willebrand factor (vWF), able to inhibit interaction between vWF multimers and platelets.

Aims: To describe caplacizumab effectiveness in a non-responsive patient, previously treated with several PE and immunosuppressive therapy.

Methods: Case Report.

Results: A 56 years female patient was admitted to emergency department forhigh suspicious of TTP, showing Plasmic Score = 7/7. PE and steroid therapy were promptly started, but platelet response was scare and transient (day 5th from starting PE: 42.000/mm^3; day 7th: 9000/mm^3).She therefore developed neurological symptoms (epileptic crisis and loss of consciousness) that required admission to intensive care unit for assisted ventilation.ADAMTS-13 activity was < 3%, with high title of enzyme inhibitor (> 93U/mL), therefore Rituximab was started and Caplacizumab, not yet registered in Italyat that time,was requestedas "expanded access program" and administered on day 16th. Platelet raised from 10.000/mm^3 on day 15th to 254.000 on day 22th. PE was withdrawn on day 26th while Caplacizumab was maintained since ADAMTS-13 activity was still < 3%. The patient was extubated on day 31th and discharged from ICU on day 33th. Rituximab therapy was extendedup to six administrations. On day 34th the patient showed signs of cutaneous andgastroenteric bleeding, without significant anemia.No invasive procedureswere performed before stopping Caplacizumabafter obtaining a stable ADAMTS-13 activity (22%). A gastroscopy (day 54th) showed hyperemic gastritis. The patient was discharged from Hospital on day 65th.

Conclusions: This case showed safety and effectiveness of Caplacizumab in acquired TTP poor responsive to standard therapy. No major bleedings, according to ISTH definition, were observed. ADAMTS-13 measurements supported diagnosis and therapy management.

PB1864 | Current Possibilities of ADAMTS 13 Diagnostics - Advantages and Limitations

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Background: The time when TTP was diagnosed mainly by clinical findings ended with the implementation of ADAMTS13 activity below 10% as a diagnostic criterion.

We currently have reliable ELISA methodologies for both ADAMTS13:ac and ADAMTS13:inh determination, new chemiluminescence methods (CLIA) and a rapid screening test.

Aims: The aim of our work was to assess the applicability of methods in real practice in terms of availability for individual workplaces, time demands and quality of results.

Methods: 21 assumed TTP samples were examined by all methods and the results were evaluated. We used ELISA methods to determine ADAMTS13: ac and ADAMTS13: inh as a gold standard for comparison with CLIA and screening test. Inhibitor determination in the CLIA method was performed according to Bethesda methodology and the screening test was supplemented with an instrument detection.

Results: 21 results were evaluated for the effectiveness of the examination. There was a consensus diagnosis using ELISA and CLIA methods in all samples, only in the obtained TTP it was necessary to complete the incubation step for Bethesda inhibitor determination in CLIA method.

The inconsistency of the screeng samples was suppressed by the implementation of the instrumentation measurement of the colometric reaction.

Conclusions: TTP diagnostics using the ADAMTS13 activity assay and inhibitor remains a challenging laboratory issue and requires considerable workplace experience and personnel training. However, new technologies open up the possibility of diagnosing TTP to a wider range of sites. However, the procedures should be validated in detail and the diagnostic procedure carefully evaluated.

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PB1865 | Relapsing or Refractory Thrombotic Microangiopathies: The Key Role of ADAMTS13 Activity and Complement Testing

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Background: Refractory and relapsing thrombotic microangiopathy (TMA) is characterized by increased morbidity and mortality. Physicians are reluctant to use novel efficacious treatments, such as caplacizumab or eculizumab, due to increased cost and lack of validated laboratory testing in many hospitals.

Aims: We evaluated ADAMTS13 activity and complement testing in refractory and relapsing TMA patients.

Methods: We enrolled consecutive TMA patients with samples referred to our Center (01/2018-2020). Severe ADAMTS13 deficiency

(< 10%) established thrombotic thrombocytopenic purpura (TTP) diagnosis. ADAMTS13 was also measured in refractory and relapsing patients. If ADAMTS13 > 10%, complement testing was performed in patients with an indication for complement inhibition. ADAMTS13 activity and the complement marker soluble C5b-9 were measured with commercially available ELISA kits (Technozym and Quidel, respectively). Clinical data were retrospectively collected from referring centers, responsible for treatment decisions.

Results: We studied 80 TMA patients. The majority of patients with relapsing TTP (25/28) had already received rituximab treatment and presented with ADAMTS13 < 10% at relapse. During remission, 6/10 patients with available measurements had ADAMTS13 < 50% and 2/10 ADAMTS13 < 20%, suggesting an imminent relapse.

Patients with secondary TMAs (ADAMTS13 > 10%) received etiologic treatment, achieving TMA resolution. C5b-9 levels were elevated (median 525 ng/ml, range 313-913 ng/ml) in patients without secondary causes (ADAMTS13 > 10%); confirming HUS diagnosis. Among HUS, 2 were shiga-toxin associated and resolved with symptomatic treatment in pediatric patients. In atypical HUS, only 1/5 patients received eculizumab and achieved TMA resolution. The other 4 patients succumbed to TMA-related complications. In transplant-associated TMA, 8/16 patients not responding to first-line treatment received eculizumab due to elevated C5b-9 levels (median 253 ng/ml, range 281-1252 ng/ml) and achieved TMA resolution.

Conclusions: Our real-world data confirm that ADAMTS13 and complement testing are valuable tools in relapsing and refractory TMAs, but also highlight the unmet need of using available novel prognostic tools and treatments in clinical practice.

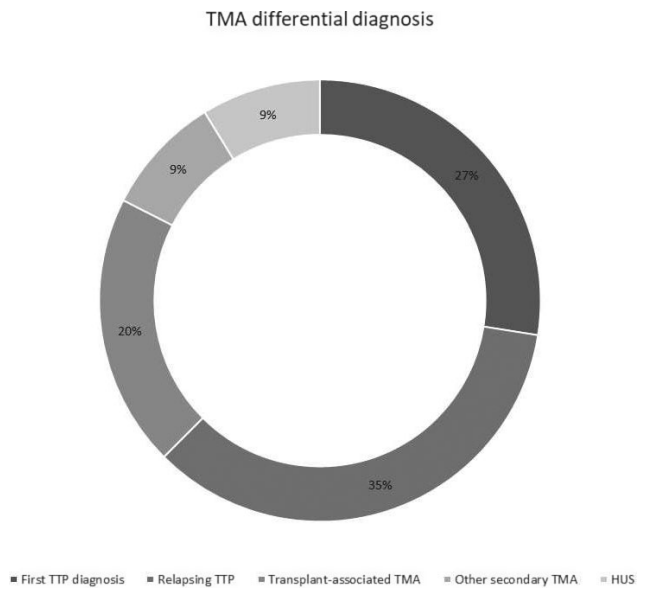

FIGURE 1 TMA differential diagnosis

PB1867 | Clinical Analysis of 83 Patients with Thrombotic Thrombocytopenic Purpura

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare thrombotic microangiopathy characterized by severe thrombocytopenia, microangiopathic hemolytic anemia, and organ ischemia ascribable to platelet-rich microthrombi. TTP is related to a severe deficiency in ADAMTS13,the specific von Willebrand factor-cleaving metalloprotease.

Aims: To analyze the clinical characteristics, treatment and outcome of patients with TTP.

Methods: We retrospectively analyzed 83 patients with TTP treated in Chinese Academy of Medical Sciences Hematology Hospital from 1998 to 2019.

Results: Among 83 patients, there were 27 males and 56 females, with a median age of 39 (10~68) years. 41 cases showed pentad sign and 79 cases showed triple sign. 78.0% (46 / 59) of the patients had a PLASMIC score of ≥ 6. TTP gene mutation was detected in 5 of 10 patients. The activity of ADAMTS13 was detected in 10 patients before plasma exchange (PEX), 9 patients had an ADAMTS13 activity level ≤ 10%. 83 patients were treated with PEX/ plasma infusion+glucocorticoid, or combined with rituximab or immunosuppressants. The overall survival rate was 78.3%, the effective rate was 81.9%, the remission rate was 63.9%, and the recurrence rate was 21.7%. There was significant difference in survival rate among different treatment groups. Compare the survival group with the death group, there were significant differences in age, hemoglobin, glutamic pyruvic transaminase / glutamic oxaloacetic transaminase, lactic dehydrogenase, total bilirubin, free hemoglobin, haptoglobin and severe neuropsychiatric symptoms. Age and severe neuropsychiatric symptoms were independent factors affecting the prognosis of TTP.

Conclusions: The diagnosis of TTP needs to be obtained by combining a variety of clinical data. The detection of TTP gene and ADAMTS13 is helpful to the diagnosis of the disease. Plasma therapy

Treatment	Number of patients	Response			Outcome		Remission		Relapse	
		Complete response	partial response	unresponse	survival	death	Yes	No	Yes	No
PEX/PI+ glucocorticoid	48	34	1	13	32	16	29	19	9	39
PEX/PI+ glucocorticoid + rituximab	11	11	0	0	11	0	8	3	4	7
PEX/PI+ glucocorticoid + immunosuppressants	20	16	2	2	18	2	14	6	4	16
PEX/PI+ glucocorticoid + rituximab + immunosuppressants	4	4	0	0	4	0	2	2	1	3

FIGURE 1 Treatment and outcome of 83 patients with TTP. PEX, plasma exchange; PI, plasma infusion

combined with glucocorticoid is the first choice, which combined with rituximab and immunosuppressant therapy can reduce mortality. Age and severe neuropsychiatric symptoms contribute to the prediction of clinical outcome.

PB1868 | PLASMIC Score Predicts Outcome of Transplanted Patients with Severe Thrombotic Thrombocytopenic Purpura

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Background: Thrombotic thrombocytopenia purpura (TTP) still presents a significant clinical challenge for physicians and despite increased awareness TTP remains a serious life-threatening illness with current mortality rates estimated at 10%-20%. The recently introduced PLASMIC score enables rapid identification of TTP based on readily available clinical and was suggested to correlate with patient outcome. However, additional validation of this practical clinical prediction tool is warranted to determine its applicability in prediction of patient outcome.
Aims: Determine whether the PLASMIC score correlates with outcome of TTP patients as a whole and specifically in patients who experienced TTP following a prior allogeneic stem cell transplantation (allo-SCT).
Methods: This was a retrospective analysis consisting of all consecutive adult TTP patients treated in the Chaim Sheba Medical Center in 2008-2018. Baseline clinical and laboratory data were analyzed for the cohort as a whole and then specifically for patients who underwent a prior allogeneic stem cell transplantation (allo-SCT).
Results: In all, our cohort consisted of 89 patients with a median age of 53 years (range 18-73 years), 52 patients (58%) underwent a prior allo-SCT. Plasma exchange therapy was initiated in 32 patients (36%), rituximab was given to 16 patients (18%), and aspirin was administered to 12 patients (14%). Transplanted patients were significantly more likely to experience relapse and TTP related mortality compared to non-transplanted patients, 74% vs. 46% (p = 0.013), and 81% vs. 28% (p < 0.0001), respectively. Transplanted patients had a significantly lower mean PLASMIC score compared to non-transplanted patients, 4.76 versus 5.5 (p < 0.0001) whereas patients with lower PLASMIC scores experienced increased TTP related mortality only in the post-transplant setting (p = 0.001).
Conclusions: Our findings reaffirm the prognostic role of PLASMIC score in predicting outcome of TTP patients including in the setting of post-transplant TTP.

PB1869 | Pregnancy and Non-Pregnancy Related Acquired Thrombotic Thrombocytopenic Purpura in Women of Reproductive Age: Clinical Course and Outcomes

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Background: Acquired thrombotic thrombocytopenic purpura (TTP) is commonly encountered among women of reproductive age, with pregnancy as a known precipitating factor for its occurrence.
Aims: Given the paucity of literature, we aimed to investigate the effect of pregnancy on the clinical course and outcomes of acquired TTP in this subset of patients.
Methods: A multicenter retrospective review of all reproductive-age women diagnosed with acquired TTP during 2006-2019 in six university hospitals in Israel.
Results: Overall, 42 women of childbearing age were diagnosed with acquired TTP during study period (confirmed by ADAMTS13 activity < 5% coupled with the presence of anti-ADAMTS13 antibodies). Twelve (28.6%) cases were pregnancy-related. At the time of presentation, the laboratory profile including hemoglobin (median 8.4 vs 8.0 g/dL, P = 0.69), platelet count (12,000/mm^3 vs. 12,500/mm^3, P = 0.76), and levels of bilirubin (1.6 vs. 1.8, P = 0.36), lactate dehydrogenase (1615 U/L vs. 1702 U/L, P = 0.59), and creatinine (0.83 mg/dL vs. 0.86 mg/dL, P = 0.76) did not differ in those with pregnancy- and non-pregnancy related TTP. The proportion of women with either renal, neurologic, cardiovascular or hepatic involvement was similar between the groups (91.6% vs. 86.6%, P = 0.99). The number of courses of plasma-exchange therapy (median 5 vs. 5, P = 0.25) was also comparable between the groups. Parenteral corticosteroids were used in all cases, and similar proportion of women in both groups received other adjunctive treatments (33.3% vs. 56.6%, P = 0.30). While no mortality cases were observed in those with non-pregnancy related event, two cases (16.7%) with pregnancy-related TTP died during the acute episode.
Conclusions: This historical cohort describing the course and outcomes of acquired TTP among reproductive-aged women demonstrates that while the clinical and laboratory characteristics might be similar regardless of the relation to pregnancy, the management of pregnancy-related TTP continues to be challenging with a considerable rate of mortality.

PB1870 | Retrospective Study of 30 TTP Patients from 2017 to 2019 in a Single Center of Suzhou, China

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Background: Thrombocytopenic purpura (TTP) is a thrombotic microangiopathy, characterized by severe deficiency in ADAMTS13. Clinical manifestations include microangiopathic hemolytic anemia, peripheral thrombocytopenia, and multiple organs dysfunction.
Aims: We retrospectively analyzed the clinical characteristics, treatments and outcomes, and follow-up of 30 TTP patients admitted to our center from 2017 to 2019.
Methods: We collected the data of clinical symptoms, laboratory tests, and therapies of TTP patients, then evaluated the treatments outcome and followed up these patients.
Results: In thirty TTP patients, eighty percent had rental dysfunction, presenting increased creatinine levels, proteinuria or microscopic hematuria. Two or more organs were affected in more than 90% of patients. PLSMIC score were calculated in 27 patients with TTP. Twenty-six patients were in the high-risk group, and only one patient had a score of 5 in the intermediate risk group. Infections were the most common complications in TTP patients. Pulmonary infection was the most common, accounting for more than 40%.
The first line treatments for iTTP include PEX, glucocorticoid and rituximab. Cyclosporine and bortezomib are second line drugs. N-acetylcestine is also used as complementary therapy. Two patients were treated with bortezomid and obtained CR. One was refractory TTP patients, with no response to rituximab and cyclosporine.
All TTP patients obtained complete remission (CR), and no patient died in the initial episode of TTP. Only one patient relapsed after 2 months of discharge, and got CR again after plasma confusion. During the followed-up, 12 (63.2%) patients with acquired TTP discontinued any drugs for TTP 6 months after discharge. One patient died of severe pneumonia related to pneumocystis carinii infection.
Conclusions: TTP is a systemic disease with multiple organs dysfunction, which most likely affected kidney function. PLSMIC score system was applicable to Chinese TTP patients. Infections occurred in the whole course of TTP, especially after the use of immunosuppressive agents.

TABLE 1 Clinical charater of 30 TTP patients

Item	TTP
Total number	30
Sex (case)	
male	15
female	15
Age (median, range)	44 (14-70)
Classification (case)	
cTTP	2
aTTP	28

TABLE 2 Complications in TTP patients

Complication	Case
Infection	22
Autoimmune diseases	8
Venous thrombosis	4
Miscellaneous	3
Total	28

PB1871 | Development and Validation of a Prediction Model (AHC) for Early Identification of Refractory Thrombotic Thrombocytopenic Purpura Using Nationally Representative Data

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Background: Acquired thrombotic thrombocytopenic purpura (TTP) is a rare and life-threatening hematologic emergency. Despite proven efficacy of therapeutic plasma exchange plus corticosteroids, a considerable number of patients remain refractory to this treatment and require early intensive therapy. However, a method for the early identification of refractory TTP is not available.
Aims: To develop and validate a model for predicting the probability of refractory TTP.

TABLE 1 Multivariable logistic regression model of the predictors of refractory TTP

	β	SE	OR (95% CI)	P value	Points
Age ≥ 45 (years)	3.79	1.15	44.24 (4.63, 422.61)	0.001	2
Hemoglobin < 60 (g/L)	3.89	1.11	48.73 (5.49, 432.59)	<0.001	2
Creatinine ≥ 106 (μmol/L)	2.09	0.71	8.12 (2.04, 32.41)	0.003	1

Methods: The model was based on 265 acquired TTP patients with severe ADAMTS13 deficiency from 17 different tertiary medical centers between Jan 2009 and Nov 2019 in China. They covered four main Chinese geographic regions: Northern China, Eastern China, Middle & Western China and Southern China. We externally validated the model by geographical validation.

Results: In the multivariable logistic analysis of derivation cohort, three candidate predictors entered the final prediction model: age ≥ 45 years, hemoglobin < 60 g/L and creatinine ≥ 106 μmol/l. It was named AHC on the basis of the first letters of the included parameters. We assigned point values to these items according to the β coefficient obtained from the multivariable regression (table 1). We defined three categories of risk for refractoriness: an AHC score of 0 denoted low risk, a score of 1 or 2 denoted intermediate risk, and a score of 3-5 denoted high risk. There was increasing risk of refractory TTP with increasing AHC risk group (figure 1). AHC model had an AUC of 0.862 (95% CI 0.643-0.943) in the internal validation cohort and 0.818 (95% CI 0.611-0.947) in the external validation cohort. The calibration plots showed a high agreement between the predicted and observed probability.

Conclusions: We developed and validated an accurate prediction model for the early identification of refractory TTP at diagnosis. The AHC model contributes to guide tailored therapy and is a step towards more personalized medicine.

PB1872 | Follow-up Evaluation of Patients with Hereditary Thrombotic Thrombocytopenic Purpura (hTTP) from the International Hereditary TTP Registry: Incidence of Acute Disease Episodes with or without Regular Prophylactic Plasma Infusions

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Background: Hereditary Thrombotic Thrombocytopenic Purpura (hTTP) is an ultra-rare autosomal-recessively inherited disorder characterized by recurring acute episodes of thrombotic microangiopathy. Patients have a variable disease course and little is known about the annual rate of acute episodes.

Aims: This study reports the incidence of acute episodes in hTTP patients enrolled in the International Hereditary TTP Registry during follow-up.

Methods: At the end of 2019, we had enrolled 139 confirmed hTTP patients. Prospective follow-up data of 87 hTTP patients from enrollment until 12/2019 were analyzed.

Results: The 87 hTTP patients were followed for 4.2 years (median; range 0-15 years). The median age at overt disease onset and at

clinical diagnosis was 4.6 years (range 0-70) and 18 years (range 0-70), respectively.18 female and 25 male patients received regular prophylactic plasma infusions, 11 females and 14 males did not. Prophylaxis was temporarily withheld in 12 females and 6 males (Figure).

109 acute episodes were reported during follow-up in 41/87 patients: 74 in patients with and 35 in patients without prophylaxis. This results in annual episode rates/patient-year of 0.44 (95%CI 0.33-0.58) and 0.16 (95%CI 0.10-0.24) in female and male patients under prophylaxis, respectively; and of 0.38 (95%CI 0.24-0.57) and 0.23 (95%CI 0.12-0.41) in females and males not under prophylaxis, respectively.

We prospectively followed 13 pregnancies in 5 patients: 6 in 3 women under plasma prophylaxis installed before pregnancy, and 7 in 2 women receiving plasma infusion only during pregnancy. Five acute episodes occurred resulting in one abortion.

Conclusions: Current prophylactic plasma infusions (about 1-3 plasma units every 1-4 weeks) are often insufficient to prevent acute episodes. Patients under prophylaxis have a similar incidence of acute episodes as those not under prophylaxis. Females seem to suffer more frequent acute episodes than males, not attributable to pregnancy alone. Further prospective follow-up will provide robustness of our estimates.

PB1873 | Multicentric Multicountry Evaluation of the Fully Automated Adamts13 Activity Assay

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Background: Inmune thrombotic thrombocytopenic purpura (iTTP) is a rare life- threatening thrombotic microangiopathy (TMA) characterized by the severe deficiency of ADAMTS13 activity (< 10%). Rapid ADAMTS13 testing is key for early diagnosis and optimal management of TTP patients and other TMAs. A fast chemiluminescence technique for the determination of ADAMTS13 activity has just been commercialized.

Aims: To evaluate the performance of the rapid, fully automated HemosIl ® Acustar ADAMTS13 activity assay.

Methods: A retrospective, multicentric, multicountry study was performed from july-october/2019 to compare the new chemiluminescence assay (CLIA) with an in-house FRETS-VWF73 assay and two commercial ELISA assays (Technoclone and DG-EIA).

239 frozen samples from 215 patients with TMA with known ADAMTS13 activity were tested (114 diagnostic and follow-up iTTP samples, 3 congenital TTP, 16 HUS and 82 samples from other TMAs). Sample and reagents distribution between centers is shown in table1. Agreement between methods was assessed by Passing-Bablok regression with focus on ADAMTS13 activity < 10%.

Results: The CLIA showed good correlation with FRETS-VW73 (r 0,94), Technoclone-ELISA (r 0,94) and DG-ELISA (r 0,87) assays.

Slope of the Passing-Bablok regression was 1,12 for FRETS-VWF73, 1,13 for Technoclone-ELISA and 1,26 for DG-ELISA (Fig-1). Agreement was lower in high ADAMTS13 activity values.

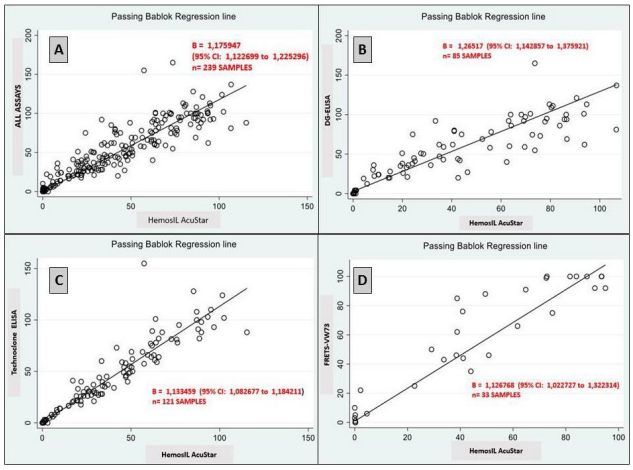

FIGURE 1 Agreement ADAMTS13 activity by all assays (A), DG-ELISA (B), Technoclone ELISA (C) and FRETS-VW73 (D)

TABLE 1 Samples distribution by center and by assay

Hospital Center	Local ADAMTS13 activity assay	ADAMTS13 CLIA beta Kit n= 123	ADAMTS13 CLIA Comercial Kit n= 116	Total samples number n= 239
HGUGM	HGUGM DG- ELISA	34	33	67
CHUAC	HGUGM DG- ELISA	18		18
HUCSC	TECHNOCLONE- ELISA	18	35	55
COIMBRA HOSPITAL	TECHNOCLONE- ELISA	16	17	33
LA FE HOSPITAL	TECHNOCLONE- ELISA	17	16	33
CLINIC HOSPITAL	FRETS-VW73	20	13	33

The study also revealed high agreement with FRETS-VWF73 (kappa 0.92), Technoclone-ELISA (kappa 0.93) and DG-ELISA (Kappa 0,86) in classifying patients with ADAMTS13 activity < 10% (TTPs).

Conclusions: This is the first validation of the commercial version of the HemosIL AcuStar ADAMTS13 activity assay.

This assay showed high agreement with other laboratory methods, especially with FRETS-VWF73 and Technoclone-ELISA. Because of its short turnaround time and full automation, it might become the assay of choice to rapidly test ADAMTS13 and thus establish the diagnosis of acute iTTP in emergency settings. A prospective validation study would be necessary to evaluate the discrepancies found between the activity assays.

PB1874 | Genetic Study of von Willebrand Factor Variants in Congenital Thrombotic Thrombocytopenic Purpura Patients

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Background: Von Willebrand factor (VWF) is a glycoprotein that plays a critical role in haemostasis and thrombosis. The size of VWF multimers is regulated by ADAMTS13, which cleaves VWF at single peptide bond, Y1605-M1606, in the A2 domain. The VWF is encoded by a large and polymorphic gene in which many polymorphisms have been reported as thrombogenic.

Aims: This observational study investigated the presence of polymorphic variants in VWF gene in congenital Thrombotic Thrombocytopenic Purpura (cTTP).

Methods: DNA was extracted from EDTA samples of 63 cTTP patients from the UK TTP Registry. VWF exons from 28 to 32, and exon 45, were amplified by polymerase chain reaction (PCR), with corresponding introns primers. PCR products were sequenced by GENEWIZ and sequences data were analysed using Sequencher 5.4.6 (Gene Codes Corporation).

Results: The study population was composed by 75% (n=47) female and 25% (n=16) male, median age 34 years (range: 2-74). The investigated exons correspond to the A1, A2, A3 and C4 domains, where binding sites for GPIb, ADAMTS13, collagen and GPIIb/IIIa are respectively located. VWF polymorphisms were identified in 47.6% (n=30) cTTP patients: two missense variants in exon 28, both located in A1 domain, p.T1381A (rs216311) and p.D1472H (rs1800383) and one in exon 45, p.F2561Y (rs35335161). Sixteen percent of patients (n=10) T/T1381 homozygotes and 46% (n=29) A/T1381 heterozygotes were identified. The D1472H and F2561Y heterozygous variants were detected respectively in 28.6% (n=18) and 6% (n=4) of patients.

Conclusions: The homozygous T/T1381 variant has been already investigated to enhance the affinity of VWF for the platelet receptor GPIbα. Similarly, the F2561Y variant has been demonstrated to increase platelets aggregates size. The D1472H polymorphism has shown to decrease ADAMTS13 cleavage. In conclusion, VWF variants were a common finding in cTTP cases and may correlate with an increased thrombotic risk for cTTP patients.

PB1875 | Patients with Acute Thrombotic Thrombocytopenic Purpura (TTP) Should Be Managed in a Critical Care Setting - A UK Single TTP Referral Centre Experience

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Background: TTP is a thrombotic microangiopathy caused ADAMTS13 deficiency, leading to microvascular thrombosis and critical organ ischaemia.

Aims: There is a growing consensus that patients presenting with TTP should be admitted to the intensive care unit (ICU), we aim to evaluate our cohort.

Methods: A retrospective review of patients admitted with acute TTP to a regional specialist centre (June 2016-December 2019) was conducted. Twenty four patients had acute TTP over this 42 month period. All patients had an ADAMTS13 < 10% at presentation.

Results: The mean age was 51 years and 67% of patients were female. 21/24 patients were admitted directly to ICU. In 6/24 episodes, patients required intubation, with the indication in all cases being reduced conscious level/seizures. Of those patients requiring intubation, 3 were intubated prior to transfer to the centre and 3 within 24 hours of admission (mean time to intubation=7.8 hours).

In 19/24 episodes, a central line was inserted for plasma exchange. The median platelet count at the time of line insertion was $15x10^9$/L (range 8-$59x10^9$/L); 1 report of slight oozing, with no other bleeding complications reported. There were 15/24 episodes of raised troponin, 12/24 acute kidney injury and 14/24 neurological symptoms on admission. The median duration of ICU stay was 6.5 days (range 2-25 days). There was 1 patient death due to cardiac arrest.

Conclusions: Acute TTP patients are at high risk of sudden deterioration. Our data highlights at least 25% require intubation either prior to or shortly after acute transfer. Central venous line placement in ICU, despite profound thrombocytopenia, appeared to be safe. Despite the number requiring organ support at presentation, >95% patients were successfully stepped down from ICU and discharged from hospital. These findings support recommendations that patients with acute TTP should be admitted directly to ICU, and provide a platform for local and national policy frameworks.

PB1876 | Performance Evaluation of a New Fully Automated Fluorogenic ADAMTS13 Activity Assay

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Background: ADAMTS13 activity is a unique marker for thrombotic thrombocytopenic purpura (TTP). Thus, laboratory assays must be especially accurate and precise in the pathological range (below 0.1IU/mL). Additionally, a rapid TTP diagnostic improves patient management and clinical outcomes, which is a challenge with most currently available assays. The automated method presented herein will help the clinician to easily and accurately assess ADAMTS13 activity in 25 minutes.

Aims: To demonstrate the performance characteristics of a rapid and fully quantitative automated ADAMTS13 activity assay suitable for use in routine laboratories.

Methods: The ADAMTS13 activity of 35 samples, including 2 TTP cases and 2 ADAMTS13 depleted plasmas, was assessed using a chromogenic ELISA method and the new automated method employing a lyophilized FRETS-VWF73 Fluorescence-Quenching Substrate on the new automated analyzer.

Results: Calibrators supplied with the assay span the range 0.0-1.01 IU/mL and are traceable to WHO international standard 12/252. Using samples with ADAMTS13 activity above 1.01IU/mL diluted in ADAMTS13 deficient plasma, linearity of the calibration curve within and beyond that range was calculated as >98%. The analytical sensitivity of the assay exhibited a LOB of 0 IU/mL and the LOD at 0.008 IU/mL using the standard assay sample dilution.

Correlation of the automated FRETS assays with the established ELISA assay for 35 samples was linear (r>0.95) with an intercept of < 0.01IU/mL. Samples with an activity above the 1,01IU/mL were diluted with an ADAMTS13 deficient plasma..Using samples with level spanning the measuring range 0.02-1.01IU/mL, imprecision was determined to be < 5%.

Conclusions: This performance evaluation study evidences that the FRETS assay performed on the automated analyzer enables quick and accurate assessment of ADAMTS13 activity levels suitable for use in clinical diagnostic laboratories.

PB1877 | Efficacy of Preemptive Subcutaneous Rituximab in Immune-Mediated Thrombotic Thrombocytopenic Purpura: A Study of 12 Cases

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Background: Immune thrombotic thrombocytopenic purpura (iTTP) is a life-threatening disease mainly caused by immune-mediated severe deficiency in ADAMTS13. During clinical remission, persistently ADAMTS13-activity < 10% is highly associated to clinical relapse. Rituximab is currently used as preemptive treatment. Recently, a subcutaneous (SC) formulation of rituximab has been approved in lymphomas. This formulation has not been evaluated in iTTP.

Aims: Efficacy and tolerance of preemptive SC rituximab treatment in patients in clinical remission of iTTP, displaying severe ADAMTS13-activity deficiency < 10% during follow-up or at TTP onset.

Methods: Prospective monocenter study. All enrolled patients received 1400mg SC rituximab. Clinical (clinical relapse rate, number of rituximab administrations to maintain a detectable ADAMTS13 activity and adverse events) and biological (ADAMTS13 activity and CD19+ B-lymphocytes count) data were collected after 1-month and then every 3 months follow-up, and expressed as median (25th - 75th percentile).

Results: We enrolled 10 women and 2 men (43 years old, 34-48) between Aug.2015 and Sep.2019. All patients had a unique SC rituximab administration. One month after administration, ADAMTS13-activity was of 67% (44-89% n=12), with normal activity in 6 cases. B-lymphocytes were undetectable in all cases. After 3 months, ADAMTS13-activity was of 66% (64-82%) and all patients had a detectable activity. After 9 months (5-18 months), 11 patients displayed detectable ADAMTS13-activity without further rituximab administration. One patient had a second administration of SC rituximab after 2 years, due to an undetectable ADAMTS13 activity during follow-up. Another patient was unresponsive after 4 rituximab intravenous perfusions, and had SC rituximab every 2 months during 22 months with subsequent a persistently detectable ADAMTS13 activity. No patient presented clinical relapse. No safety concerns were noted.

Conclusions: Preemptive SC rituximab seems to have similar efficacy than intravenous perfusion as a preemptive treatment for iTTP and

would provide greater comfort for patients, notably when repeated administrations are needed.

PB1878 | Risk Factors Associated with Severe Neurological Symptoms in Thrombotic Thrombocytopenic Purpura (TTP)

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Background: Neurologic complications are present in up to 60% of thrombotic thrombocytopenic purpura (TTP) episodes and contribute to adverse clinical outcomes and death. Although the central nervous system is the leading target organ in TTP, risk factors associated with neurological complications are not established.

Aims: To evaluate parameters associated with severe neurological complications during TTP episodes. We also evaluated the association of these complications with treatment outcomes, such as number of plasma exchange procedures, days in hospital and death.

Methods: We included a cohort of TTP patients treated at the Clinical Hospital of the University of Campinas - Brazil between 1995 and 2016. Clinical parameters at baseline were considered as potential risk factors for severe neurological complications and were evaluated in regression models.

Results: A total of 67 TTP episodes in 51 patients were included. Forty-one (80%) patients were women, and the mean age was 35 years (SD 12). Neurological symptoms occurred in 78% of TTP episodes: headache only (15%); confusion, personality change, sensorimotor loss or seizures (31%) and coma (33%). First TTP episode, lower platelet and higher reticulocyte count and higher PLASMIC score were detected in TTP with severe neurological complications as compared to TTP with no such complications. The parameters associated with the risk of severe neurological complications were first TTP episode (OR 2.45 95%IC 1.39 - 5.59, P=0.01) and the PLASMIC

TABLE 1 Clinical characteristics of TTP episodes at diagnosis and clinical evolution during hospitalization

N	67
First episode (%)	50 (74.6)
PLT count x109 per L (mean (SD))	20158.21 (16857.75)
Reticulocyte % (mean (SD))	9.00 (5.59)
Creatinine mg dL-1 (mean (SD))	1.27 (1.52)
PLASMIC SCORE 6 or 7 (%)	57 (85.1)
Neurological = Y (%)	52 (77.6)
Ischemic stroke during PTT episode (%)	N: 31 (46.3) Y: 6 (9.0) NA: 30 (44.8)
Days in hospital (mean (SD))	26.45 (13.98)
Death during hospitalization = Y (%)	5 (7.5)

TABLE 2 Clinical features of TTP episodes by neurological complications

	N (neurological complications)	Y (neurological complications)	P
Order of episode (%) 1 2 3 4 5 6 8 9 10	12 (50.0) 4 (16.7) 1 (4.2) 2 (8.3) 1 (4.2) 1 (4.2) 1 (4.2) 1 (4.2) 1 (4.2)	38 (88.4) 3 (7.0) 1 (2.3) 1 (2.3) 0 (0.0) 0 (0.0) 0 (0.0) 0 (0.0) 0 (0.0)	0.063
PLT count x109 per L (mean (SD))	26208.33 (22049.31)	16781.40 (12155.09)	0.027
Reticulocyte % (mean (SD))	6.95 (5.05)	10.11 (5.61)	0.040
Creatinine mg dL-1 (mean (SD))	1.72 (2.44)	1.01 (0.41)	0.069
PLASMIC SCORE (%) 4 5 6 7	2 (8.3) 6 (25.0) 9 (37.5) 7 (29.2)	1 (2.3) 1 (2.3) 23 (53.5) 18 (41.9)	0.016
Start of simptoms and star of tratament (days) (mean (SD))	10.84 (10.44)	17.70 (13.48)	058
Ischemic stroke during PTT episode (%) N Y NA	6 (25.0) 0 (0.0) 18 (75.0)	25 (58.1) 6 (14.0) 12 (27.9)	0.001
Days in hospital (mean (SD))	22.04 (11.37)	28.91 (14.80)	0.053
Death during hospitalization = Y (%)	1 (4.2)	4 (9.3)	0.778

score (OR 2.39 95% IC 1.12 - 5.75, P=0.03). Severe neurological complications were associated with higher in-hospital stay, with a hospitalization period 7 days longer when compared to no neurological complications (mean difference 6.87 95% CI -0.1 - 13.8, P=0.05).
Conclusions: First TTP episode and higher PLASMIC score values are associated with severe neurological complications. PLASMIC score may represent a prognostic tool from a neurological point of view. Awareness of the risk of neurological complications may improve TTP treatment.

PB1879 | Influence of Personality and Life Conditions on Depression, Anxiety and Cognitive Performance in 104 Patients Having Survived Acute Autoimmune Thrombotic Thrombocytopenic Purpura (iTTP)

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Background: iTTP is a potentially life-threatening, relapsing disease in which an acquired deficiency of the enzyme ADAMTS13 leads to generalized microvascular thrombosis in various organs. We (Falter et al.; Transfusion 2017;57:1152) and others reported that survivors of iTTP have a high prevalence of self-reported significant depression and impaired cognitive function.
Aims: The aim of the present study was to determine whether life circumstances and personality have an influence on the development and severity of depression and anxiety in iTTP patients.
Methods: We examined with validated questionnaires the prevalence of depressive (PHQ-9) and anxiety symptoms (GAD-7) in 104 iTTP patients, as well as parameters of subjective cognitive deficits (FLei), quality of life (QLQ-C30), attitude to life (LOT-R) and resilience (RS-11) at two observation points one year apart. At the second observation time, an age- and sex-matched healthy control group (n=134) was simultaneously interviewed.
Results: Survivors of acute iTTP had significantly more depressive symptoms ($p< 0.001$), a clear tendency to anxiety disorders ($p=0.035$) and a significantly worse cognitive performance ($p=0.008$), as compared to healthy controls. Sex, age, physical activity, and partnership status had no significant influence on depression, whereas the number of comorbidities had (diabetes mellitus, cancer, hypertension, coronary heart disease etc.). Worse scores of resilience, attitude to life and quality of life were reported by patients as compared to controls. A positive inverse correlation was found between severity of depressive symptoms and results on resilience and quality of life.
Conclusions: In summary, iTTP patients have a reduced quality of life, worse attitude to life and lower resilience than control subjects. The prevalence of depressive and anxiety disorders and disturbed physical and mental function is higher in patients than in controls. It remains to be investigated whether psychological or psychiatric counseling in these long-term patients will help to improve neuropsychiatric disturbances during long-term follow-up.

PB1880 | The Effect of Liver Transplantation on ADAMTS13 Activity and Antigen Levels

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Background: The liver plays a central role in the promotion and regulation of hemostasis. The VWF-cleaving protease - ADAMTS13 - is mainly produced by hepatic stellate cells. Deficiency of ADAMTS13 activity results in accumulation of unusually large VWF multimers, leading to platelet adhesion and aggregation in the microvasculature and organ ischemia causing thrombotic microangiopathy (TMA).
Aims: The aim of this study was to characterize the effect of liver transplantation (LTX) on ADAMTS13 activity and ADAMTS13 antigen.
Methods: In this prospective observational study, 25 patients (9 females, 16 males) were included who were consecutively transplanted between April 2017 and December 2017 in our center. A laboratory "TMA panel" including ADAMTS13 activity, ADAMTS13 antigen levels, hemoglobin, haptoglobin, LDH and fragmentocytes in

addition to routine laboratory liver transplant testing was performed before, 2-4 days, 7-9 days and during the first outpatient visit (median 14 weeks after LTX) after liver transplantation.

Results: After LTX, in 16 patients a decreased ADAMTS13 antigen level as well as a decreased ADAMTS13 activity (median 43.88% [IQR 37.03-46.03, min 22.67, max 49.08]) was detectable.

Three patients showed signs of a TMA (platelets < 100/nl, hemoglobin (females < 12g/dl/ males < 14g/dl, haptoglobin < 0.35g/l, increased LDH levels >254U/l and fragmentocytes >5‰). One patient died as a result of septic shock with subsequent multi-organ failure.

All surviving patients had an ADAMST13 activity and antigen values of median 81.75% [IQR 58.60-97.80, min 52.2] and median 0.768ng/ml [IQR 0.499-0.884] at the first outpatient visit.

Conclusions: In summary, the occurrence of TMA during liver transplantation is rare, whereas a moderate decrease of ADAMTS13 activity and ADAMTS13 antigen occurs in more than half of liver transplant recipients. Nevertheless, in patients after liver transplantation with decreased platelets and hemolytic anemia, TMA should be considered and ADAMTS13 activity should be analysed to rule out TMA.

PB1881 | Is Therapeutic Plasma Exchange (TPE) Still Necessary for the Treatment of Thrombotic Thrombocytopenic Purpura (TTP)?

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Background: TPE is still indicated for TTP, a severe disease caused by autoantibodies to ADAMTS13. However, exacerbations, refractory courses, and 10% mortality from organ failure are well known. Although caplacizumab reduced the need for TPE and improved platelet recovery and survival, it is indicated only in addition to TPE. The increasing clinical experience with caplacizumab suggests that platelet increase occurs much faster than with TPE alone.

Aims: Recently, successful treatment of single patients with TTP only with caplacizumab and immunosuppression, but without TPE, have been reported.

Methods: We report the cases of 3 patients with TTP 1 male, 2 female, age 35-59 yrs, severe clinical organ dysfunction (high troponin, stroke, confusion). All had severe ADAMTS13 deficiency and detectable inhibitors. These patients were treated with reduced intensity TPE.

Results: Two patients received TPE prior to the start of caplacizumab, one was treated completely without TPE. After the first iv dose of caplacizumab, platelet counts increased within some hours, and TPE was stopped. Therefore, the number of TPE treatments was 0-1 before the first dose of caplacizumab, and only 0-3 treatments thereafter. In addition, all patients received early rituximab for immunosuppression.

Platelet counts remained in the normal range during caplacizumab, no exacerbations or relapses occurred. Organ dysfunction resolved completely within a few days. No bleeding events were observed. Caplacizumab therapy was continued until ADAMST13 activity recovered.

Conclusions: In conclusion, an increasing number of cases suggests that during caplacizumab treatment the need of TPE can be considerably reduced, even the complete TTP management without TPE is possible. We suggest the initiation of a clinical trial to clarify whether TPE is still needed for TTP in the era of caplacizumab.

PB1882 | Acquired Thrombotic Thrombocytopenic Purpura: Key Findings of Patients from Southern Italy

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Background: Clinical features of patients with acquired thrombotic thrombocytopenic purpura (aTTP) are heterogeneous. Identifying those patients who are at risk for recurrence is difficult. Here, we describe clinical history, symptoms, laboratory and treatment data of patients with aTTP referred to 5 Italian Centres.

Aims: To add knowledge on natural history of patients with aTTP.

Methods: From January 2012 to September 2019, 70 patients were investigated for clinical and laboratory signs of Thrombotic Microangiopathy (TMA). Sixteen patients were excluded from the present study because of a diagnosis other than aTTP. Thus, 54 patients with a confirmed diagnosis of aTTP disease are described. We report clinical manifestations, potential triggers, and co-morbidities. Demographic and clinical information were collected.

ADAMTS13 activity was measured by means of a chromogenic assay (Technoclone, Austria) and the presence of inhibitor against ADAMTS13 using a classical Bethesda method. Patients were followed for at least 6 months.

Results: Demographic, laboratory data and clinical information are reported in table 1. In 43 patients (78%), aTTP started with neurologic symptoms, in 18 (32.7%) skin manifestations, 11 (20%) kidney impairment [(GFR median=37 ml/min (range 35-74)]. Three patients (5.5%) showed abdominal symptoms, 3 (5.5%) cardiovascular manifestations (1 myocardial infarction and 2 hearth failure). Co-morbidities were: malignancy (7.4%), autoimmune diseases (3.7%), psychiatric disease (11%), pregnancy (1.8%), infections (5.5%).

TABLE 1 Demographic, laboratory data and clinical information

Variables	Data
Median age (years), (range)	50 (21-69)
Female, n (%)	38 (70.3)
ADAMTS13 activity (U/dL), mean±SD	1.7±0.9
ADAMTS13 activity (U/dL) during follow-up, mean±SD	69±12
ADAMTS13 inhibitor (Bethesda Unit/mL), median (range)	3 (0.6-28)
Platelet count at first acute event, (10^9/L), median (range)	13 (6-29)
Relapse, n (%)	10 (18.5%)
Death, n (%)	4 (7.4%)

Potential triggers were oral contraceptives (n=2, 3.7%), antidepressants (n=6,11.0%), antiinflammatory drugs (n=5 (9%), anti-epileptics (n=1 1.8%) and clopidogrel (n= 1, 1.8%) . All acute events were treated with plasma-exchange [(median number=13, (range 4-43)] and steroids. Rituximab was also used in 16 (29.5%). Four patients (7.4%) died during acute event. We calculated a recurrence rate of 3 per year (observation period 40 months).

Conclusions: Our data add knowledge to natural history of aTTP and confirm a relatively high mortality and recurrence.

PB1883 | Lombardy Network for the Study and Treatment of Patients Undergoing Apheresis

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare, life-threatening thrombotic microangiopathy (TMA) requiring rapid initiation of treatment. Standardization of diagnosis and optimal management is challenging.

Aims: To study the role of ADAMTS13 in patients experiencing acute TMA requiring plasma-exchange (PEX).

Methods: A cohort study was performed in the frame of the project "Set-up of a Lombardy network for the study and treatment of patients undergoing apheresis", including 11 transfusional centers in Lombardy (Italy). Consecutive patients with acute TMA requiring PEX in 2014-2016 were enrolled. Centralized ADAMTS13 activity testing (Technozym Activity assay, Technoclone) was performed at the Hemophilia and Thrombosis Center of Milan within 24 hours after sample receival. Clinical manifestations, laboratory parameters and outcomes (mortality, exacerbation) of acute TMA events with ADAMTS13 activity < 10% or ≥10% were compared with Fisher's Exact test or Mann-Whitney t-test.

Results: Forty-three acute TMA patients (44 events) were enrolled, 35 (81%) with ADAMTS13 activity < 10%, 8 (9%) with ADAMTS13 activity≥10% (Table 1). Patients with severe ADAMTS13 deficiency were younger and mostly women, with higher prevalence of autoimmune disorders and lower prevalence of cancer. Patients with ADAMTS13 < 10% showed a higher proportion of bleeding (mostly muco-cutaneous) and cardiovascular manifestations. Conversely, neurological and renal manifestations were more frequent in patients with ADAMTS13≥10% (Table 2). Platelet levels significantly differed between the two groups. PEX treatment, initiated in all patients, was discontinued in most cases without severe ADAMTS13 deficiency. Immunosuppressive drugs were more frequently used in patients with ADAMTS13 < 10% (corticosteroids: 89% versus 75%; rituximab: 40% versus 0%). Among patients with severe ADAMTS13 deficiency, 1 (3%) died and 14 (42%) exacerbated. Among patients without severe deficiency, 2 (25%) died and nobody exacerbated.

Conclusions: Eighty-one percent of TMA patients requiring PEX resulted to be TTP. Severe ADAMTS13 deficiency distinguished two groups of TMA patients characterized by different presentation and disease course.

TABLE 1 Baseline characteristics of patients

	ADAMTS13 activity <10% (n=35)	ADAMTS13 activity ≥10% (n=8)
Female sex, n (%)	23 (66)	3 (38)
Age at 1st episode, years, median (IQR)	48 [29-60]	58 [50-79]
Age at enrollment, years, median (IQR)	50 [40-63]	58 [50-79]
First acute TMA event, n (%)	22 (61)	8 (100)
BMI, kg/m2, median (IQR)	25 [22-28]	25 [23-26]
Cancer, n (%)	0 (0)	4 (50)
Autoimmune disease, n (%)	11 (31)	1 (13)

TABLE 2 Clinical and laboratory characteristics at acute TMA patients

Clinical manifestation/laboratory parameter	ADAMTS13 activity <10% (n=35)	ADAMTS13 activity ≥10% (n=8)	Difference of proportions/medians (95%CI), p value
Systemic (headache, fever, asthenia, jaundice), n (%)	31 (89)	7 (87)	2 (-25 to 27), 1.00
Bleeding, n (%)	15 (43)	1 (13)	30 (-5 to 66), 0.22
Neurological, n (%)	15 (43)	4 (50)	-10 (-52 to 38), 1.00
Renal, n (%)	4 (11)	3 (38)	-27 (-69 to 17), 0.11
Cardiovascular, n (%)	3 (9)	0 (0)	9 (-8 to 26), 1.00
Platelet count, 10^9/l, median (IQR)	13 [10-25]	43 [34-51]	-28 (-37 to -4), 0.01
Hemoglobin, g/dl, median (IQR)	8.5 [7.1 - 11.1]	7.6 [6.9-9.1]	0.8 (-1.1 to 2.8), 0.40
LDH, IU/l, median (IQR)	1065 [845-1818]	1197 [836-1956]	-96 (-850 to 467), 0.76
eGFR, ml/min, median (IQR)	81 [53-108]	50 [30-107]	17 (-28 to 56), 0.38

PB1884 | Correlation between Phenotype-genotype versus *in-silico* Prediction of Novel and Known Disease-causing Variants in Upshaw-Schulman Syndrome Patients in a Single Institution of Argentina

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Background: Upshaw-Schulman syndrome (USS) is a disorder due to deficiency of ADAMTS13 activity and accounts for approximately 2-4% of cases. Its mode of inheritance is autosomal recessive.

Aims: To show relationship between phenotype-genotype versus *in-silico* prediction tools in 16 patients with clinical and laboratory phenotype of USS and with 18 disease-causing variants (DCV) (11 novel and 7 previously reported).

Methods: The 29 exons and intron-exon boundaries of the ADAMTS13 were amplified by PCR and sequenced (Sanger method). Eighteen DCV were found; 9 were novel (N). A total of 14 in-silico prediction tools were used to evaluate to deleterious effect (PolyPhen-2; SIFT; Panter; Mutation-taster; Meta-SNP; CADD; SNP&GO; FATHMM; PhD-SNP; Provean; BDGP; HSF; ASSP and I-Mutant to evaluate the stability of the protein).11 available for missense DCV and only 3 for nonsense, 4 for frameshift and 4 for splicing site. Informed consent and institutional ethical approval was obtained from the patient.

Results: Following DCV were described

Missense DCV: p.Asn146Tyr; p.Arg193Trp; p.Arg692Cys; p.Arg409 Trp; p.Ala893Pro; p.Arg1060Trp; p.His1077Pro; p.Asp1240Tyr; p.Asp 1362Val.

Frameshift DCV: c.774_775insccgcgcc (p.Gly259Profs*133); c.794_803del (p.Arg267Cysfs*2); c.2321del (p.Gly774Alafs*4); c.4050del (p.Glu1351Argfs*9).

Splice site DCV: c.539+1G>A; c.987+5G>A; c.2610+5G>C.

Nonsense DCV: p.Tyr617*; p.Arg910*.

TABLE 1 In-silico prediction study: domain affected and percentage of pathogenicity of missense DCV in USS

DCV		Domain affected	Percentage of pathogenicity
p.Asn146Tyr	**novel**	**Metalloprotease**	**Deleterious:11; (100%)**
p.Arg193Trp	known	Metalloprotease	Deletereius:11; (100%
p.Arg409Trp	known	TSP1-1	Deleterious:10; (90.9%)
p.Arg692Cys	known	TSP1-2	Deletereius:8; (72.7%)
p.Ala893Pro	novel	TSP1-3	Deleterious:3; (27.3%)
p.Arg1060Trp	known	TSP1-7	Deleterious:8; (72.7%)
p.His1077Pro	novel	TSP1-7	Deleterious:7; (63.6%)
p.Asp1240Tyr	novel	CUB-2	Deleterious:7; (63.6%)
p.Asp1362Val	known	CUB-2	Deleterious:8; (81.8%)

TABLE 2 In-silico prediction study: domain affected and percentage of pathogenicity of frameshift, splicing site, non-sense DCV in USS

DCV		Domain affected	Percentage of pathogenicity
p.Gly259Profs*133	**known**	**Metalloprotease**	**Deleterious:3; (75%)**
p.Arg267Cysfs*2	novel	Metalloprotease	Deleterious:4; (100%)
p.Gly774Alafs*4	novel	TSP1-3	Deleterious:3; (75%)
p.Glu1351Argfs*9	novel	CUB-2	Deleterious:4; (100%)
c.539+1G>A	novel	Metalloprotease	Deleterious:4; (100%)
c.987+5G>A	novel	Disintegrin	Deleterious:4; (100%)
c.2610+5G>C	novel	TSP1-4	Deleterious:4; (100%)
p.Tyr617*	novel	spacer	Deleterious:3; (100%)
p.Arg910*	known	TPS1-5	Deleterious:3; (100%)

The analysis of *in-silico* prediction results according to the DCV is shown in table 1 and table 2.

Conclusions: Using variants of known pathogenicity, we assessed a variety of predictive algorithms in *ADAMTS13*. Optimum predictions were found by different tools in all nonsense and splicing sites DCV, and in most of missense and frameshift DCV. A good relationship between phenotype-genotype versus *in-silico* prediction was achieved. In the context of individual DCV, the alignment quality and score are important in achieving more accurate predictions of pathogenicity.

These methods demonstrated themselves to be useful; however, in a punctual case they were not comparable with each possibility due to the difference in information and algorithms they used for predicting deleteriousness.

PB1885 | Association of Plasmic Score with Neurological Symptoms in Patients with Thrombotic Thrombocytopenic Purpura (TTP)

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Background: The PLASMIC score was recently developed for rapid diagnosis of thrombotic thrombocytopenic purpura (TTP) and therapeutic decision as ADAMTS13 is frequently unavailable. Although the PLASMIC score is validated to predict ADAMTS13 values, its role as a predictor of adverse clinical outcomes is not established.

Aims: The primary aim of this study was to evaluate whether the PLASMIC score is associated with neurological complications during TTP episodes.

Methods: In the present study, we retrospectively applied the PLASMIC score at the time of diagnosis of TTP episodes treated at the UNICAMP Clinical Hospital (Brazil) between 1995 and 2016. We divided the episodes according to the PLASMIC score, and compared the occurrence of neurological symptoms and other clinical manifestation between the PLASMIC score groups. A total of 50 episodes of TTP were identified, of these 47 episodes, and 34 patients, were included in the study.

Results: At the diagnosis, the mean PLASMIC score was 6. In 74.5% of the episodes, the patients presented with some neurological symptoms. The incidence of neurological symptoms was higher in PLASMIC score 7 (n= 14 [87, 5%]) and 6 (18 [81, 8%]) when compared with scores 5 (n=1, [16.7%) and 4 (n= 2 (66.7%]). The severity of the neurological complications was also higher in PLASMIC scores 6 and 7. Stupor or coma were reported only in PLASMIC scores 6 [n=7 (31%]) and 7 (n= 9 [56%]). Splenectomy and deaths during hospitalization occurred only in cases with PLASMIC score 6 or 7.

Conclusions: The frequency and severity of neurological injuries increased with a higher PLASMIC score. This observation suggests

TABLE 1 Clinical characteristics of TTP episodes at diagnosis and clinical evolution during hospitalization

	All TTP episodes	PLASMIC score 4	PLASMIC score 5	PLASMIC score 6	PLASMIC score 7	P value
Age at diagnosis (mean (SD))	35.73 (12.71)	35.38 (9.74)	37.98 (19.16)	36.38 (10.73)	34.08 (13.91)	0.921
Neurological (eg headache, confusion, personality change, sensorimotor loss, seizures, com = Y %	35 (74.5%)	2 (66.7%)	1 (16.7%)	18 (81,8%)	14 (87,5%)	0.005
Days in hospital (mean (SD))	27.04 (14.38)	23.67 (18.58)	16.67 (9.52)	30.36 (14.00)	27.00 (14.88)	0.218
Infection = Y (%)	25 (53.2%)	1 (33.3)	1 (16.7%)	7 (31.8%)	9 (56,2%)	0.042
Fever = Y (%)	27 (57.4%)	0 (0.0)	1 (16.7%)	14 (63.6%)	9 (56.2%)	0.194
Steroids = Y (%)	43 (91.5%)	1 (33.3)	0 (0.0)	13 (59.1)	14 (87.5)	0.060
Vincristine = Y (%)	23 (48.9%)	1 (33.3)	0 (0.0)	3 (13.6)	9 (56.2)	0.063
In hospital splenectomy = Y (%)	8 (17.0%)	0	0	5 (22.7%)	3 (18.8%)	0.496
Death in hospital = Y (%)	4 (8.5%)	0	0	3 (13,6%)	1 (6,2%)	0.640

TABLE 2 Type of neurological complication

	All TTP episodes	PLASMIC score 4	PLASMIC score 5	PLASMIC score 6	PLASMIC score 7	P value
Type of neurological complication						
Headache only = Y (%)	23 (48.9%)	1 (33.3%)	0	2 (9,1%)	2 (12,5%)	0.07
Confusion = Y (%)	18 (38.3%)	1 (33.3%)	1 (16.7%)	11 (50%)	5 (31,2%)	0.418
Personality Chance = Y (%)	6 (12.8%)	0	0	2 (9.1%)	4 (25%)	0.292
Sensorimotor loss = Y (%)	22 (46.8%)	0	1 (16.7%)	11 (50%)	10 (62.5%)	0.090
Seizures = Y (%)	8 (17.0%)	0	0	3 (13,6%)	5 (31,2%)	0.229
Stuporous or Comatose = Y (%)	16 (34.0%)	0	0	7 (31.8%)	9 (56,2%)	0.042

that TTP patients with PLASMIC score 6 or 7 need more attention regarding neurological complications. It is possible also that these patients benefit from intensive treatments. Therefore, the PLASMIC score may be an important tool not only to predict ADAMTS13.

PB1886 | The Changing Face of Thrombotic Thrombocytopenic Purpura (TTP) in South Africa in the Human Immunodeficiency Virus (HIV) Era

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Background: Thrombotic thrombocytopenic purpura (TTP) is a life-threatening condition, due to congenital or acquired deficiency of a protease, ADAMTS-13, resulting in ineffective proteolysis of von Willebrand Factor which binds platelets obstructing the micro-circulation. TTP is

rare with an incidence of ~1 per million of the population. A TTP-like syndrome is prevalent in human immunodeficiency virus (HIV) infected patients in South Africa and partially responds to therapeutic plasma exchange (TPE) but the pathophysiology is unclear.

Aims: Investigate HIV-TTP pathophysiology: measure ADAMTS-13 antigen/activity and neutralizing auto-antibody levels in patients presenting to a quaternary hospital in Johannesburg, South Africa.

Methods: Ethics approval was obtained (M0160134). ADAMTS-13 antigen/activity and inhibitory auto-antibody levels were measured before and after TPE.

Results: 18 patients with HIV-associated TTP were admitted over 12-months (2018-19) of whom 13 were antiretroviral therapy (ART) naïve with mean CD_4 T-cell counts (reference range 332-1 362 cells/µl) and HIV-viral loads of 212 cells/µl (range 87-421) and 1 062 760 copies/ml (range 50-9 800 000) respectively. The mean ADAMTS-13 antigen and activity levels (% of normal) were 5 and 1 respectively prior to TPE and improved to 39 and 28 after TPE and were similar in the ART naïve and exposed groups. 3 patients in the cohort did not have clinically significant ADAMTS-13 inhibitory auto-antibodies although baseline ADAMTS-13 levels were < 20%. 6 patients demonstrated no significant recovery in ADAMTS-13 levels despite on average 11 days of TPE but ADAMTS-13 auto-antibodies remained significant in only 3 of these patients after TPE.

Conclusions: HIV associated TTP/TTP-like syndrome is prevalent in the South African HIV infected population occurring at all stages of the HIV disease with variable responses to TPE and inconsistent ADAMTS-13 auto-antibody titres. Additional studies are indicated to elucidate the causative factors including the contribution of endothelial dysfunction and non-inhibitory auto-antibodies to facilitate capacity planning in Africa.

PB1887 | Inhibitory Anti Adamts13 Antibodies with a New Rapid Fully Automated Clia Assay

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Background: Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disorder, characterised by severe deficiency of ADAMTS13 activity associated with autoantibodies to ADAMTS13. Noninhibitory and inhibitory autoantibodies can be detected with an enzyme-linked immunosorbent assay (ELISA); Bethesda Assay can identify the subset of inhibitory autoantibodies. Recently, a chemiluminescence (CliA) ADAMTS13 activity (HemosIL Acustar ADAMTS13 activity, IL, USA) because of its short turnaround time, full automation and good performances has been demonstrated to be the first choice for the diagnosis of TTP in emergency settings.

Aims: Aim of this study was to analyze the performances of CliA ADAMTS13 activity assay detecting inhibitory anti-ADAMTS13 antibody by using Bethesda Assay.

Methods: A method comparison study was performed on 32 samples stored by 4 Italian hospital between 2014 to 2019: 8 acute TTPs, 9 TTPs follow-up, 4 TTPs relapse, 1 congenital TTP, 4 HUS, 1 atypical HUS, 4 suspected but not confirmed TTPs. We retrived results of test previously run in ELISA for both activity and autoantibodies (Technozym Adamts13 Activity and INH, Technoclone Gmbh, Austria). We then rerun at the same time new tests on thawed frozen samples: ELISA and CliA activity, ELISA autoantibodies, and ELISA and CliA Bethesda assays.

Results: We found a very good correlation between ELISA and CliA activity assays results (r=0.96) and between archived ELISA and CliA activity results (r=0.93). Both correlation were better than the one obtained between historical and newly performed ELISA results (r=0.92). Agreement between the anti-ADAMTS13 assays performed ranged from moderate to very good (Table1).

Table 1. Kappa ...

TABLE 1 Kappa Cohen coefficients obtained by comparing anti-ADAMTS13 antibodies

	ELISA autoantibodies newly performed	ELISA autoantibodies newly performed	CliA Bethesda Assay
ELISA autoantibodies historical archive data	-	-	-
ELISA autoantibodies newly performed	0.50	-	-
CliA Bethesda Assay	0.68	0.86	-
ELISA Bethesda Assay	0.58	0.80	0.93

Conclusions: CliA and ELISA Bethesda assays showed very good agreement with samples run at the same time using ELISA anti-ADAMTS13 autoantibodies assay. CliA Bethesda assay allowed identification of inhibitory anti-ADAMTS13 within almost same turnaround time of ELISA, but with better automation; the only manual operations needed were sample mixing and two incubations.

PB1888 | Evaluation of a Rapid, Automated Chemiluminescent ADAMTS-13 Activity Assay for the ACL AcuStar

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Background: Rapid diagnosis of thrombotic thrombocytopenic purpura (TTP) is important because untreated mortality is 90%, which can be reduced with prompt plasma exchange (PEX). A severely reduced ADAMTS-13 activity of < 10 IU/dL (< 10%) confirms TTP diagnosis. Without a rapid ADAMTS-13 activity assay, some cases of possible TTP may have inappropriate PEX administered. A reliable ADAMTS-13 activity result is also needed to diagnose atypical HUS (aHUS).

Aims: To compare a rapid, automated quantitative chemiluminescent assay (HemosIL® ADAMTS-13 Activity, Instrumentation Laboratory) to a commercially available ADAMTS-13 activity ELISA (Technozym® ADAMTS-13 Activity, Technoclone).

Methods: Frozen citrated plasma samples (n=205) from patients with possible TTP were analysed using both methods. Based on ELISA results, there were 59 samples with ADAMTS-13 activity < 10 IU/dL, 76 with ELISA results 10-40 IU/dL and 70 with ELISA results > 40 IU/dL.

Results: Correlation between the two methods was 0.929. The mean sample results was 32 IU/dL and 37 IU/dL (p < 0.0001) for ELISA and AcuStar, respectively. All 59 samples with ELISA results < 10 IU/dL also had AcuStar < 10 IU/dL. Amongst the 76 samples with ELISA results 10-40 IU/dL, 4 samples (ELISA results 10.8, 10.9, 10.9 and 24.9 IU/dL) had AcuStar results of 5-10 IU/dL.These differences would not have impacted on patient management. No samples with ELISA >40 IU/dL had AcuStar results < 22 IU/dL. Analysis time with the HemosIL ADAMTS-13 Activity assay on the AcuStar is < 40 minutes giving a turnaround time from receipt of sample to issue of result of < 1 hour with AcuStar compared to 4 hours for ELISA.

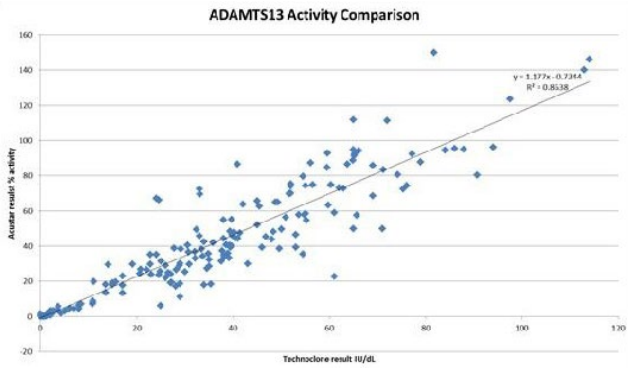

FIGURE 1 ADAMTS-13 activity comparison data

Conclusions: The rapid, automated chemiluminescent HemosIL AcuStar ADAMTS-13 activity assay is suitable for routine use. The technical simplicity and assay time < 40 minutes has led to the introduction of a 24/7 on-demand ADAMTS-13 activity service in our centre.

PB1889 | Clinical and Economic Impacts of a New Rapid ADAMTS-13 Assay for the Diagnosis of Suspected Thrombotic Thrombocytopenic Purpura

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Background: Acquired thrombotic thrombocytopenic purpura (TTP) is a microangiopathic disease caused by immuno-mediated ADAMTS13 severe deficiency typically characterized by hemolytic anemia, thrombocytopenia and neurological symptoms. The clinical presentation overlaps with others microangiopathic disorders and ADAMTS13 assay is mandatory for differential diagnosis. Because ADAMTS13 assays currently in use are not readily available and are time-consuming, the first line treatment with plasma exchange (PEX) typically starts before assay results are available.

Aims: To evaluate clinical and economic impacts of a new rapid (33min) assay to detect ADAMTS13 activity (HemosIL AcuStar ADAMTS13 Activity, IL, USA) to promptly recognize and treat patients with acquired TTP.

Methods: All costs associated with both PEX and assay were evaluated for 4 Italian hospitals. These include cost of plasma units, CVC placement, average time required to carry out each activity necessary to perform PEX and to run the assay, consumables, and the depreciation cost of instrumentations. This analysis was applied to a real scenario as we retrospectively analyzed requests for suspect TTP patients during 2019 (n=84) and the number of PEX procedures or plasma infusion performed before receiving ADAMTS13 activity results. The retrospective turnaround time (TAT) was also evaluated.

Results: The ADAMTS13 TAT ranges from 4 to 170 hours. The total cost for the first PEX varies from 1755 to 2098€ depending on which hospital performed the procedure. Only 7% of the patients analyzed showed a confirmed TTP.

Conclusions: Only a small portion of the patients with suspected of TTP have the disease. Having a rapid assay to promptly screen patients allows for reduction of unnecessary invasive procedures for patients and considering alternative diagnosis and treatments with also a potential cost saving. Data will be analyzed (EUnetHTA 3.0 protocol) for a HTA evaluation. The assay's cost is widely covered by reduction of unnecessary plasma infusion and PEX.

PB1890 | Genetic Variants in *ADAMTS13* in Patients with Acquired TTP (aTTP)

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Background: Thrombotic thrombocytopenic purpura (TTP) is a life-threatening illness due to deficiency in ADAMTS13 activity; this can be either congenital (inherited), or acquired (autoimmune), being this last condition the 95% of cases.

Aims: To report the finding of two novel genetic variants (GV) and one known variant in five patients with diagnosis of aTTP.

Methods: ADAMTS13-activity (normal value [nv]=40-130%), IgG (nv< 15U/uL), IgM (nv< 2.5 arbitrary units/uL[au/uL]), IgA (nv< 1.5au/uL) anti ADAMTS13 and neutralizing ADAMTS13-inhibitor (nv< 30%) were evaluated by ELISA.

The 29 exons and intron-exon boundaries of *ADAMTS13* were amplified (PCR) and sequenced (Sanger method).

Twelve *in-silico* prediction tools were used: PolyPhen-2; SIFT; Panter; Mutation-tester; I-Mutant; Meta-SNP; CADD; SNP&GO; FATHMM; PhD-SNP; Provean.

Informed consent and institutional ethical approval was obtained from the patient.

P1: Female; diagnosis at 28 years.
P2: Female; diagnosis at 29 years.
P3: Female; diagnosis at 13 years.
P4: Female; diagnosis at 25 years.
P5: Female; diagnosis at 36 years.

Results: Plasmatic parameters of ADAMTS13 during acute crisis and GV found in the patients are shown in the table 1. All GV were found in heterozygous state.

Conclusions: p.Val195Ile and p.Pro952Ser were predicted as deleterious in 54.5% and 81.8% of *in-silico* tools, respectively; whereas p.Arg1096His was predicted as neutral by 100% of *in-silico* tools, but decreased stability of protein.

p.Arg1096His has been described as polymorphism (rs61751476) and as like benign GV in clinical database (ClinVar and VarSome), and also associated with a reduction in functional activity by 50%.

The finding of heterozygous GV in these patients could suggest that their presence would be associated with changes in specific epitopes in domains of ADAMTS13 to develop of neutralizing ADAMTS13-inhibitor (P1), IgM (P2), IgG (P3, P4), IgA (P5); however, further studies and patients are needed to confirm this relationship.

TABLE 1 Plasmatic parameters and genetic study of ADAMTS13

	Plasmatic parameters of ADAMTS13					
Patient	Activity (%)	IgG (U/mL)	IgM (au/uL)	IgA (au/uL)	Neutralizing inhibitor (%)	Genetic Variant
P1	8	9	1.9	0.35	90	p.Val195Ile (Novel)
P2	6	3	4.1	0.43	0	p.Pro952Ser (Novel)
P3	<5	109	1.54	0.46	0	p.Arg1096His.
P4	<5	55	1.04	0.56	11	p.Arg1096His.
P5	12	12	0.12	3.66	24	p.Arg1096His.

PB1891 | Impact of Ultralarge von Willebrand Factor (VWF) Multimers and Platelets in Cultured Endothelial Cells in Acute Immune-mediated Thrombotic Thrombocytopenic Purpura

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Background: In the absence of functional ADAMTS13, ultralarge VWF multimers and platelets lead to the formation of platelet-rich microthrombi within small arterioles inducing immune-mediated Thrombotic Thrombocytopenic Purpura (iTTP). Under physiological conditions, VWF is secreted from endothelial cells as ultralarge

multimers, and ADAMTS13 progressively reduces the size of these VWF multimers as they circulate in the blood.

Aims: We studied the impact of VWF in platelet-dependent primary haemostasis in reduced/absence ADAMTS13 activity under flow conditions in acute thrombocytopenic iTTP patients.

Methods: A shear flow-based assay with Human Umbilical Vein Endothelial Cells (HUVECs) was developed. After histamine stimulation of endothelial cells, plasma with the addition of platelets at a concentration of 0.5-1 $x10^8$/mL was perfused through gelatin coated μ-slides VI (Ibidi, Germany) at a constant wall shear stress of 2.5 dynes/cm^2. Cells were then immune-labelled for VWF and CD41 after being fixed under flow with 4% formaldehyde. Platelet recruitment and VWF coverage were quantified.

Results: We investigated twenty acute iTTP presentation patients with severe deficiency (< 10%) of ADAMTS13 activity. Median presenting anti-ADAMTS13 antibodies levels was 67% (range, 2%-140%) and median presenting ADAMTS13 antigen levels was 3.0% (range, 0.7%-47.6%). Pre-treatment median levels of VWF antigen and VWF activity were respectively 1.71IU/mL (range, 0.72 IU/mL-3.16 IU/mL) and 0.84 IU/mL (range, 0.15 IU/mL-2.10 IU/mL). With the shear flow-based assay with HUVECs, significant increase of platelet recruitment (p< 0.0001) and VWF coverage (p=0.0035) were observed in patients compared to pool normal plasma. Significant increase in median string number was reported in patients (n=83, range 46-184) compared to pool normal plasma (n=38, range 20-66) (p< 0.0001). Reduced ADAMTS13 activity increased the percentage of the longest (>100μm) strings in patients (p=0.0011).

Conclusions: Investigation of the interaction between VWF and platelets on HUVECs under shear flow may help to understand the pathophysiological mechanism of iTTP and could contribute in disease severity evaluation.

PB1892 | Treatment of Acquired Thrombotic Thrombocytopenic Purpura in Rituximab Era: A Single Center Experience from India

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Background: Acquired thrombotic thrombocytopenic purpura (TTP) is caused by development of antibodies against ADAMTS 13, a VWF multimer cleaving protease. Treatment involves urgent institution of plasma exchange. Rituximab, a monoclonal anti CD 20 antibody, has shown efficacy in the treatment of relapsed and refractory cases of TTP. It also decreases number of plasma exchanges required to attain remission. There is paucity of data regarding treatment and outcome of TTP from developing countries using rituximab in upfront therapy.

Aims: To evaluate treatment and outcome of acquired TTP in patients treated with plasma exchange and rituximab

Methods: Retrospective review of adult patients treated as acquired TTP in hematology department in Sir Ganga Ram Hospital New Delhi India from Jan 2016 to Dec 2019.

Results: A total of 8 patients were treated during above period (female 5 male 3), median age 50 years (32 - 57), mean Hb 7.4gm/dl (6.0-8.7), median platelet count 14 (10-26), schistocyte index 11.6% (4.3-21.8), serum LDH 1277 (411-2580). One patient had underlying HIV infection but was a treatment defaulter with very high viral load. ADAMTS 13 activity was less than 3 in all patients with mean inhibitor activity of 4.68 BU (1.6-12.1). Mean number of plasma exchanges needed were 8 (4-12). Two patients didn't respond to plasma exchange and died after 5 days including one with HIV associated TTP. Six patients achieved complete remission and also received all 4 weekly courses of rituximab. None of the six patients have relapsed after a 28 months of median follow up (6-48 months).

Conclusions: Acute acquired TTP is frequently misdiagnosed as immune thrombocytopenic purpura resulting in delayed diagnosis resulting in significant early mortality. There is a need to diagnose TTP early and refer them to appropriate center for treatment with plasma exchange and rituximab for improving the outcome.

PB1893 | Varied Presentations of TTP

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare and life-threatening thrombotic microangiopathy characterized by

- Microangiopathic hemolytic anemia,:
- Thrombocytopenia, often with purpura but not usually severe bleeding:
- Acute renal insufficiency:
- Neurologic abnormalities, usually fluctuating:
- Fever:

Only 20-25% of patients present with the above classical pentad: Recently developed, high probability PLASMIC score supports treatment with immediate plasma exchange where as low PLASMIC score suggests withholding plasma exchange and search for alternative diagnosis.

Aims: Series of Thrombotic thrombocytopenic purpura presentation and course of disease.

Methods: This is a retrospective study conducted in department of clinical haematology in a tertiary hospital in india. we are reporting five cases of TTP, data were retrieved from MRD department, two cases were pregnancy related and other three were sporadic TTP. One case presented with expressive aphasia.

Results: All 5 cases were evaluated, for all cases PLASMIC score was done, all 5 received plasma exchange, 3 cases were successfully treated only with single episode but two cases had recurrence. These reccurences were post rituximab therapy which were successfully treated with ciclosporin. All five cases had ADAMTS13 level estimation, No mortality was seen in our series.

Conclusions: TTP is uncommon and potentially fatal disease if not having high index of suspicion and early plasma exchange, classical Pentad may not be useful for diagnosing. PLASMIC score help us to early initiate the plasma exchange.

ANTIPHOSPHOLIPID SYNDROME

PB1894 | Obstetric Antiphospholipid Syndrome: ADAMTS-13 Tested at Pregnancy Diagnosis Is Associated with the Occurrence of Early-Onset Pre-Eclampsia Despite Treatment

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Background: Late pregnancy complications are still frequent in treated pregnant women with the obstetric antiphospholipid syndrome (oAPS). Available data strongly suggest ADAMTS13 autoantibodies and ADAMTS13 dysfunction globally occurring in APS, particularly in oAPS during preeclampsia (PEcl) and hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome.
Aims: To study ADAMTS13 and ADAMTS13 antibodies and their prognostic value in newly pregnant oAPS women.
Methods: The study was performed in the 513 oAPS women included into the NOH-APS cohort, who began a new pregnancy treated with enoxaparin 40 mg + aspirin 100 mg daily (Blood 2014; 123: 404-13). Frozen plasma aliquotes obtained just before the beginning of LMWH injections were tested for ADAMTS13 activity and antigen, ADAMTS13 antibodies (ELISA, Technoclone, Vienna, Austria), PGF and sFlt1 concentrations (ELISA, R&D Systems, Abingdon, UK) blindly from pregnancy outcomes. The relationship between biological data and the occurrence of placenta mediated complications was investigated through univariate then multivariate logistic regression analysis.
Results: Women who developed PEcl had lower ADAMTS13 activities, higher ADAMTS13 antibodies and sFlt1 concentrations, categorization into tiertiles showing a concentration-effect relationship on the clinical risk. This culminated in severe PEcl cases, in early-onset PEcl cases
(< 34 weeks), in eclampsia cases and in HELLP syndrome cases. The final models evidenced high concentrations of ADAMTS13 antibodies and of sFlt1, and low concentrations of ADAMTS activities, to be independent risk factors for the occurrence of early-onset PEcl, also of HELLP syndrome, together with a triple positivity for antiphospholipid antibodies.
Conclusions: In newly pregnant women with oAPS conventionally treated, the early evaluation of ADAMTS13 can help to indicate the clinical risk of late complications with high morbidity potential. ADAMTS13 might be a clinically-relevant therapeutic target.

PB1895 | Role of Anti Phosphatidylserine/Prothrombin Antibodies on Thrombin Generation and Protein C Resistance in Carriers of Antiphospholipid Antibodies

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Background: aPS/PT antibodies confer additional risk for thrombembolic events and pregnancy loss to the usual aPL profile (LA, aCL, and anti- β2GPI antibodies). A role of aPS/PT in modifying thrombin generation (TG) parameters is not yet established. However, most of APS patients are on oral anticoagulant treatment that per se causes alteration of thrombin generation.
Aims: To study the role of aPS/PT antibodies on thrombin generation and its inhibition by thrombomodulin in carries of aPL antibodies not on oral anticoagulant treatment.
Methods: Asymptomatic carriers positive for aPL antibodies on 2 occasions at least 12 weeks apart were recruited in an outpatient setting. LAC, aCL, and anti- β2GPI antibodies were tested according to current guidelines. TG in tissue factor-triggered platelet-poor plasma was measured with the calibrated automated thrombogram method. A comparison of TG in the presence or absence of thrombomodulin (20 nM) enables to assess the function of the activated protein C system.
Results: Fifty-five individuals resulted positive for at least one test exploring the presence of aPL and confirmed after at least 12 weeks. Among them, 37 were positive for aPS/PT and 18 were negative for aPS/PT antibodies. Triple positivity (18) and isolated LAC (14) were more represented in aPS/PT + group, while double positive for aCL

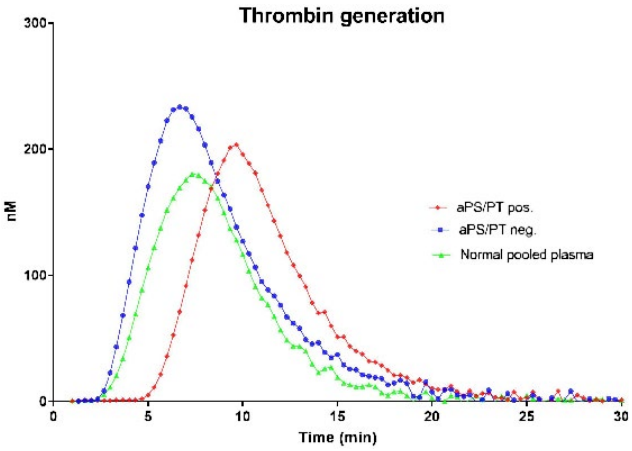

FIGURE 1 A representative graph of thrombin generation in healthy volunteers, in aPS/PT positive and negative individuals

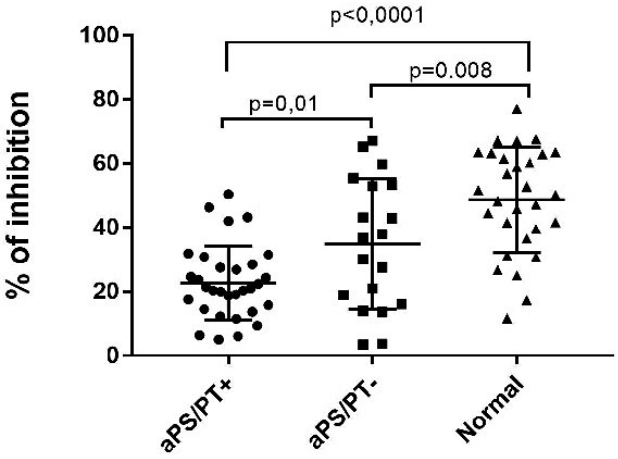

FIGURE 2 Inhibition of thrombin generation by thrombomodulin

and aβ2GPI (7), and isolated aβ2GPI (8) were more represented in aPS/PT - group. In aPS/PT+ group, Lag Time is significant longer (5.37min (4.07; 7.27) vs 3.36min (2.97;4.54),p< 0.0001); time to peak is significantly delayed (9.59min (8.10;10.96) vs 7.68min (6.78;8.78), p=0.0011). ETP, Peak, Vel.Index are lower in aPS/PT+ carriers but didn't reach statistics significance (figure 1). Protein C resistance measured by the inhibition of ETP was significantly lower in aPS/PT+ then in aPS/PT- group (22.81%±11.5 vs 34.89%±20.44, p=0.01, figure2).

Conclusions: aPS/PT antibodies significantly reduce TG and showed significant resistance to thrombomodulin activated endogenous protein C.

PB1896 | Tetra Positive Thrombotic Antiphospholipid Syndrome: Major Contribution of Anti Phosphatidyl-Serine/Prothrombin Antibodies to Lupus Anticoagulant Activity

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Background: The concurrent presence of lupus anticoagulant, anti-cardiolipin and anti β2-glycoprotein I antibodies ((LA+, aCL+, aβ2GPI +, triple positive profile) identifies patients at high-risk of thromboembolic events. These patients are often positive for anti phosphatidyl-serine/prothrombin antibodies (aPS/PT+, tetra positive profile).

Aims: To identify which antibody between aβ2GPI and aPS/PT is responsible for lupus anticoagulant activity (LA).

Methods: aβ2GPI antibodies were purified from 14 tetra positive APS patients by immune-affinity chromatography. Both eluate and flow through from the purification system were spiked into normal pooled plasma to test LA activity. Flow through of aβ2GPI purification system was used to purify aPS/PT antibodies through a prothrombin affinity column, eluate of which was measured by aPS/PT ELISA and also spiked in to normal pooled plasma for LA testing.

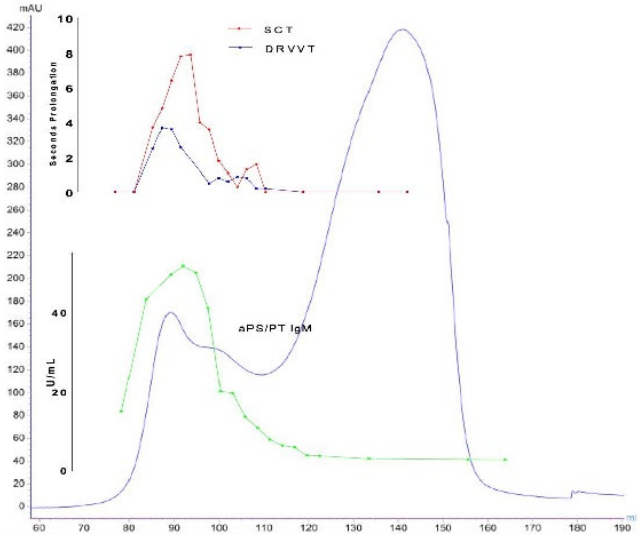

FIGURE 1 Size-exclusion chromatography (Sephacryl S-300) of β2GPI affinity column flow through

Results: Immuno-affinity purified aβ2GPI antibodies from 14 tetra APS patients did not prolong the diluted Russell Viper Venom Time (dRVVT) and Silica Clotting Time (SCT) (median ratio 0.98, IQR 0.9-1.06 and 1.0, IQR 0.91-1.03, respectively) while flow through of column deprived of aβ2GPI antibodies showed LA positivity (dRVVT 1.88, IQR 1.58-2.77, and SCT 1.75, IQR 1.17-2.9, respectively). Material purified by prothrombin affinity column from three flow through showed strong positivity in aPS/PT ELISA, with the concentration ranging from 54 to 91 mg/mL. aPS/PT prolonged the coagulation time of normal pooled plasma: the dRVVT ratio in the three patients were 2.09, 1.21, 1.35 and that of SCT were 2.05, 1.5, 2.13. Figure 1 shows a representative experiment (patient #4) showing that both LA and IgM aPS/PT antibodies co-eluted in the same fractions.

Conclusions: Anticoagulant activity in tetra positive antiphospholipid syndrome patients may largely be attributable to anti phosphatidyl-serine/prothrombin antibodies.

PB1897 | Effect of Immune-Purified aβ2-Glycoprotein I (aβ2GPI) and Anti Phosphatidylserine/Prothrombin (aPS/PT) Antibodies on Thrombin Generation

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Background: aPS/PT antibodies confer additional risk for thrombembolic events and pregnancy loss to the usual aPL profile (LA, aCL, and anti- β2GPI antibodies). The relative contribution of aβ2GPI and aPS/PT antibodies in determining anticoagulant activity in APS patients is unknown.

Aims: We aimed at separation of aβ2GPI and aPS/PT antibodies from tetra positive APS patients, to test their contribution to anticoagulant activity by thrombin generation (TG).

Methods: aβ2GPI antibodies were purified from 24 and aPS/PT antibodies were from 4 tetera positive (LA+, aCL+, aβ2GPI +, aPS/PT+) APS patients by immune affinity chromatography. The antigenic specificity of eluted antibodies was determined using ELISA. TG in tissue factor-triggered platelet-poor plasma was measured with the calibrated automated thrombogram method and purified aβ2GPI and aPS/PT antibodies and tris buffer were spiked into normal plasma to obtain TG curves.

Results: The mean concentration of purified aβ2GPI is 65.79±34.48 μg/mL, aPT is 37.4 μg/mL. As shown in figure 1, NNP+ aPS/PT TG curve showed anticoagulated characteristics in all five parameters, compared to NNP+ aβ2GPI and NP+TRIS, NNP+ aPS/PT showed longest lag time (4.5 ±1.3 VS 3.5±0.6 VS 2.6±0.3 min, p< 0,0001), highest ETP (1450±79 VS 1427±123 vs 1338±87 nM per min, p=0,0090), lowest peak (143±11 vs 187±31 vs 166 ± 20 nM, p=0,0008), longest time to peak (8.9 ± 1.7 vs 6.1 ± 0.8 vs 5.9 ± 0.8 min, p=-0.002), lowest velocity index (33.5±8.2 vs 76.1±24.9 vs 52.1 ± 13.7 nM/min, p < 0,0001). Compare to NNP+ aβ2GPI group, NNP+ aPS/PT group showed significantly lower peak (p=0.003), longer time to peak (p=0.004), and lower velocity index (p=0.0005).

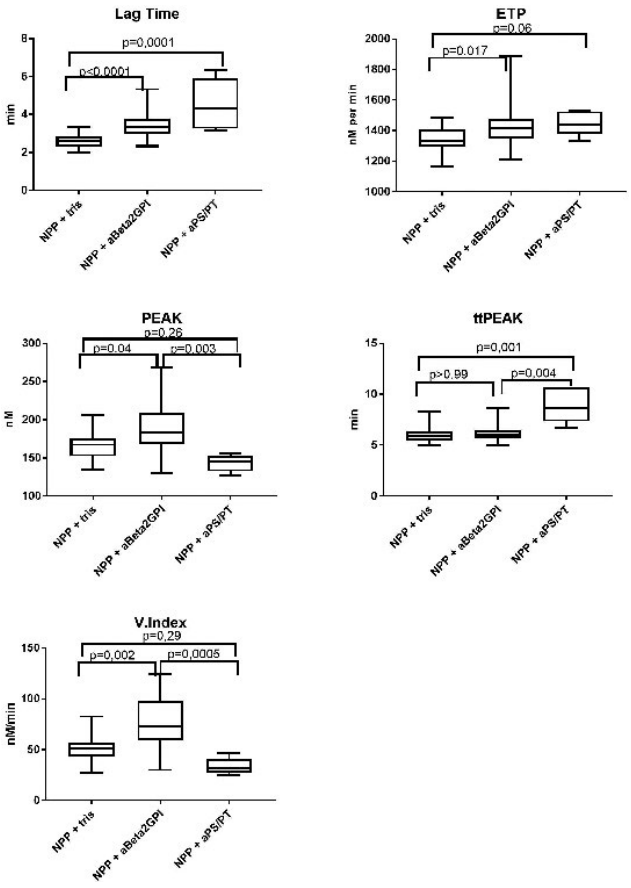

FIGURE 1 Comparison of parameters of TG curves by Tris buffer, purified aβ2GPI and aPS/PT spiked into same normal plasma pool

Conclusions: Immune purified aPS/PT and aβ2GPI cause significantly reduced thrombin generation of normal pooled plasma in vitro. The anticoagulant effect of APS/PT+ antibodies was significantly stronger than that of aβ2GPI antibodies.

PB1898 | Lupus Anticoagulant and Antiphospholipid Antibodies: A Real-World Study

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[1]Erasmus University Medical Center, Hematology, Rotterdam, the Netherlands; [2]Erasmus University Medical Center, Internal Medicine and Immunology, Rotterdam, the Netherlands; [3]Erasmus University Medical Center, Vascular Surgery, Rotterdam, the Netherlands; [4]Erasmus University Medical Center, Obstetrics and Gynecology, Rotterdam, the Netherlands

Background: Antiphospholipid antibodies (aPL) are a heterogeneous group of antibodies that can interact with phospholipids, phospholipid-binding proteins, or both and its presence is associated with increased risk of thrombosis. aPL are routinely measured in daily clinical practice for diagnostic purposes in autoimmune diseases or to detect risk factors after obstetric complications or thrombosis. Data on incidence of aPL positivity and characteristics of patients with positive aPL are scarce.

Aims: To identify patients with positive aPL, including lupus anticoagulant, anticardiolipin antibodies and anti-β2GPI antibodies, and to determine the association with antiphospholipid syndrome (APS) and thrombosis.

Methods: On average 770 patients are tested annually for aPL at the Erasmus MC. In this single center retrospective study all first positive aPL samples between June 2015 till April 2018 were collected as were the patients' clinical characteristics.

Results: Out of 2147 patients, 284 patients had ≥1 positive aPL test result. 93 patients met the revised Sapporo criteria for APS, with significantly higher titers of all aPL than patients who did not meet these criteria. APS patients were treated by internists (63.4%), gynecologists (22.6%) and neurologists (14.0%). 54.8% of the APS patients were newly diagnosed. APS patients were single positive for aPL (N=36), double positive (N=15), triple positive (N=17) or had incomplete results (N=25). Of the non-APS patients, 20 were single, 8 double, and 3 triple positive, and 157 had incomplete aPL results. Triple positive aPL and presence of anti-β2GPI IgG were the strongest predictors for thrombosis. 69 APS patients were treated with different anticoagulant therapy, of whom 6 with DOAC.

Conclusions: This study identifies characteristics of patients with positive aPL. Triple positive aPL was also found in patients without APS. Triple positive aPL and anti-β2GPI IgG were the strongest predictors for thrombosis. Variation in anticoagulant therapy in patients with APS necessitates increase of the awareness.

PB1899 | Increased Baseline Platelet Activation and Decreased Platelet Reactivity in Patients with the Antiphospholipid Syndrome

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Background: Patients with the antiphospholipid syndrome (APS) are at high risk of developing venous and arterial thromboembolism. The role of platelets in the pathogenesis of these prothrombotic conditions is not yet fully understood. Platelet proteome analysis showed protein abundance changes of platelets in patients with APS indicating a causative role of platelets in the development of thrombosis in APS. Additional studies have shown that antiphospholipid antibodies interact with platelets, leading to platelet activation and aggregation.

Aims: The aim was to gain deeper insights into the role of platelets in the APS by investigating differences in platelet activation and responsiveness between patients with APS and healthy controls.

Methods: Platelets from 33 patients with APS (81.8% females, median age: 47 years, interquartile range (IQR): 39-50.5) and 33 healthy controls (81.8% female, median age: 48 years, IQR: 35-53.5) were analysed for differences in P-selectin and PAC-1 (activated glycoprotein IIb/IIIa) positivity (%) and surface expression of P-selectin and PAC-1 (mean fluorescence intensity (MFI)) *in vivo* and response to *ex vivo* stimulation with different agonists by whole blood flow cytometry.

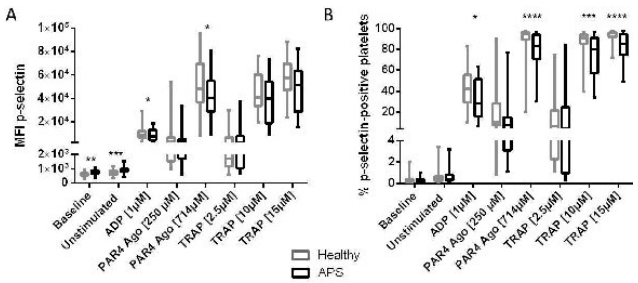

FIGURE 1 Surface expression of P-selectin in patients with APS compared to healthy controls

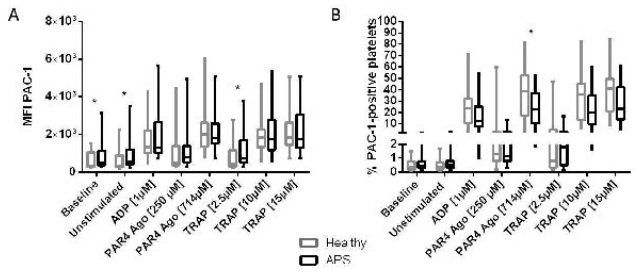

FIGURE 2 Surface expression of PAC1 in patients with APS compared to healthy controls

Results: Increased baseline but few significant changes to *ex vivo* stimulation in platelet surface expression of p-selectin (Figure 1A) and PAC-1 (Figure 2A) were detected in patients with APS compared to controls. No difference of baseline p-selectin and PAC-1 positivity could be found, however, platelets from patients with APS were less reactive to *ex vivo* stimulation with agonists measured on P-selectin (Figure 1B) and PAC1 (Figure 2B) positive platelets.

Conclusions: Patients with APS expose increased baseline platelet activation but decreased platelet reactivity, presumably as a consequence of pre-activation. Together with our previous findings that patients with APS have an altered "prothrombotic" platelet proteome (Hell et al. *Exp Mol Med*, 2020), the present results support the hypothesis that platelets are involved in thrombotic complications in patients with APS.

PB1900 | Observational Study of Outcomes in Patients with Antiphospholipid Syndrome Anticoagulated with Rivaroxaban

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Background: In response to the TRAPS study, the MHRA have recommended avoidance of DOACS in all patients with antiphospholipid syndrome.

Aims: We performed a retrospective study of thrombotic and haemorrhagic outcomes in patients with APS and VTE receiving rivaroxaban in our centre.

Methods: Patients newly diagnosed with APS without prior exposure to warfarin between November 2016 and August 2019 and treated with rivaroxaban were included. APS was diagnosed based on persistent positivity of LA and/or ACA Ig over a 12 week period. We reviewed clinical records for evidence of thrombotic and haemorrhagic events whilst on rivaroxaban with reference to ISTH criteria of thrombosis and major, clinically relevant non-major bleeding (CRNMB) and minor bleeding episodes.

Results: 31 cases were identified. 30 cases were single positive, 1 double positive. Amongst 30 single positives, the presenting clinical feature was DVT in 53.3% (n=16), PE in 36.6% (n=11) both PE and DVT in 6.6% (n=2) and ischaemic stroke in 3.3% (n=1). No new venous or arterial thrombotic events were reported throughout the study period. Median treatment duration with rivaroxaban was 723 days and total follow up period was 21,691 patient days. No major bleeding occurred, 6.6% (n=2) patients reported CRNMB. Rate CRNMB was approximately 1 in 10,000 days.

Conclusions: In our study no new thrombotic events occurred in 21,691 days of treatment in patients with newly diagnosed single positive APS treated with rivaroxaban compared with 12% in 59 patients followed for median 569 days in TRAPS. Rates of major bleeding and CRNMB were also lower than reported in TRAPS. This small retrospective study suggests that the event rate in non-triple positive APS patients on rivaroxaban is less than that seen in TRAPS. Our results suggest further large prospective studies are required

to address whether the TRAPS data is generalisable to non-triple positive APS.

PB1902 | Laboratory versus Point-of-Care INR Measurement in Patients with Lupus Anticoagulant - A Retrospective Cohort Analysis

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Background: Patient self-testing (PST) and self-management (PSM) using point-of-care (POC) devices, have been found to improve the quality of anticoagulation, reduce thromboembolic events and all-cause mortality, and is the recommended mode of VKA monitoring by the American society of hematology. Accrued evidence supports the accuracy of LAC insensitive thromboplastins for INR testing in patients with lupus anticoagulant (LAC), however this may not be generalized for patients monitored by POC devices.
Aims: To investigate the accuracy of POC devices for measuring INR in LAC positive and negative patient cohort followed at our outpatient clinic.
Methods: We retrospectively analyzed INR results of consecutive VKA treated patients (with and without evidence of LAC) followed at our clinic and had their INR checked simultaneously by both POC device (CoaguChek® XS) and our laboratory. We used linear regression analysis to evaluate the correlation between INR results from the POC device versus laboratory INR in patients with and without evidence for LAC. We then compared the regression lines by ANCOVA.
Results: We identified 472 paired samples from 102 patients of which 44 (39.2%) were LAC positive treated with VKAs mainly for venous and arterial thrombosis with a median age of 45 years. The ANCOVA full model analysis showed a very good correlation between INR results obtained by the POC device and laboratory INR results in both LAC negative and LAC positive patients (figure). The presence of LAC had no effect in the model on the correlation between POC and lab INRs (p=0.365) while the POC INR results had a very significant effect (p=0.0001) and explained 77% of the variance in lab INR results.
Conclusions: The correlation between INR results obtained by the POC device and the laboratory was comparable for both LAC positive and negative patients, suggesting POC devices may be used for PST and PSM regardless of LAC status.

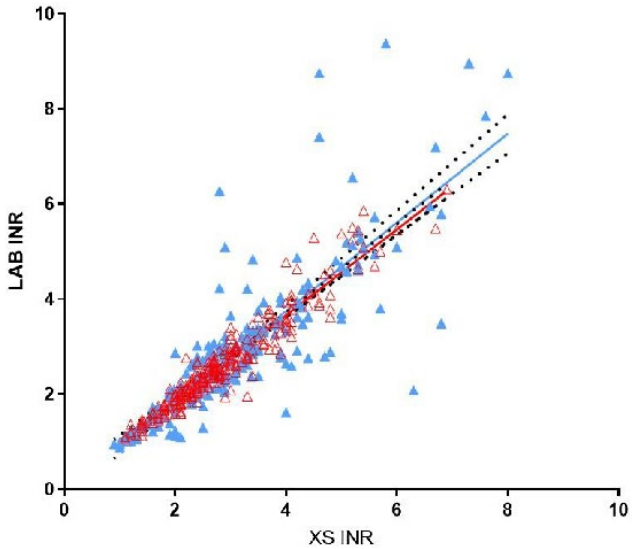

FIGURE 1 Regression analysis, Laboratory INR versus POC device INR, LAC positive (full symbols) and LAC negative (open symbols) patients

PB1903 | The Prothrombin/MHC II Complexes on Cell-Surface of Monocytes Is the Antigenic Targets in Antiphospholipid Syndrome

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[1]*Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Department of Rheumatology, Endocrinology and Nephrology, Sapporo, Japan;* [2]*Research Institute for Microbial Disease, Osaka University, Department of Immunochemistry, Suita, Japan*

Background: Antiphospholipid syndrome (APS) is an autoimmune disease characterized by thrombotic events and pregnancy complications with persistently positive antiphospholipid antibodies (aPL). Phosphatidylserine-dependent antiprothrombin antibodies (aPS/PT) recognize the phosphatidylserine/prothrombin (PS/PT) complex and are strongly correlated with APS. Recently, the complexes of misfolded proteins with major histocompatibility complex class II (MHC II) molecules are considered as major targets of autoantibodies in autoimmune diseases.
Aims: This study was investigated weather misfolded prothrombin (PT) are transported to the cell surface by MHC II in procoagulant cells, consequently being targeted by aPS/PT.
Methods: 1) The interaction between PT and MHC II alleles was analyzed by flow cytometry (FCM) using the cells co-transfected with PT and different MHC II alleles.
2) Synthesis of PT in monocytes was evaluated in phorbol-12-myristate-13-acetate (PMA) treated THP-1 cells by western blotting and FCM.
3) Cell-surface transport of synthesized PT was investigated by FCM on THP-1 cells treated with PMA and interferon gamma.
4) The presence of cell-surface PT was evaluated on monocyte between APS patients and healthy controls by FCM.
Results: 1) Overexpressed PT/MHC II complexes were detected on THP-1 cell-surface by a mouse monoclonal aPS/PT. We confirmed the dependency of MHC II allele differences on aPS/PT binding.
2) PMA treated THP-1 cells synthesized PT, which showed stronger binding with aPS/PT compared with non-pathogenic monoclonal anti-PT antibody.

3) aPS/PT binding to PT/MHC II complexes was confirmed in co-stimulated THP-1 cells.

4) Cell-surface PT was detected on monocyte in APS patient and aPS/PT binding to the monocyte was confirmed.

Conclusions: We found that PT was synthesized under some conditions and transported to the cell-surface on monocyte in APS. The PT/MHC II complexes on cell-surface of monocytes might be considered as one of the antigenic targets for pathogenic aPS/PT.

PB1904 | Assessment and Comparison of Acquired Activated Protein C Resistance with Two Thrombin Generation Methods, CAT and ST-Genesia, in APS and SLE Patients

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Background: Acquired resistance to the anticoagulant actions of activated protein C (APCr) may contribute to thrombotic risk. Using the thrombin generation (TG) system and the CAT machine, we showed that antiphospholipid syndrome (APS) patients with previous venous thromboembolism and systemic lupus erythematosus (SLE) patients exhibit increased APCr.

Aims: The aim of this study was to evaluate the prevalence of APCr using the ST-Genesia system in the presence/absence of thrombomodulin (TM) in APS and SLE patients and to compare APCr results obtained with the CAT machine using rhAPC and Protac®.

Methods: In this cross-sectional study, patient groups tested comprised: 75 normal controls (NC), 68 thrombotic (single or double aPL positive) APS patients, 38 SLE non-thrombotic (17 aPL+, 20 aPL-), 23 APS pregnancy morbidity (PM), 15 triple aPL+ patients and 36 thrombotic controls were tested. Patients on anticoagulation were tested in 1:1: mixture with pooled normal plasma. Samples were tested with the ST-Genesia system using STG-ThromboScreen with/without TM and the CAT system using PPP-reagent (5-picomolar TF) (Stago) in the presence of rhAPC or Protac®. APCr defined as %inhibition of ETP below the 99th centile of NC; rhAPC (56%); Protac® (63%), -/+TM (38.9%).

Results: % inhibition of ETP in the presence of TM was significantly lower in APS patients (median, confidence intervals): 48.2% (40.7-55.5%) compared to thrombotic controls (66.3%, 61.7-72.3%, $p < 0.0001$), but there were no differences between APS and SLE patients. PM (36.9%, 21.9-49.0%) exhibited higher APCr (lower % inhibition of ETP) compared to thrombotic APS patients (p=0.03). APCr results with the ST-Genesia in the presence of TM were comparable to those obtained with the CAT machine with rhAPC or Protac® for all patient groups.

Conclusions: In APS and SLE patients, TG performed with CAT and with ST-Genesia provided similar results and highlighted potentially important differences in APCr between different clinical phenotypes.

PB1905 | Hydroxychloroquine Effect on the Immune System, Coagulation and Lipid Profile in Patients with Thrombotic Primary Antiphospholipid Syndrome

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UNICAMP, Campinas, Brazil

Background: Antiphospholipid syndrome (APS) is a pro-thrombotic autoimmune disease characterized by the occurrence of thrombosis or gestational complications associated with the presence of

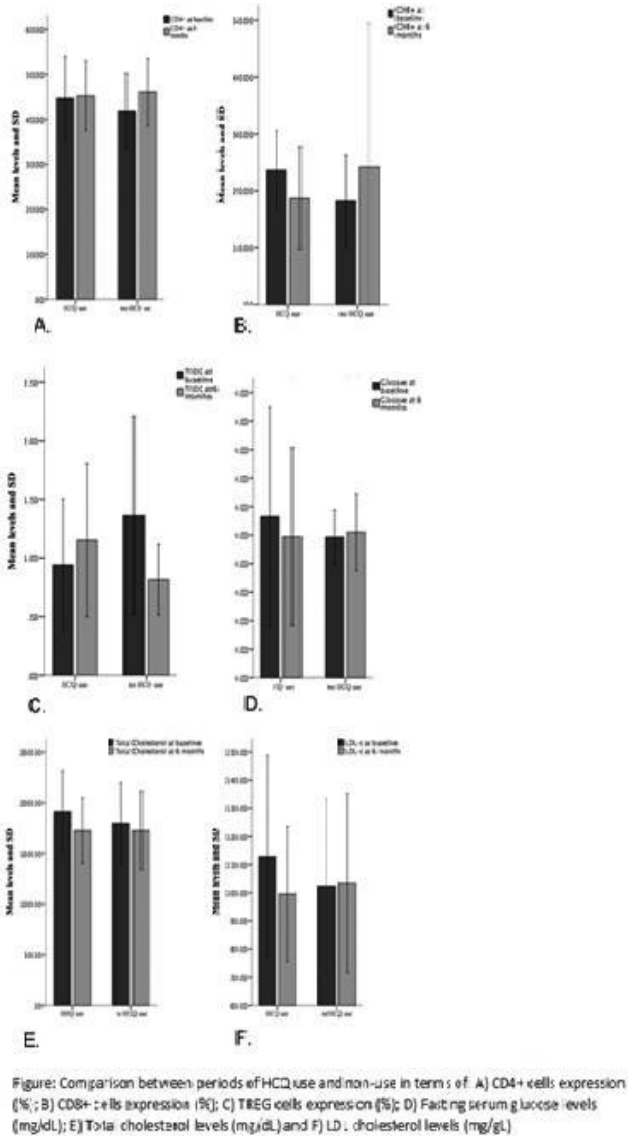

Figure: Comparison between periods of HCQ use and non-use in terms of A) CD4+ cells expression (%); B) CD8+ cells expression (%); C) TREG cells expression (%); D) Fasting serum glucose levels (mg/dL); E) Total cholesterol levels (mg/dL) and F) LDL cholesterol levels (mg/gL)

FIGURE 1 Comparison between periods of HCQ use and non-use

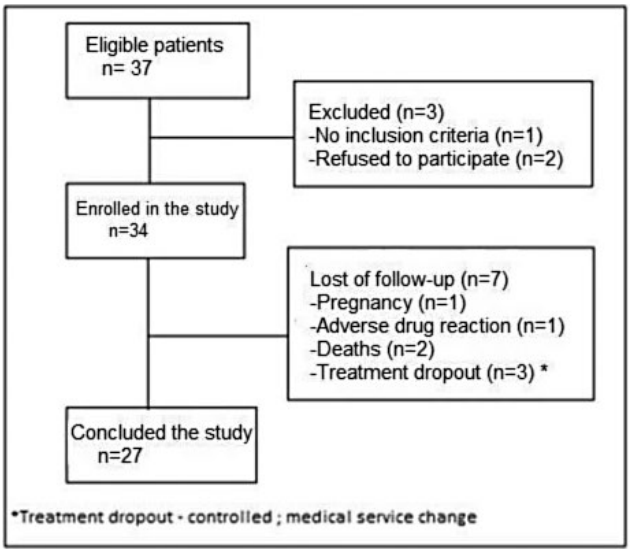

FIGURE 2 Patient Selection

antiphospholipid antibodies. The current treatment is based on secondary prophylaxis of thrombotic events with anticoagulant therapy. However, it is possible that treatments that alter the immune response have a role in controlling the manifestations of the disease.
Aims: HCQ could form an alternative treatment for t-PAPS if HCQ is proven to reduce the risk of thrombosis.
Methods: HCQ at 400 mg/day was given to anticoagulated t-PAPS patients for 6 months. After HCQ withdrawal and a wash-out period of 6 months, the same patients were followed for further 6 months. Blood samples taken at baseline and at the end of each 6-months follow-up period were analyzed employing flow cytometry and biochemical assays. The informed consent was obtained and the study was approved by UNICAMP ethics committee.
Results: Twenty-seven patients were included, mean age was 44 years, 44% had hypertension, 44% dyslipidemia, 77% had venous thrombosis, and 33% recurrent thrombosis. Lymphocyte counts decreased during HCQ use (mean change -318/mm3; 95%CI -438 to -198) and did not changed during non-HCQ use period. CD8+ and CD4+ cells decreased during HCQ treatment and remained unchanged during non-treatment period. In contrast, TREG (FoxP3+) cells increased during HCQ treatment (mean change 0.2U; 95%CI 0 to 0.4) and decreased in the period of non-HCQ treatment (mean change -0.6U; 95%CI -0.9 to -0.1). Serum glucose, total cholesterol and low density lipoprotein cholesterol decreased during HCQ use (7%, 9% and 12% respectively) and did not change substantially during non-HCQ period.
Conclusions: HCQ reduces cytotoxic T lymphocytes, increases regulatory T lymphocytes and decreases LDL cholesterol and serum glucose levels in t-PAPS. These findings may support that HCQ may contribute to reduce the risk of thrombosis.

PB1906 | IVF Outcomes in Women with Antiphospholipid Antibodies Circulation

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Background: Antiphospholipid antibodies (aPLa) have a multifaceted effect on the hemostatic system, damaging its entire protective links: the endothelial barrier, the function of natural anticoagulants, endogenous fibrinolysis, activating the platelet link of hemostasis and are associated with many obstetric complications and infertility. But there is little data about the frequency of aPLa circulation in women with in vitro fertilization (IVF) failures and is aPLa associated with a worse reproductive outcome?
Aims: To evaluate the frequency of aPLa in women undergoing assisted reproductive technologies (ART).
Methods: 267 women aged 23 to 45 years who were in the IVF program were examined. Of these, 178 women had IVF failures (one or more failed IVF attempts, the number of attempts ranged from 1 to 9) (group I) and 89 women with pregnancy after the IVF program (group II).
Results: aPLa circulation was diagnosed in 42.1% of patients of the group I. Attention is drawn to the high percentage of antibodies to β2-GPI-31.4% and to annexin V-24.7%. aPLA circulation was also detected in the group II in 19.1%. At the same time, lower aPLA titers were observed compared to group I (see table 1). All women received aspirin low doses before starting IVF procedure and LMWH during protocol.

TABLE 1 aPLA profile in women undergoing IVF program

aPLA profile	Women with IVF failure (I group), n=178, % (n)	Pregnancy after IVF (II group), n=89, % (n)	Control group, N=80, %(n)
APA (total)	42.1 (75)	19.1 (17)	3.4 (3)
Antibodies to cardiolipin (IgG/M)	8.9 (16)	3.4 (3)	1.3 (1)
LA circulation	19.6 (35)	4.5 (4)	1.3 (1)
Antibodies to annexin V (IgG/M)	24.7 (44)	10.1 (9)	0
Antibodies to β2GPI (IgG/M/A)	31.4 (56)	14.6 (13)	3.8 (3)
Antibodies to prothrombin (IgG/M/A)	13.5 (24)	7.9 (7)	0

Conclusions: Women with aPLA circulation demonstrate a higher rate of IVF failure and a worse reproductive outcome compared to women without aPLA. We believe that the circulation of aPLA is a temporary contraindication to ART and attribute the successful onset of pregnancy in group II, despite the presence of antiphospholipid antibodies, to the timely initiation of therapy. We consider that it is necessary to examine women who are planning a pregnancy using IVF for the presence of aPLA, as well as the need to start anticoagulant therapy before IVF protocol.

PB1907 | Monocyte Activation and Acquired Protein S Deficiency Promote Disseminated Intravascular Coagulation in a Patient with Antiphospholipid Antibodies

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Background: Acquired (i.e. antibody-mediated autoimmune) protein S (PS) deficiency is a rare and potentially life-threatening disorder. Concomitant occurrence of acquired PS deficiency and antiphospholipid antibody (aPL)-induced tissue factor (TF) expression in an individual has not yet been reported.
Aims: We describe the case of a 76-year-old woman with disseminated intravascular coagulation (DIC) associated with elevated aPL and acquired PS deficiency.
Methods: Serum IgM and IgG were purified by immunoprecipitation and analyzed for PS inhibitory activity by spiking normal human plasma (NHP). In addition, the stimulatory effect of patient-derived IgM and IgG on peripheral blood mononuclear cells (PBMCs) isolated from healthy controls was investigated. TF-specific procoagulant activity (PCA) of PBMCs and plasma microvesicles (MVs) was measured by single-stage clotting and chromogenic FXa generation assay, respectively.
Results: The patient initially presented with a painful reticular erythema indicating microvascular thrombosis and a consumptive coagulopathy (Table 1). Surgical and antibiotic treatment of concurrent sigmoid diverticulitis did not resolve DIC. Overt malignancy was excluded. The coagulopathy was controlled by continuous anticoagulation with low-molecular-weight heparin or a direct oral FXa inhibitor. Titers of IgM-aCL and IgM-anti-β2GPI were elevated, while tests for ANA and LA were negative. Free and total PS antigen were normal, but PS activity was largely reduced. Exclusion of FV gene mutation Leiden was consistent with acquired APC resistance. PS activity was significantly inhibited, when NHP was incubated with patient IgG, indicating a function blocking IgG autoantibody. In the patient's plasma, robust MV-associated TF PCA was detected, and ex-vivo stimulation with purified patient IgG, but not IgM, activated PBMCs from healthy donors with increased TF production and MV shedding.
Conclusions: In this case, autoimmune-mediated induction of monocyte TF in combination with acquired APC resistance and depletion of protein S activity caused a systemic coagulopathy, which could only be controlled by continuous anticoagulation.

PB1908 | Lupus Anticoagulant-Hypothrombinemia Syndrome (LAHS): A Case Report

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Background: About a hundred cases of lupus anticoagulant-hypothrombinemia syndrome have been reported. More common in children and women with systemic lupus erythematosus; sometimes no underlying condition is found.
Aims: Report of a case of infrequent Lupus Anticoagulant syndrome.
Methods: Clinical data available in the medical reports and laboratory findings are revised.
Results: We report a case of a female patient of 72 years referred to a haematology consult with prolonged prothrombin clot timing

TABLE 1 Key laboratory parameters

Parameter	Value	Reference range	Parameter	Value	Reference range
Platelet count	166×10^{9}/L	150-350 x 10^{9}/L	IgM-aCL	140 U/mL	< 10 U/mL
Prothrombin time	45.5%	80 - 130%	IgG-aCL	0.7 U/mL	< 10 U/mL
Fibrinogen	0.5 g/L	1.8 - 4.0 g/L	IgM-anti-β_2GPI	12 U/mL	< 7 U/mL
D-dimer	34 mg/L	< 0.5 mg/L	IgG-anti-β_2GPI	0.6 U/mL	< 7 U/mL
Free PS antigen	96.0%	60 - 130%	LA	negative	
Total PS antigen	73.0%	55 - 125%	ANA	negative	
PS activity	13.9%	55 - 125%	APC resistance	1.7	> 2.0
Total PC antigen	108.2%	65 - 140%	FV gene mutation	negative	

TABLE 1 Laboratory findings

PT ratio	1.4 (0.8-1.2)
PT mixing study	14.9 seconds, ratio 1.4; (after one hour incubation: 14.7 seconds, ratio 1.37)
aTTP	34 s (24-38)
aTTP ratio	1.1 (0.8-1.2)
Russell	2.54 (>1.20 positive)
Silica	1.16 (>1.20 positive)
Factor II	60% (70-130)
Factor x	67% (70-130)
Anticardiolipin antibody (IgM)	Positive
Anti β2 GPI antibody (IgM)	Positive

since May 2016. The patient was asymptomatic and the consult was previous to a knee prosthesis surgery. Previous medical conditions were two normal pregnancies, localized breast cancer with adjuvant hormonal therapy with letrozole, dyslipidemia, chronic venous insufficiency and hypertension. No previous haemorrhagic or thrombotic events were reported.

Laboratory test performed shows a normal activity for clotting factor VII. Immediate and incubated mixing study indicated the presence of an inhibitor. Russell anticoagulant was strongly positive with no interferences documented and anticardiolipin and beta-2-glycoprotein antibodies were both positive.

Reducing the thrombotic risk after the surgery and referral to a rheumatologist was recommended to the patient. No rheumatologic condition was diagnosed and the surgery performed in December of 2019 had no immediate complications. The patient was discharged after three days of hospitalization, with daily prophylactic enoxaparin 60 mg.

Conclusions: Lupus anticoagulant is associated with thrombotic events but some cases of bleeding have been reported in LAHS. There is no specific treatment other than treating the underlying condition and supportive care in case of bleeding.

Our patient had several risk factors for thrombosis: hypertension, dyslipidemia, hormonal therapy with letrozole, chronic venous insufficiency and knee surgery. Prophylaxis with enoxaparin 60 mg once a day was effective to prevent thrombotic events with no reported bleeding events other than minimum haematoma in the operated knee which is common after surgery.

PB1909 | Cohort of Patients with Triple Antiphospholipid Antibodies: Differences between Asymptomatic and Symptomatic Individuals

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Background: Antiphospholipid antibodies are associated with the antiphospholipid syndrome (APS) but are also found in asymptomatic individuals. The risk of complications increases with the number of positive antibodies; however, some asymptomatic individuals have Lupus anticoagulant (LA), Anticardiolipin (ACA) and Anti β2 Glycoprotein I (aB2GPI) antibodies or "triple positivity" (TP). The reason why some remain asymptomatic and others develop APS is unknown. We hypothesized that in asymptomatic individuals the antibodies exhibited a different behavior (i.e.: lower titers, remained positive for a shorter time).

TABLE 1 Patients characteristics and antibodies at diagnosis and at the end of follow-up

Group	Reasons for referral	Sex, N (%)	Age, years (SD)	Laboratory*	ACA IgG (95% CI)	ACA IgM (95% CI)	aB2GPI IgG (95% CI)	aB2GPI IgM (95% CI)	LA (%)
Asymptomatic patients	Prolonged APTT or false positive VDRL	Female: 17 (94) Male: 1 (6)	40.3 (12.3)	At diagnosis	40 (18- 63)	68 (44- 91)	19 (5- 32)	111 (74- 148)	18 (100)
				At end of follow-up	39 (16-62)	77 (22-132)	29 (11-46)	89 (53- 125)	15 (88)
Thrombotic APS	Venous or arterial thrombosis	Female: 10 (71) Male: 4 (29)	48 (13.5)	At diagnosis	49 (23- 74)	81 (30-131)	33 (10- 56)	81 (48- 113)	14 (100)
				At end of follow-up	41 (16-66)	71 (40- 102)	25 (1-50)	66 (23- 110)	13 (93)
Obstetrical APS	Adverse pregnancy outcomes	Female: 7 (100)	38.8 (5.4)	At diagnosis	6 (1-12)	57 (31-83)	4 (1- 8)	62 (37- 87)	7 (100)
				At end of follow-up	6 (1-12)	59 (48- 70)	6 (1-17)	51 (23- 79)	6 (86)

* In each group: ACA IgM vs IgG and aB2GPI IgM vs IgG: $p < 0.05$

Aims: To assess clinical characteristics and antibody behavior during follow-up in a cohort of TP patients. To compare asymptomatic versus thrombotic and obstetrical APS patients.

Methods: We evaluated all patients who tested TP between January 2015 and December 2019. Inclusion criteria: LA and high-titer ACA and aB2GPI at least two lab determinations. Patients' characteristics and reasons for referral were recorded. Mean follow-up and difference in antibodies titers or positivity at diagnosis and at the end of follow-up were calculated and compared (t test).

Results: During the study period, 126 patients were diagnosed TP; 24 were lost for follow-up after the first determination and 63 did not remain TP in the second test (mean time between labs: 160 days). Thirty-nine patients met the inclusion criteria, 35 were female. Mean age: 42 (SD 14) years. Mean follow-up time: 3.5 (SD 1.8) years. Table 1 shows patients characteristics according to clinical presentation and lab at diagnosis and at the end of follow-up. 28% (5/18) of asymptomatic individuals had an autoimmune disorder. In all groups, IgM isotypes (ACA and aB2GPI) were significantly higher than IgG isotypes, at diagnosis and at the end of follow-up.

Conclusions: No significant differences in antibodies titers or behavior were observed between APS patients and asymptomatic individuals. Higher IgM isotypes observed in all groups might suggest an inflammatory or infectious component.

PB1910 | Does the von Willebrand Factor: ADAMTS-13 Axis Differ between Antiphospholipid Syndrome and Systemic Lupus Erythematosus?

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Background: Dysfunction in the VWF:ADAMTS13 axis plays a critical role in thrombosis, and has been proposed to be dysregulated in antiphospholipid syndrome (APS) and systemic lupus erythematosus (SLE) patients. However, possible differences between APS and SLE and associations with thrombotic history remain undefined.

Aims: To establish plasma VWF, ADAMTS13 and ADAMTS13 autoantibodies levels in both disorders and possible differences; and associations with antiphospholipid antibody (aPL) status and thrombotic history in a cross-sectional study.

Methods: Plasma VWF and ADAMTS13 antigen levels were measured by ELISA in 97 APS, 49 SLE (aPL-), 29 thrombotic controls (TC) and 66 healthy controls (HC). Samples with low ADAMTS13 antigen (< 100 ng/mL) were tested for IgM anti-ADAMTS13 antibodies and ADAMTS13 activity. Clinical history and aPL status were accessed from medical records.

Results: In this ongoing study, VWF antigen levels were significantly higher in APS (median 1.3 IU/ml; 95% CI 1.1-1.5; $p< 0.0001$), SLE (1.8 IU/ml; 1.6-2.0; $p< 0.0001$) and TC (1.4 IU/ml; CI 1.1-1.8; $p< 0.0001$) compared to HC (mean: 0.8 IU/ml; 0.7-1.0). SLE patients demonstrated higher VWF levels than APS ($p=0.001$). ADAMTS13 levels were significantly lower in APS (138.9; 123.7-157.1; $p< 0.0001$) and SLE (175.3; 154.2-205.3; $p=0.01$) patients compared to HC (234.8; 197.7-283.3), and significantly lower in APS compared to SLE patients ($p=0.0002$). 58% (11/19) of APS patients tested so far were positive for IgM anti-ADAMST13 antibodies. No differences in plasma VWF:Ag and ADAMTS13 antigen levels were observed between aPL+ and aPL- patients. No differences in VWF:Ag levels were observed between APS patients with venous (n=61) and arterial (n=25) thrombosis. Notably, APS patients with venous thromboembolism (VTE) had higher VWF:Ag compared to obstetric morbidity ($p=0.01$).

Conclusions: The mechanisms underlying VWF:ADAMTS13 dysregulation appear to differ between SLE and APS and in APS with VTE or obstetric morbidity, with these differences possibly explained by differential underlying pathogenic mechanisms.

PB1911 | Antiphospholipid Antibodies "Multi-Positivity" in Women with Thromboembolism

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Background: Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by vascular thrombosis and/or pregnancy morbidity in the presence of circulating antiphospholipid antibodies (aPL) against β2-glycoprotein I (β2GPI) and cardiolipin (aCL) together with the functional assay lupus anticoagulant (LA). Among the three criteria aPL test, LA has been appointed as the strongest predictor of clinical events, raising the risk of thrombosis by approximately 4-fold. It's well known that triple positivity (LA+anti-B2GpI+aCL) increases the risk of thrombosis. We supposed that the presence of other aPL is also clinically and laboratory valuable approach.

Aims: To evaluate the importance of so called non criteria aPL.

Methods: We examined 124 women with thromboembolism. In 22% thrombosis was during pregnancy, 30% - postpartum, 38% - after oral contraceptives or IVF protocol, 10% - after surgical intervention. In 82% of DVT -it was the left leg involvement. In 9.7% - DVT of lower leg, 12% - common femoral vein, 70% - iliofemoral DVT, 5.6% - inferior vena cava, 0.8% - cerebral veins, 0.8% - mesenterial vessels, 0.8% - hepatic veins, 1.6% - retinal veins, 1.6% - upper extremities

veins and 4% - DVT-associated massive pulmonary embolism (PE) and 1.6% - with recurrent PE. All patients we examined for the presence of different aPL.

Results: LA was in 22.9%, anti-β2GPI - 26.6%, aCL - 18.5%, anti-annexin V - in 9.7%, anti-prothrombin - in 12.1%. In 7 women (5.6%) was the combination of LA and aCL, in 3.2% - both LA and anti-β2GPI abs. Triple positivity - in 8.9%. In 5.6% we diagnosed the circulation of all examined antibodies.

Conclusions: Triple positive patients display the highest vascular hazard. But sometimes the screening only for the routine aPL can miss and delay diagnosis of APS and treatment. May be in future the criteria of APS will be probably re-evaluated and the new antibodies included.

PB1912 | Thrombotic Outcomes in Pregnant Women with Antiphospholipid Antibodies in a Tertiary Referral Centre

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Background: Antiphospholipid syndrome (APS) is defined by the presence of antiphospholipid antibodies (aPL) with thrombosis and/or obstetric morbidity. aPL-associated thrombosis can affect the venous and arterial systems and also the microvasculature. We have an established antithrombotic management in pregnancy protocol aiming to prevent maternal thrombosis and obstetric morbidity.

Aims: To evaluate thrombotic outcomes in pregnancy and post partum among women with antiphospholipid antibodies followed in a tertiary care referral centre with a set protocol.

Methods: This is a retrospective observational study. Data were collected from the Lupus & Thrombosis units at St. Thomas' Hospital, London, UK. Women were identified from clinic lists during 2010-2017. Women persistently positive for antiphospholipid antibodies were included if complete pregnancy outcome data were available.

Results: Five-hundred-and-twenty-one pregnancies in 374 women were included in the study. Thrombotic complications were encountered in 20 (4%) pregnancies, including 19 venous events and one arterial thrombosis. Five (25%) occurred in the first trimester, four (20%) in the second trimester, five (25%) in the third trimester. Six (30%) presented in the post-partum period (median 4.5 (range 1-12) weeks): two cases occurred at 8 weeks post-partum (after cessation of 6 weeks thromboprophylaxis) and one at 12 weeks post-partum (after 6 weeks of post-partum thromboprophylaxis). History of prior thrombosis was noted in 141 (27.1%) pregnancies, and was significantly associated with recurrent thrombosis despite anticoagulation therapy as compared to those with no prior thrombotic history (7.7% vs. 2.4%, P=0.009). Table 1 describes the 20 thrombotic cases encountered.

Conclusions: This study demonstrates that despite optimal and vigilant care, up to 4% of women with antiphospholipid antibodies developed thrombosis during the antenatal and postpartum course. Women with prior thrombotic history were identified as a high-risk group for recurrent thrombosis despite anticoagulation treatment. Further studies are warranted to better delineate the optimal strategy to reduce thrombosis rate in this subset of women.

TABLE 1

CASE #	Maternal age	Time of Thrombosis	Dose of LMWH at the time of event	Type of Thrombosis	Which imaging was used to confirm
1	43	3rd Trimester	Prophylactic	PE	VQ-Spect
2	37	2nd Trimester	Not given	DVT	Duplex
3	39	2nd Trimester	Prophylactic	DVT	Duplex
4	24	3rd Trimester	Not given	DVT	Duplex
5	25	Post Partum, 1 week	Not given	PE	CT
6	22	2nd Trimester	Not given	DVT	Duplex
7	32	1st Trimester	Prophylactic	cerebral APS	None
8	40	1st Trimester	Not given	PE	CT
9	40	1st Trimester	Prophylactic	PE	VQ-Spect
10	30	Post Partum, 8 weeks	Not given	DVT	Duplex
11	28	Post Partum, 8 weeks	Warfarin*	DVT	Duplex
12	36	Post Partum, 1 week	Prophylactic	IVC and left adrenal vein	CT
13	36	1st Trimester	Not given	SVT left leg in GSV	Duplex
14	35	Post Partum, 1 week	Prophylactic	DVT	Duplex
15	33	3rd Trimester	Not given	DVT	MRI
16	35	3rd Trimester	Not given	PE	VQ Spect
17	38	1st Trimester	Prophylactic	PE	VQ Spect
18	26	3rd Trimester	Not given	Sagittal sinus	MRI
19	39	Post Partum, 12 weeks	Prophylactic	DVT	MRI
20	36	1st Trimester, just after LMWH stop	Not given	DVT	Duplex

*Out of range INR-1.5, at the time of event
Abbreviations: APS-antiphospholipid syndrome; CT-computed tomography; DVT-deep vein thrombosis; GSV-great saphenous vein; IVC-inferior vena cava; LMWH-low-molecular weight heparin; MRI-magnetic resonance imaging; PE-pulmonary embolism; SVT- superficial vein thrombophlebitis

PB1913 | MicroRNAs as Potential Biomarkers in Thrombotic Primary Antiphospholipid Syndrome

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Background: The presence of antiphospholipid autoantibodies (aPL) is mandatory for the diagnosis of antiphospholipid syndrome (APS) but not sufficient to predict the risk of APS-related thrombosis. The identification of additional biomarkers is warranted and microRNAs, which are small non-coding simple chair RNA molecules, may play a role in this context.

Aims: The aim of this study was to determine microRNAs differentially expressed in thrombotic primary APS (t-PAPS) in comparison with controls and to evaluate to discriminate capacity of these microRNAs.

TABLE 1 Clinical and laboratory features of t-PAPS n=21

Age in the diagnosis, mean (standard deviation)	33 (16)
Non-provoked thrombosis,n (%)	15 (71,4)
Venous thrombosis, n (%)	11 (52,4)
Multiple thrombosis, n (%)	6 (28,6)
Time elapsed since the last thrombosis in months, mean (standard deviation)	34.3 (28.4)
Lupus anticoagulante, n (%)	21 (100,0)
Anticardiolipin antibody IgM (%)	3 (14,3)
Anticardiolipin antibody IgG (%)	9 (42,8)
Anti-β2-glycoprotein I antibody (%)	16 (76,2)
Triple positivity for aPL, n (%)	9 (42,8)

Methods: In this exploratory study, patients and controls were enrolled at the Hematology Center at University of Campinas, Brazil. Differentially expressed miRNAs were identified by MicroArray analysis in Affymetrix workstation. Cut-off values were determined by specificity and sensitivity analysis. A prediction model was built with the most differentially expressed microRNAs between t-PAPS and controls. The discriminate capacity of the model was tested using Receiver-Operating Characteristic test (ROC).

Results: 21 t-PAPS and 21 controls we included. The mean age was 40 (SD 12) in controls and 37 (SD 13) in patients, 52% of patients and controls were women and cardiovascular risk factors were more prevalent among t-PAPS (43% versus 10%). Clinical and laboratory features of t-PAPS are shown in Table 1. From the 24 miRNAs differentially expressed between the two groups, 5 were selected to compose the prediction model. The Beta regression coefficients were 0.227 for hsa-miR-149-3p; 3.536 for hsa-miR-1273 g-3p; -1.252 for hsa-miR-4687-3p; 2.968 for hsa-miR-4497 and 1.848 for hsa-miR-4516. The area under the curve was 0.933 (95% IC 0.854-1.000) for the model, as shown in Figure 1.

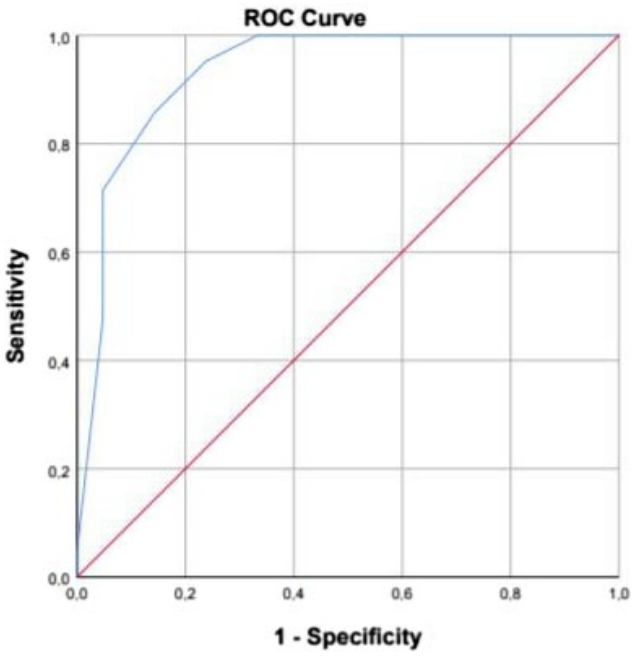

FIGURE 1 ROC plot of the 5 miRNAs differentially expressed in t-PAPS as compared to controls.Area under curve 0.933 (95% IC 0.854-1.000)

Conclusions: We identified a cluster of 5 miRNAs with discriminatory ability to identify t-PAPS in relation to controls. This study demonstrates that miRNAs have potential role as biomarkers for t-PAPS. However, the results must be validated in a larger and external population.

PB1914 | Identification of microRNAs Potentially Involved in Primary Antiphospholipid Syndrome Related Thrombosis

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Background: The mechanisms behind the severe hypercoagulable state in Antiphospholipid Syndrome (APS) are not fully elucidated. Some individuals with persistent antiphospholipid antibodies (aPL) remain asymptomatic whereas others develop thrombotic complications, which suggests that the presence of aPL is not sufficient to trigger thrombosis.

Aims: The aim of this study was to evaluate the association between miRNAs, which are small non-coding simple chair RNA molecules, with thrombosis in patients with primary APS (t-PAPS).

Methods: In this exploratory study, t-PAPS patients and controls were enrolled at the Hematology Center at University of Campinas, Brazil. Plasma miRNAs were extracted using miRNeasySerum/Plasma Kit (Qiagen). Differentially expressed miRNAs were identified by MicroArray analysis in Affymetrix workstation. Functional analysis were performed using Transcriptome Analysis Console ™, Ingenuity Pathway Analysis™ and Cytoscape softwares. Local Ethical Committee (CAAE: 83102317.5.0000.5404).

TABLE 1 Clinical and laboratory features of t-PAPS n=21

Age in the diagnosis, mean (standard deviation)	33 (16)
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Anticardiolipin antibody IgG (%)	9 (42,8)
Anti-β2-glycoprotein I antibody (%)	16 (76,2)
Triple positivity for aPL, n (%)	9 (42,8)

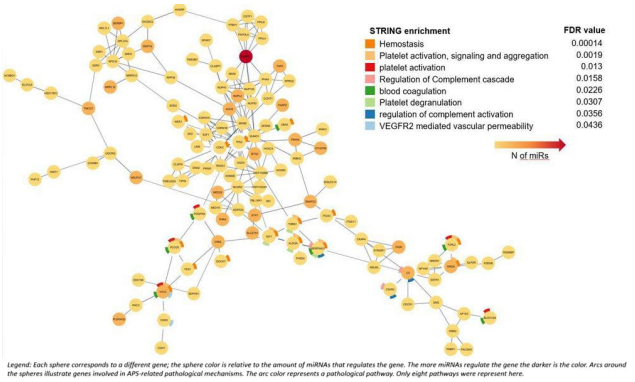

FIGURE 1 Predicts interactions between microRNAs target genes in biological context (confidence index > 0.8)

Results: 21 t-PAPS and 21 controls we included. The mean age was 40 (SD 12) in controls and 37 (SD 13) in patients, 52% of patients and controls were women and cardiovascular risk factors were more prevalent among t-PAPS (43% versus 10%). Clinical and laboratory features of t-PAPS are shown in Table 1. 24 miRNAs were differentially expressed between the two groups; 19 were up-regulated and five were down-regulated in t-PAPS as compared to controls. Only one (has-miR-4516) has been described in APS before. Together, the miRNAs have 1978 validated targets. Figure 1 shows the potential biological interactions between the miRNAs, their targets and pathways that can explain APS-related thrombosis. Particularly, five miRNAs regulate the expression of natural anticoagulants (tissue factor pathway inhibitor, antithrombin, thrombomodulin) and five regulate the expression of complement inhibitors.

Conclusions: We identified 24 miRNAs differentially expressed between t-PAPS and controls that are potentially involved in the regulation of coagulation and complement cascade. Presence of miRNAs and their and potential pathological mechanisms will be confirmed in a larger PAPS population.

PB1915 | Secondary Thromboprophylaxis in Antiphospholipid Syndrome Patients with Arterial Thrombosis: A Systematic Review and Meta-analysis

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Background: Arterial thrombosis is one of the thrombotic complications in antiphospholipid syndrome (APS). Antithrombotic options as a secondary thromboprophylaxis in these patients remain controversial.

Aims: We assessed the effective and safety of the secondary thromboprophylaxis in APS patients who presented with arterial thrombosis.

Methods: We conducted a systematic review and network meta-analysis by searching MEDLINE, EMBASE and Cochrane library databases from inception to December 2019. Randomized-controlled trials (RCT), prospective or retrospective trials investigating APS with arterial thrombosis were included. Patients must receive either of following treatments: antiplatelet, warfarin, combined antiplatelet and warfarin or direct oral anticoagulants (DOACs). A network meta-analysis was performed with a frequentist method. The outcomes were recurrent overall thrombosis, arterial thrombosis, venous thrombosis and major bleeding.

Results: Ten studies were included. There were 4 RCTs, 1 prospective and 5 retrospective studies, comprising 605 participants. Twelve stepwise comparisons from 8 studies were included in the network meta-analysis of recurrent overall thrombosis. When compared with antiplatelet, combined warfarin and antiplatelet was associated with decreased recurrent overall thrombosis (odds ratio [OR] 0.41, 95%CI 0.19 to 0.89), Figure 1A. From 8 studies with 10 pairwise comparisons, DOAC and moderate-intensity warfarin were associated with increased risk of recurrent arterial thrombosis comparing to antiplatelet with OR 10.84 (2.41 to 48.67) and OR 4.81 (1.73 to 13.40) respectively, Figure 1B. From 6 studies with 8 stepwise comparisons, none of treatment was significantly superior to antiplatelet in prevention of recurrent venous thrombosis, Figure 1C. From 5 studies with 7 pairwise comparisons, there was no significant difference of the rate of major bleeding from DOAC, moderate-intensity warfarin and combined warfarin and antiplatelet when compared to antiplatelet, Figure 1D.

Conclusions: Combined warfarin and antiplatelet was associated with reduced recurrent overall thrombosis. Whereas, DOAC and moderate-intensity warfarin were associated with an increased risk of recurrent arterial thrombosis compared to antiplatelet.

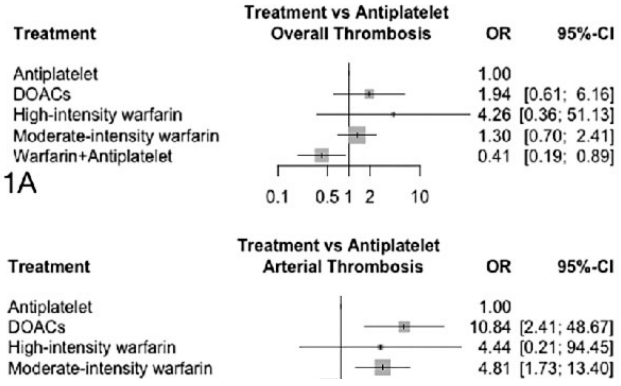

FIGURE 1 Forest plots of network meta-analysis for overall thrombosis (1A), arterial thrombosis (1B), venous thrombosis (1C) and major bleeding (1D)

PB1917 | What to Expect about Antiphospholipid Positivity in Otherwise Healthy Patients: Is There a Real Risk of Thrombosis?

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Background: Definition of antiphospholipid syndrome (APS) comprises the presence of antiphospholipid antibodies (aPL) associated with thromboembolic events (TE) or obstetric morbidity (OM). Persistent aPL are occasionally found in individuals without previous TE, thus raising uncertainty about its clinical significance. The risk of first TE in asymptomatic aPL carriers is still unknown. So far triple positivity (lupus anticoagulant (LA), anticardiolipin antibodies (ACA) and anti-β2-glicoprotein I (aβ2GPI) positiveness) has been categorized as high-risk profile.
Aims: Evaluate the incidence of TE in asymptomatic aPL carriers.
Methods: We evaluated retrospectively/prospectively 88 individuals (69 women, 19 men), with a median age at diagnosis of 34 years-old (range 3-80) and laboratorial diagnosis of aPL positivity. Patients were excluded if aPL positivity was not confirmed in a second sample or in follow-ups lessen than 2 years.
Results: Studied population's characteristics are depicted on Table 1. Profiles of aPL are described in Table 2. Our follow-up was of 757 individuals/year with average duration of 8.6 years (2-30 years). Reviewing data, 5 patients had TE during this period. Two LA+ patients with high cardiovascular risk had a stroke; the three remaining developed deep vein thrombosis after surgery (1 patient LA+, 1 patient triple positive, 1 patient LA+ and aβ2GPI IgG+).
Conclusions: In our cohort, nearly half of the patients had autoimmune diseases, which we cannot dissociate from the positivity found for aPL.
LA is considered the most important risk predictor for TE in APS. However, its isolated positiveness seems not to correlate with an increased thrombotic risk.
In our population, the incidence of TE was approximately 0.66%/year, not differing that much from the average Caucasian population of the same age (around 0.4%/year)[1].
Regarding triple positive profile's significance, our results are against the majority of the literature, suggesting that triple positivity alone might not be the determining risk factor for TE.
[1]Pengo V, Blood 2015.

TABLE 1 Population's characteristics

Sex (Female/Male)	69/19
Median age at diagnosis (years-old)	34 (3-80)
Auto-immune diseases (n=45)	
SLE/others	30/15
aPL profile	
Isolated LA positivity	38
Triple positivity	33
ACA and/or aß2GP positivity*	17
Mean follow-up (years)	8.6 (2-30)

aPL: antiphospholipid antibodies; LA: lupus anticoagulant (LA); ACA: anticardiolipin antibodies; aß2GPI: anti-ß2-glicoprotein I antibodies; SLE: Systemic Lupus Erythematous; *ACA IgG / ACA IgM / aβ2GPI IgM / ACA IgM and aβ2GPI IgM (6/2/7/2)

PB1918 | Treating Antiphospholipid Syndrome Patients with Vitamin K Inhibitors in Real Life; Long Term Outcomes from a Single Centre Retrospective Study of 101 Patients

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Background: Antiphospholipid syndrome (APLS) is characterised by venous/arterial thrombosis with sometimes devastating effects. Vitamin K antagonists (VKAs) remain the mainstay for secondary prevention of thrombosis in APLS patients(pts). Higher-intensity VKAs (target INR: 3-4) though used in high risk APLS pts do not seem to further reduce the risk of recurrent thrombosis in randomized controlled trials.
Aims: We sought to distinguish APLS pts on VKAs for >4 months and record their outcomes.
Methods: We identified 101 (female:71/male:30) APLS pts in our anticoagulant database. Median age at VKA initiation was 47.5

TABLE 2 Characterization of the different aPL profiles

	Isolated LA positivity (n=38)	Triple positivity (n=33)	ACA and/or aß2GP positivity (n=17)
Sex (Female/Male)	28/10	27/6	14/3
Median age (years-old)	48 (8-80)	32 (7-75)	33 (3-61)
Context of aPL detection	19 extended aPTT / 6 AID / 2 OM	4 extended aPTT / 21 AID / 4 OM	8 AID / 5 OM
ASA prophylaxis (Yes/ No)	15/23	26/7	13/4
AID	16	21	8
LA intensity	1.6	1.9	does not apply
Mean follow-up (years)	9.2	8.3	7.9

ASA: acetylsalicylic acid; AID: autoimmune disease; OM: obstetric morbidity; SLE: systemic lupus erythematous.

(18-89)years. The index events to offer anticoagulation were arterial (50/101),venous (30/101) and mixed arterial/venous (21/101). 19 pts were triple positive while 32 suffered recurrent thrombosis;33 pts had a coexisting autoimmune disease including 19 suffering from lupus. The majority (64%) were put on VKA with a target INR of 2.5. 12/101 pts were offered dual anticoagulation (VKA+low dose aspirin/clopidogrel). Median follow-up time was 46 (4-259) months while the median time within therapeutic range (TTR) was 57%.

Results: Recurrent thrombosis occurred in 13/101 (11.9%)pts with median time of 38.5 (34.25- 68.5)months. The majority of thromboses were arterial (stroke:5,myocardial infarct:2). At the time, 12/13pts were on anticoagulation with an INR target ≥3.0; 8/13pts had a TTR< 65%. Post-recurrence, increasing the INR target was the most common therapeutic decision (5/13pts). Still, 3/13pts suffered a further event. Catastrophic APLS was recorded in 2 cases. Bleeding occurred in 15/101pts (14.9%), major bleeds were recorded in 11pts (10.7%) and 6/11 had an INR target ≥3.0. Median time to major bleeding was 52 (27.5-73.75)months. Overall, the 5-year probability for recurrent thrombosis was recorded at 7.8% while major bleeding risk was 4.9% respectively.

Conclusions: Our results confirm that in APLS, quality of anticoagulation (TTR) is probably more important than setting a higher INR target. APLS pts represent a heterogeneous group with different clinical phenotypes; we are still some way from identifying the optimal anticoagulation strategy.

PB1919 | Anti-β2-Glycoprotein I and Anti-Prothrombin Antibodies Cause Lupus Anticoagulant by Interfering with Factor Xa-Mediated Factor V Activation

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Background: The presence of lupus anticoagulant (LA) is strongly correlated with an increased risk of thrombosis. LA is a prolongation of the clotting time that can be attenuated by addition of an excess of phospholipids and is caused by autoantibodies against β2-glycoprotein I (β2GPI) or prothrombin. The mechanism behind the paradoxical association of thrombosis in vivo and prolonged clotting times in vitro remains unclear. In order to solve this paradox, we need to understand how these autoantibodies cause LA.

Aims: To elucidate the mechanism of action of LA caused by anti-β2GPI and anti-prothrombin antibodies.

Methods: The effects of a panel of monoclonal anti-β2GPI and anti-prothrombin antibodies on coagulation were analyzed in plasma and with purified coagulation factors. Results were confirmed in plasma from patients positive for LA.

Results: Addition of anti-β2GPI or anti-prothrombin antibodies to plasma caused a phospholipid-dependent increase in clotting time in FXa-initiated coagulation tests. In contrast, they did not prolong clotting times in plasma containing only active Factor V (FVa), or when coagulation was initiated with preformed prothrombinase complex. Next, clotting tests were performed in plasma from patients positive for LA, before and after treatment with the FV activating snake venom enzyme RVV-V. RVV-V-mediated FV activation strongly attenuated the LA phenomenon. Combined, these data support a role for FV activation in the mechanism of action of antiphospholipid antibodies. Analysis of FXa-mediated activation of FV with purified coagulation factors confirmed that both anti-β2GPI and anti-prothrombin antibodies attenuate FV activation at limiting phospholipid concentrations, but have no effect on FV activation at high phospholipid concentrations. Binding studies showed that β2GPI-antibody complexes directly interacted with FV, whereas prothrombin-antibody complexes did not.

Conclusions: Anti-β2GPI and anti-prothrombin antibodies cause LA through interference with the activation of FV by FXa through different mechanisms of action.

PB1920 | Patients with Antiphospholipid Syndrome Display a Marked Resistance to the Profibrinolytic Activity of Anticoagulants

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Background: Despite strict anticoagulant treatment, a proportion of patients with antiphospholipid syndrome (APS) experiences recurrent thrombotic episodes. Treatment with anticoagulants has two main effects: it reduces thrombin generation and, as a consequence, attenuates thrombin-mediated anti-fibrinolytic effects, thereby enhancing endogenous fibrinolysis.

Aims: To evaluate the anticoagulant and profibrinolytic responses to warfarin and activated protein C (APC) in patients with APS.

Methods: Twenty-three APS patients (20 triple and 3 double aPL-positive) and 26 aPL-negative patients under stable warfarin treatment, along with 20 healthy controls, were studied. Anticoagulation was measured by thrombin generation assay and fibrinolytic resistance as the lysis time of plasma clots by rt-PA. The two assays were also performed after addition of APC. PAI-1 levels were determined by ELISA, and myeloperoxidase (MPO) by chromogenic assays.

Results: Warfarin treatment induced a strong reduction of thrombin generation in both aPL-positive and aPL-negative patients, peak

TABLE 1 Anticoagulant and profibrinolytic responses (median & IQR) in patients under warfarin treatment

	aPL-positive (n=23)	aPL-negative (n=26)	P (aPL+ vs aPL-)	Healthy controls (n=20)
ETP (nMxmin)	287 (129-534)	344 (253-500)	n.s.	1078 (950-1242)
Peak thrombin (nM)	25 (7.9-64)	60 (38-104)	0.041	178 (133-255)
Lysis time (min)	67 (64-74)	52 (42-59)	<0.0001	76 (69-83)
PAI-1 (ng/ml)	28 (23-47)	62 (47-74)	0.0008	38 (22-54)
APC-induced ETP reduction (%)	27 (10-100)	100 (57-100)	0.002	52 (42-59)
APC-induced lysis time reduction (%)	9.0 (5.7-15)	33 (21-54)	<0.0001	36 (32-47)
MPO (mOD)	160 (87-500)	85 (60-136)	0.048	68 (38-81)

thrombin activity being even lower in the former (table 1). Using a clot lysis assay that is very sensitive to thrombin generation, we found longer lysis time in aPL-positive group. This fibrinolysis impairment could not be explained by PAI-1 levels, which, surprisingly, were lower in aPL-positive patients. The latter, moreover, showed a strong resistance to the anticoagulant and even more so to the profibrinolytic effect of APC addition to plasma. Finally, MPO, a marker of neutrophil activation and NETosis, was significantly increased in aPL-positive group.

Conclusions: In APS patients, the marked inhibition of thrombin generation induced by warfarin treatment is not accompanied by the expected enhancement of fibrinolysis, notwithstanding the lower levels of PAI-1. This phenomenon, along with the strong resistance to the profibrinolytic effect of APC, may contribute to recurrent thrombosis in APS patients under warfarin treatment. The role of neutrophil activation in fibrinolysis impairment warrants further investigation.

PB1921 | Penile Mondor's Disease Associated with Anticardiolipid Antibodies: A Very Rare Case of Antiphospholipid Syndrome

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Background: Thrombosis of the dorsal vein of the penis is a rare disorder that affects males in the age range between 20-70 years.

Aims: We present the first case report in literature involving a man diagnosed with Penile Mondor's disease (PMD) and antiphospholipid syndrome (APS).

Methods: A 38y man developed persistent pain in his penis after intense sexual activity and he visited an urologist. Clinical and ultrasound examination revealed thrombosis of the superficial dorsal vein of the penis. The patient then was referred to our hemostasis unit for management.

Results: The patient had negative personal history, but his brother was heterozygous in Prothrombin G20210A mutation with two DVTs.

His blood count, coagulation values, antithrombin, protein C and S were normal and inherited thrombophilia was discovered. An APAs panel test was performed. Elevated titres of IgM aCL (31.1 MPL, normal< 20MPL) were found which were confirmed 12 weeks later. Neither IgG aCL antibodies nor lupus anticoagulant nor IgG/M antiβ2 GPI antibodies were found.

We managed him by administering LMWH in prophylactic dose (Bemiparin 3,500 IU/day). One month later repeat ultrasound examination revealed complete recanalization of the lesion and rivaroxaban was suggested to the patient.

Conclusions: PMD is a rare male disease. Transection of the vessel during surgery or any type of trauma such as external compression may trigger its possible development.

This disease usually is self-limiting. In the acute phase, sexual activity should be restricted in addition to the use of anticoagulation. Creams containing heparin and anti-inflammatory drugs are used in the chronic phase. No permanent sequel has been defined in long-term. Thrombectomy and resection of the superficial penile vein are applied surgically in patients refractory to the medication.

To our knowledge this is the first documented case report of a patient with PMD and documented APS, making his therapeutic approach and monitoring a real challenge.

PB1922 | Neutrophil Activity in Patients with Antiphospholipid Syndrome-Related Thrombosis

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Background: The release of neutrophil extracellular traps (NETs) has been described to be at the basis of immune-mediated thrombosis.
Aims: We investigated whether neutrophil activity and NETs are associated with thrombosis in APS (t-APS).
Methods: 50 secondary t-APS (t-SAPS), 103 primary t-APS (t-PAPS) and 131 individuals without thrombosis (controls) were included. The following markers of NETs were evaluated: gene expressions of PADI4, ELANE, and MPO by qPCR, citrullinated histone H3 (H3cit), circulating levels of myeloperoxidase - DNA complexes (MPO-DNA) and circulating free dsDNA.
Results: Mean age was 37.5 (SD 14.7) in t-SAPS; 42.1 (SD 15) in t-PAPS and 41.8 (SD 13.9) in controls; most of the subjects were female. The mean expression of MPO was 1.05 (SD 1.02) in controls; 1.56 (SD 1.71) in t-PAPS and 3.62 (SD 5.56) in t-SAPS. The mean difference in MPO expression between t-SAPS and controls was 2.57 (95% CI 1.31; 3.84). The mean expression of ELANE was 2.23 (SD 3.11) in controls; 3.46 (SD 14.25) in t-PAPS and 13.36 (SD 36.72) in t-SAPS. The mean difference in ELANE expression between t-SAPS and controls was 11.13 (95% CI 2.48; 19.78). PADI4 expression was similar between APS and controls. Circulating levels of dsDNA and MPO-DNA did not differ between t-SAPS (22.27 and 0.18) t-PAPS (22.36 and 0.19) and controls (25.4 and 0.16, respectively). Levels of H3cit was decreased in t-SAPS when compared to controls (Tabe 2).
Conclusions: The expression of genes associated with neutrophil activity was up-regulated in patients with SAPS. Molecular and plasma markers of NETs, however, were not associated either with t-PAPS nor t-SAPS, which suggests that the observed neutrophil activity is not related to the production of NETs.

TABLE 1 Differences in the levels of NETs markers between controls, patients with thrombotic PAPS and SAPS

	Mean Levels (SD)	Mean difference no adjustments* (95% CI)	Mean Difference adjusted for age and sex * (95% CI)
Controls (Free- dsDNA)	5.42 (16.61)	Reference	Reference
t-PAPS (Free- dsDNA)	22.36 (9.15)	-3.06 (-7.19; 1.07)	-3.05 (-7.16; 1.06)
t-SAPS (Free- dsDNA)	22.27 (10.11)	-3.15 (-7.95; 1.65)	-2.52 (-7.13; 2.27)
Controls (MPO-DNA)	0.16 (0.16)	Reference	Reference
t-PAPS (MPO-DNA)	0.19 (0.15)	0.04 (-0.01; 0.08)	0.03 (-0.01; 0.07)
t-SAPS (MPO-DNA)	0.18 (0.09)	0.02 (-0.04; 0.07)	0.02 (-0.03; 0.07)
Controls (H3-citrullinated)	2.03 (1.56)	Reference	Reference
t-PAPS (H3-citrullinated)	2.11 (1.77)	0.08 (-0.43; 0.59)	0.11 (-0.40; 0.62)
t-SAPS (H3-citrullinated)	1.18 (1.57)	-0.85 (-1.44; - 0.25)	-0.85 (-1.44; - 0.25)

TABLE 2 Differences in the expression of neutrophils-associated genes between controls, patients with thrombotic PAPS and SAPS

	Mean levels (SD)	Mean difference no adjustments* (95% CI)	Mean Difference adjusted for age and sex * (95% CI)
Controls (MPO)	1.05 (1.02)	Reference	Reference
t-PAPS (MPO)	1.56 (1.71)	0.51 (-0.47; 1.49)	0.41 (-0.58; 1.39)
t-SAPS (MPO)	3.62 (5.56)	2.57 (1.31; 3.84)	2.55 (1.26; 3.84)
Controls (ELANE)	2.23 (3.11)	Reference	Reference
t-PAPS (ELANE)	3.46 (14.25)	1.23 (-5.47; 7.92)	1.00 (-5.82; 7.81)
Controls (ELANE)	13.36 (36.72)	11.13 (2.48; 19.78)	11.41 (2.52; 20.31)
Controls (PAD4)	0.96 (0.41)	Reference	Reference
t-PAPS (PAD4)	1.10 (0.72)	0.14 (-0.07; 0.36)	0.12 (-0.09; 0.34)
t-SAPS (PAD4)	0.95 (0.56)	-0.01 (-0.28; 0.27)	0.05 (-0.23; 0.34)

PB1923 | Catastrophic Antiphospholipid Syndrome, Kikuchi-Fujimoto Disease and Adult-Onset Still Disease: An Unusual Association

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Background: In 2005, we published the case of a 30-year-old man with a rare association of Kikuchi-Fujimoto Disease (KFD) and a definitive Catastrophic Antiphospholipid Syndrome (CAPS), triple-positive at high titers for antiphospholipid antibodies (aPL). Cytomegalovirus infection was the triggering factor. The patient presented severe distal necrosis in the four extremities. Among other therapies, he was treated with recombinant activated protein C, which probably improved his altered coagulation profile. He evolved satisfactorily and has been under oral vitamin K antagonist anticoagulants (RIN=3-3.5) and glucocorticoids since then.

Aims: During the next 10 years, he suffered at least 3 relapses/year, which always included high erythrocyte sedimentation rate, liver-splenomegaly, aPL persistence and 1-2 of the characteristics shown in table 1. Strikingly, other autoantibodies were negative. All these episodes were treated successfully with a higher dose of oral glucocorticoids (20 mg/day).

Methods: According to previous reports, some KFD evolved to an autoimmune disease, particularly systemic lupus erythematous. However, in this case it was not until 2015 that the diagnosis could be achieved: the patient accumulatively presented enough characteristics to define adult-onset Still disease (AOSD) (Yamaguchi criteria). In addition, ferritin levels were increased (1800 mg/dl). Infections, malignancies and connective tissue diseases were excluded.

Results: The patient was treated with methotrexate, azathioprine, mycophenolate and/or leflunomide, but without success. Indeed, the frequency of the crisis increased to 5-6 episodes/year and were responsive to higher oral dose of corticosteroids.

TABLE 1 Clinical and laboratory characteristics of the crisis alternatively presented between 2005 - 2015

Clinical	Laboratory
- High temperature of more than two weeks of evolution (peaks)	- Leukocytosis with neutrophilia
- Maculopapular rash	- Increased liver enzymes
- 2nd and 3rd right metacarpo-phalangical synovitis; left elbow synovitis	
- Odynophagia	
- Diarrhea and vomiting	
- Poly-arthralgias	

TABLE 2 Yamaguchi criteria (1992): five features, including at least two major criteria, must be present for adult-onset Still disease diagnosis

Major criteria:
1. Fever of 39°C or higher (≥1 week)
2. Arthralgia (≥2 weeks)
3. Salmon colored maculopapular rash
4. Leukocytosis (≥10.000/ul with ≥80% granulocytes)
Minor criteria:
1. Sore throat
2. Lymphadenopathy and/or splenomegaly
3. Liver dysfunction
4. Negative rheumatoid factor and antinuclear antibody test.

Conclusions: Despite the high mortality rate of CAPS and the usual prognosis of KFD into an autoimmune pathology, this patient successfully survived and just 10 years later his pathology was properly identified as a systemic inflammatory syndrome known as AOSD. The only autoantibodies detected were persistently triple positivity of aPL, always to high titers, without thrombosis. As with current therapies the patient has a poor prognosis we are evaluating to apply a biological treatment.

PB1924 | Vitamin K Antagonist (VKA) Monitoring Using Point of Care Testing (POCT) in Patients with Antiphospholipid Syndrome (APS)

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Background: Patients with thrombotic complications of APS are at increased risk of recurrent thrombotic events and are recommended to have lifelong anticoagulation, usually with vitamin K antagonists (warfarin). Warfarin requires monitoring of the international normalised ratio (INR), since the anticoagulant effect is unpredictable. Monitoring INRs using POCT for patients with APS is often considered unreliable due to potential interactions of antiphospholipid antibodies with reagents used to calculate INR by POCT.

Aims: The aim of this study was to review reliability of POCT in APS patients whose anticoagulation is monitored at Guys and St. Thomas' (GSTT) anticoagulation clinic.

Methods: We conducted a retrospective analysis of paired POCT and venous INR results (n=70) from 32 patients with APS (aged 28-78 years, 7 male & 25 female) monitored at GSTT (July 2018-January 2020) using a Coaguchek XS device. Six-month TTR of APS POCT, APS venous and non-APS POCT cohorts was also compared.

Results: Excluding paired samples with POCT INR >= 4.5, demonstrated a concordance of 92% of samples within an INR of 0.5 (n=50).

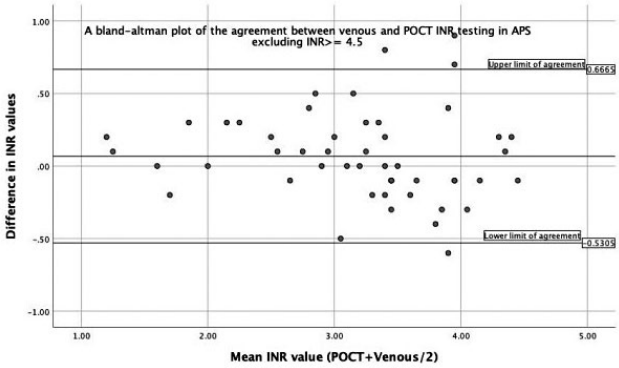

FIGURE 1 A Bland-Altman plot of the agreement between venous and POCT INR testing in APS excluding INR >= 4.5

76% of paired samples had an acceptable INR variation of within 0.5 for the APS POCT cohort. POCT manufacturers do not advise interpreting INR results >= 4.8 since they cannot guarantee accuracy above this. Bland-Altman concordance analysis (Figure 1) showed high concordance between POCT and venous testing excluding INR >= 4.5. The mean six-month TTR for the APS POCT population was 48.37% (+-23.9, n=70), compared to 56.3% (+-21.4, n=174) in our venous testing cohort and 70% (+- 23.5, n=35) in non-APS patients using POCT.

Conclusions: Our results show good concordance between INR results obtained by POCT and venous sampling methods in APS patients and similar TTRs between APS POCT and non-POCT. INR POCT is a potential alternative to venous sampling for patients with APS when INR < 4.5 but requires further study.

PB1925 | Complement Regulatory Abnormalities are Frequent in Patients with Catastrophic APS and Refractory Patients Respond Rapidly to Eculizumab Treatment

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Background: Catastrophic antiphospholipid syndrome (CAPS) is a life threatening complication of APS characterized by simultaneous intravascular and microvascular thrombosis involving three or more organs in presence of antiphospholipid antibodies. recent studies have demonstrated significant complement activation in patients with thrombotic APS and clinical manifestations of CAPS TMA is similar to complement- mediated TMA.

Aims: To evaluate the efficacy of Eculizumab in refractory CAPS patient. To perform molecular sequencing of complement regulatory genes and screening for antibodies against CFH.

Methods: Six APS patients who presented with Clinical manifestation fulling CAPS criteria The genomic DNA was used as a template for a highly multiplexed polymerase chain reaction scheme. Sequence data were aligned to the human reference genome (Hg19) to identify nucleotide variants.

Results: Six APS patients, all triple positive, five males and one female ages 36 to 71 who fulfilled criteria for CAPS with three or more organ systems involved and all with renal insufficiency and thrombocytopenia on presentation. Three were considered secondary with a diagnosis of Lupus. One with a prior diagnosis of ITP. All were treated with corticosteroids and heparin and 4 received plasmapheresis. Three were treated with Eculizumab, two with disease progression while on plasmapheresis. All three rapidly improved with complement inhibition.

Complement gene molecular variants were identified in 4 patients, two with multiple variants. Two of the tree patients treated with Eculizumab has variants identified.

Conclusions: Our finding support a role for complement dysregulation in APS patients who developed the Catastrophic form of the disease. Complement inhibition in three patients with worsening clinical manifestation resulting in clinical improvement most noted in improved renal function similar to patients with aHUS. The efficacy of heparin in CAPS may also be related to its documented anti-complement inhibitory activity in addition to its anticoagulant activity.

PB1926 | Direct Oral Anticoagulants versus Vitamin K Antagonists in Antiphospholipid Syndrome: A Meta-analysis

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Background: Anticoagulant treatment is recommended in patients with thrombosis and antiphospholipid syndrome (APS). Conflicting results have been reported on the role of direct oral anticoagulants (DOACs) in these patients.

Aims: The primary objective was to assess the efficacy of DOACs compared to VKAs to prevent recurrence of thromboembolic events in APS patients.

Methods: We performed a meta-analysis of randomized controlled trials (RCTs) focused on this issue. We searched MEDLINE and EMBASE for RCTs comparing DOACs and vitamin K antagonists (VKAs) for secondary thromboprophylaxis in patients with thrombotic APS.

Results: Three RCTs (426 patients) were included, all comparing rivaroxaban with VKAs. VKAs were associated with reduced risk for thromboembolic recurrences (2.8% vs 9.5%; OR 0.29, 95%CI, 0.11-0.71; NNT 19 patients). The incidence of venous recurrences was similar in the two treatment groups (1.4% vs 1.4% with VKAs vs. rivaroxaban; OR 0.99, 95%CI, 0.22-4.42) while arterial recurrences were more common with rivaroxaban (1.4% vs. 8.5%; OR 0.17, 95%CI, 0.05-0.54). These differences were also present independently from the index event (OR 0.23, 95%CI, 0.07-0.77 for venous index event; OR 0.23, 95%CI, 0.07- 0.80 for arterial index event) and also in patients with triple antiphospholipid antibodies positivity (OR 0.26, 95%CI, 0.09-0.78). Similar incidences of any bleeding (17.7% vs 22.7%; OR 0.72, 95%CI, 0.44-1.18), major bleeding, (4.2% vs 4.7%; OR 0.88, 95%CI, 0.35- 2.24) and of death (1.4% vs 2.8%; OR 0.52, 95%CI, 0.14-1.96) were observed.

Conclusions: The use of rivaroxaban in APS patients is associated with an increased rate of thrombotic recurrences compared to VKAs, mainly arterial, and independently from the index event.

PB1927 | Triple Positive Antiphospholipid Antibodies: A 10-year Retrospective Review of 37 Patients with Triple Positive Antiphospholipid Antibodies and their Thrombotic Outcomes and Mortality in a Single Centre in Singapore

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Background: Triple positivity (positivity for lupus anticoagulant (LAC), anti-cardiolipin (aCL) and anti-B2 glycoprotein) is associated with high risk for thrombosis and recurrence.

Aims: We evaluated the 10 year thrombotic outcomes and mortality of 37 patients with triple positive antiphospholipid antibodies detected from 2010 to 2019.

Methods: We included 37 patients from Tan Tock Seng Hospital, Singapore. Presence of LAC was confirmed with STA-Staclot and DRVV Lupus anticoagulant assay, while aCL were determined using INOVA QUANTA Lite IgG III and IgM III, anti-B2 glycoprotein (aBGP) by Euroimmun Test System (ELIZA).

Results: 37 patients had triple positivity, (14 male 23 female, age 17-87 years). All 37 had presence of LAC and anti B2 glycoprotein, 30 were positive for aCL IgG and 24 positive for aCL IgM. 14 had triple positivity with no initial thrombosis, of which 3 later developed thrombosis (after 7 months, 4 and 6 years). 14 had primary anti-phospholipid syndrome (APS) and 9 had secondary APS (6 SLE, 2 SLE/Sjogrens overlap, 1 HIV). 15 had a single thrombotic event, 4 had 2 thrombotic events, 6 had multiple thrombotic events. 10 had venous thrombosis, 8 small vessel thrombosis, 5 had arterial thromboembolism, 2 combined venous and arterial thromboembolisms. Sites of thrombosis included 16 brain ischaemic and small vessel strokes, 12 lower limb DVT, 3 adrenal haemorrhages, 3 recurrent miscarriages, 2 myocardial infarctions, 1 kidney infarcts and 1 retinal ischaemic retinopathy. 21 (56%) had concurrent thrombocytopenia upon APS antibody testing. 24 out of 26 patients with APS had long term warfarin, 2 developed major bleeding (haematuria). 3 had thrombosis related death (1 pulmonary embolism, 1 brain stroke and 1 catastrophic APS).

Conclusions: Triple positivity is associated with a high risk for thrombosis with a 10-year cumulative incidence of 70.3% (26 out of 37 patients) and a high 8.1% thrombotic associated mortality.

PB1928 | Prevalence of "Cofactor Effect" on Lupus Anticoagulant Mixing Studies: Patients and Laboratory Characteristics

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Background: "Cofactor effect" (CE) Lupus anticoagulant (LA) in mixing studies was associated to depletion of β2 glycoprotein I (β2GPI) or prothrombin (PT) that provoke longer screening tests when patient antibodies react with de "cofactor" present in the normal plama(NP). Its prevalence is unknown.

Aims:

- To detect the prevalence of CE
- To describe patients' laboratory and clinical characteristics

Methods: Descriptive retrospective cohort study (2015-2019) of patients studied for LA. Samples from external laboratories, with

TABLE 1 Patients with CE on either DRVVT or APTT characteristics

Characteristic	Prevalence
Age Mean, SD Sex (F/M)	59.0. 17.3 44/13
APS, n(%) Thrombosis/ Venous/ Arterial/ Both / Fetal Loss, n (%)/ Fetal Loss (≥ 2)	13 (22.8)/ 13 (22.8)/ 9/ 3/ 1/ 7 (12.2) 4
ASSOCIATED DISEASE n (%) SLE/ Other Autoimmune disease/ Oncohematological disease/ Neoplasm/ Other diseases/ Casual finding	11 (19.3)/ 10 (17.5)/ 9 (15.8)/ 5 (8.8)/ 14 (24.6)/ 7 (12.3)
ANA positive antibodies Prevalence (%)	22/40 (55)
Minor bleeding n (%)	8 (14%), 4 under Anti vit K antagonists

TABLE 2 Laboratory characteristics of the samples with CE

	CE in APTT 39.6% (n=57)	CE in DRVVT 75.4% (n=57)	CE in APTT and DRVVT 14% (n= 57)	Control group of samples LA+ without CE (n=663)
Mixture /patient CT ratio Mean, SD, (n)	1.18. 0.15 (22)	1.1 (0.14) (49)	–	<1.0
APTT (sec) Mean,SD	59.6, 34.6	61.6, 24.4	–	54.9, 28.0
DRVVT (sec) Mean, SD	66.8, 36.0	55.8, 24.2	–	49.9, 18.9
SCT (sec) Mean, SD	80.1, 43.8	78.6, 35.0	–	52.6, 14.9
dPT P/N ratio	1.96, 0.91	2.30, 0.75	–	1.60, 0.67
ACL IgG + (%) (% Moderate or high titer)	52.4 (47.6)	20.4 (16.3)	–	46.2
ACL IgM + (%) (% Moderate or high titer)	71.4 (61.9)	57.7 (41.3)	–	35.7
Ab2GPI IgG + (%) (% Moderate or high titer)	13.3 (13.3)	0	–	37.5
Ab2GPI IgM + (%) (% Moderate or high titer)	69.2 (53.8)	45 (29.7)	–	27.9

preanalytical problems, with heparins or direct anticoagulants were excluded.

Coagulations tests: APTT (APTT SP), Silica Clotting time (SCT), DRVVT (HemosIL dRVVT), diluted prothrombin (dPT) with recombinant thromboplastin (1/500) were performed in ACLTOP platform (Instrumentation Laboratory). Home-made NP was used and mixing study was performed even if screening test was normal. ACL and anti b2GPI were performed by ELISA Quanta Lite (INOVA). ANA were detected by immunofluorescence (HEp-2, Kallestad, BioRad).

Results: From a total of 13950 studies, 1159 were LA positive, 69 showed CE. We included 57/69 in which anticardiolipin (ACL) and anti β2GPI antibodies result were available.

Prevalence of CE was 5.9% in LA+. No patient with hypoprothrombinemia was detected, 8 presented prolonged PT (5 taking anti VitK antagonists and 3 with cirrhosis)

Patients characteristics and laboratory results are shown in tables 1 and 2, respectively.

Only 2 patients presented normal APTT, DRVVT, SCT and dPT but prolonged leading to a LA + positive result in the mixture. Both have ANA+, one ACLIgG+ and none antiphospholipid Syndrome (APS).

Conclusions: Prevalence of CE is low, associated with high prevalence of solid phase IgM antibodies. The prevalence of APS is low, and CE is mainly associated to autoimmune, oncohematological diseases or neoplasm. The extremely low prevalence of CE in patients with normal results on all screening tests discourages to perform systematically mixing tests in these samples.

PB1929 | FXI Levels as Independent Risk Factor for Antiphospholipid Syndrome: Development of a New Predictive Score, APS-FXI

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Background: The prothrombotic consequences of antiphospholipid antibodies (aPL) are well demonstrated. Antibodies (titer and type) and cardiovascular risk factors have been used as predictors of thrombotic or obstetric events (antiphospholipid syndrome, APS) in the aGAPSS score, but further factors might improve predictions. Factor XI (FXI), a target of aPL, has been involved both in thrombosis (>150%) or bleeding (< 70%). Moreover, FXI deficiency protects against thrombosis.

Aims: To evaluate FXI in patients with aPL.

Methods: 194 consecutive subjects with aPL, 112 primary APS and 82 asymptomatic (a-aPL) were enrolled. FXI was evaluated by coagulometric assays (FXI:C) using two contact activators (silica -SS- and ellagic acid -SF-), and by western-blotting. *F11* was studied by sequencing and MLPA. Statistical analysis included binary logistic regression, Mann-Whitney U test, and ROC curves.

Results: FXI:C values were significantly higher in APS than in a-aPL. Remarkably, cases with FXI>150% were 14-times higher in APS, and cases with FXI< 70% were 4-times higher in a-aPL.

Two patients had congenital FXI deficiency caused by the Ashkenazy p.Glu135* variant, however, dilution analyses confirmed the presence of inhibitors in the remaining 12 patients with FXI deficiency. Multivariate analysis confirmed dyslipidemia, aB2GPI IgG, and lupus anticoagulant (LA) as independent risk factors for APS, but FXI (both excess and deficiency) showed the highest odds ratios (OR). A predictive score for APS based on b coefficients and including for the first time FXI (APS-FXI), was performed. ROC curve showed a better adjustment for APS-FXI than aGAPSS.

Conclusions: Our study shows a strong predictive value of FXI levels in subjects with aPL. FXI>150% are associated to APS, while FXI deficiency < 70%, which may be caused by the antibodies, protects subjects with aPL from APS. We have developed a new score for predicting APS including FXI levels with high sensitivity and specificity.PI18/00598 (ISCIII y FEDER); and 19873/GERM/15 (Fundación Séneca).

TABLE 1 Characteristics of subjects with antiphospholipid antibodies (aPL). Potential risk factors associated to antiphospholipid syndrome (APS)

		APS (n: 126)	Asymptomatic aPL (n: 82)	OR	95%CI	p
Sex	Male, n (%)	58 (51,8)	28 (34,1)	2,23	1,24-4,01	0,008
	Female, n (%)	54 (48,2)	54 (65,9)	0,45	0,25-0,81	0,008
Age	Mean, years (SD)	51,3 (12,8)	50,2 (15,1)	1,01	0,99-1,03	0,59
CVRF	AHT, n (%)	50 (44,6)	25 (30,5)	1,87	1,02-3,41	0,042
	DLP, n (%)	84 (75)	43 (52,4)	2,72	1,48-5,00	0,001
	DM-2, n (%)	11 (9,8)	9 (11)	0,88	0,35-2,24	0,79
	BMI>30, n (%)	43 (38,1)	17 (20,7)	2,45	1,27-4,73	0,007
	Smoker, n (%)	66 (58,9)	44 (53,7)	1,24	0,70-2,20	0,47
	Sedentarism, n (%)	57 (50,9)	49 (59,8)	0,71	0,39-1,27	0,25
aPL profile	High risk, n (%)	88 (78,6)	59 (72)	1,43	0,74-2,77	0,29
	Low risk, n (%)	24 (21,4)	23 (28)	0,70	0,36-1,35	0,29
	Triple positive, n (%)	21 (18,8)	23 (28)	0,6	0,30-1,18	0,14
aPL type n (%)	LA	84 (75)	50 (61)	2,07	1,11-3,86	0,023
	aCL IgM	21 (18,8)	20 (24,4)	0,77	038-1,54	0,45
	aCL IgG	17 (15,2)	18 (22)	0,64	0,31-1,33	0,23
	aCL (IgM y/or IgG)	36 (32,1)	32 (39)	0,74	0,41-1,34	0,32
	aB2GP1 IgM	12 (10,7)	19 (23,2)	0,42	0,19-0,92	0,03
	aB2GP1 IgG	27 (24,1)	32 (39)	0,51	0,27-0,95	0,03
	aB2GP1 (IgM &/or IgG)	33 (29,5)	34 (41,5)	0,58	0,32-1,05	0,07
FXI	FXI-SFX (Mean (SD))	122,8 (33,4)	104,5 (27,5)	1,02	1,01-1,03	<0,001
	FXI-SS (Median (IQ))	110,9 (47)	99,1 (36,2)	1,01	1,00-1,02	0,002
FXI>150%	FXI>150 SFX, n (%)	22 (19,6)	2 (2,4)	10,11	2,30-44,36	0,002
	FXI>150 SS, n (%)	20 (17,9)	3 (3,7)	5,9	1,69-20,65	0,005
	FXI_150 Global, n (%)	18 (14,3)	1 (1,2)	14,95	1,95-114,83	0,009
FXI <70%	FXI<70 SFX, n (%)	3 (2,7)	10 (12,2)	0,20	0,05-0,76	0,018
	FXI<70 SS, n (%)	10 (8,9)	16 (19,5)	0,42	0,18-0,97	0,042
	FXI_70 Global, n (%)	3 (2,7)	10 (12,2)	0,20	0,05-0,76	0,018

Binary logistic regression	B	Sig.	Wald	OR (95%CI)	APS-FXI=[β x / β min]
Dyslipemia col>200, LDL>150, TG>150	0.708	0.033	4.531	2.029 (1.058-3.894)	+1
LA positive (VVRsd and/or SCT)	0.875	0.013	6.178	2.400 (1.203-4.786)	+1
aB2GP1 IgG >20 CU	-0.823	0.019	5.504	0.439 (0.221-0.873)	-1
FXI >150%	2.653	0.012	6.281	14.199 (1.783-113.081)	+3
FXI <70%	-1.530	0.029	4.783	0.217 (0.055-0.853)	-2

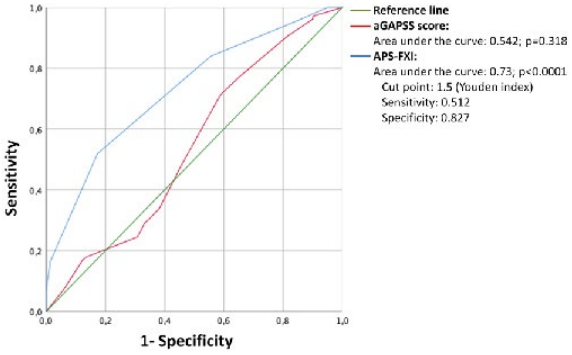

FIGURE 1 Multivariate analysis by binary logistic regression and ROC curves; APS-FXI vs aGAPSS in the prediction of APS in our cohort

PB1930 | β_2-Glicoprotein I (β2GpI) Is a Fibrinogen Binding Protein

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Background: β_2GpI is an abundant plasma protein of yet unknown function and the main autoantigen in the antiphospholipid syndrome (APS). We have recently discovered that β_2GpI may function as a mild anticoagulant, as it binds to α-Thrombin (αT) exosite-2, impairing binding to GpIbα platelet receptor [Pozzi et al., JTH (2013); Acquasaliente et al., Biochem J (2016)]. Earlier studies have shown that β_2GpI Domain-I is the major epitope for APS autoantibodies.

Aims: Check whether β_2GpI interacts with fibrinogen (Fb); identify the binding regions on β_2GpI and Fb involved in β_2GpI-Fb complex formation; study the effect of β_2GpI binding to Fb on fibrin structure.

Methods: Binding measurement were performed by fluorescence and Surface Plasmon Resonance (SPR). Fibrin structure was studied by turbidimetry, Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). Fb fragment-X and β_2GpI nicked at Domain-V were both prepared by limited proteolysis with plasmin [Furlan and Beck, 1972; Ohkura et al., Blood (1998)]. Domain-I was produced by solid-phase synthesis [Pozzi et al., Protein Sci (2010)].

Results: Fluorescence and SPR analyses indicate that β_2GpI interacts with Fb at two sites, with K_d^1 = 15±4nM and K_d^2 > 1μM. Identical results were obtained with Domain-I and Domain-V clipped β_2GpI. Binding of β_2GpI to Fb fragment-X, lacking the Ca-domains, resulted in affinity drop. Turbidimetric, DLS, and SEM measurements indicate that 4mM β_2GpI induces formation of thinner and shorter fibrin fibers.

Conclusions: Given the plasma concentrations of β_2GpI (4mM) and Fb (7mM), and the affinity of β_2GpI for Fb (K_d^1 = 15nM), we conclude that under physiological conditions β_2GpI circulates in the fibrinogen-bound form. β_2GpI-Fb complex formation is driven by β_2GpI Domain-I and Fb Ca-domains. Considered that Domain-I is also the major epitope for APS autoantibodies, our results challenge the current view on the role of β_2GpI in the pathogenesis of APS.

PB1931 | Mixing Studies in Lupus Anticoagulant Tests: A Useful Tool at the Time of Analysis

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Background: The lupus anticoagulant (LA) is classified as an antibody that is recognized in antiphospholipid syndrome (APS). Mixing plasma LA test improves the discrimination of LA in presence of an inhibitor or in deficiencies of a coagulation factor, as is the case in patients receiving vitamin K antagonists(VKAs).

Aims: The objective of this study is to review the experience over the LA tests in a our hospital.

Methods: Cross-sectional study performed from January 2018 to December of 2019 in patients with an INR above 1.5 in who a study for detection of LA was required.

Results: Samples from 68 men and 65 women were analyzed. 44 patients was diagnosis of venous thromboembolic 12 patients arterial thrombosis, the remaining 69 patients had non-thrombotic conditions.

A total of 86 samples had an INR between 1.5-3. In these patients a complete study was carried out for the detection of AL. Out of 125 samples, 39 presented an INR >3, and confirmatory AL study was not performed. In 86 remaining samples with INR between 1.5 and 3 was ordered a confirmatory AL study in a sample mixed 1:1 (patient plasma/normal plasma) using dRVVT and SCT (screen-confirm- ratio mixing) (IL instruments®). Out of 86 samples, 55 had a positive mixing test for LA and 26 were positive for both dRVVT and SCT tests (screen-confirm mixing) (IL instruments®). The 55 patients with samples with a positive LA mixing test, 11 patients were diagnosed of APS. (Table 1)

TABLE 1 Correlation between test and diagnosis

Test	Number of patients	APS
dVVRT (-) SCT (-) 1	31	1
dVVRT (+) SCT (-)	29	1
dVVRT (+) SCT (+)	26	10
Total	86	12

Conclusions: The use of dRVVT and SCT assays reports clearer results of laboratory determination of LA. In samples with INR between 1.5-3 using a AL study in a sample mixed 1:1 using dRVVT and SCT (screen-confirm- ratio mixing) can be a useful tool. In samples with INR above of 3.0 should be mandatory to perform new LA tests beyond the treatment with anticoagulants.

PB1932 | CliA Immunoassay: Better Identification of High Thrombotic Risk Triple Positive Antiphospholipid Patients

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Background: Current laboratory classification criteria for definite Antiphospholipid syndrome (APS) require the demonstration of lupus anticoagulant (LA), and/or anticardiolipin (aCL) and/or anti-b2glycoprotein I (a-β2GpI) IgG or IgM antibodies detected by immune-sorbent method. Triple positivity (LA, aCL and a-β2GpI antibodies) is associated with high thrombotic risk.

Traditionally aCL and a-β2GpI antibodies were detected by enzyme linked immunosorbent assay (ELISA), but nowadays more advanced automated platforms with reduced interlaboratory variation are available: fluorescence enzyme immunoassay (EliA), luminex and chemiluminescence assays (CliA). As reported in the literature triple positivity identification is assay dependent.

Aims: Aim of our study was to investigate whether CliA Acustar (Instrumentation Laboratories, Bedford, Massachusetts, United States) have better performances than EliA ImmumnoCAP (Thermo Fisher Scientific Phadia, Upssala Sweden) assay in triple positivity aPL detection.

Methods: We collected and studied, 30 consecutive LA positive patient samples, in accordance with the revised three-step diagnostic strategy of the ISTH, on whom an aPL determination was requested coming from Clinical Immunology or Haematology Department. aCL and a-β2GpI IgG/M antibodies were determined by our in house platform EliA ImmunoCAP and by CliA Acustar.

Results: Clinical characteristics and results are reported in Table 1 and 2. By using CliA ACL Acustar assay the percentage of triple-positive cases increased significantly in all LA positive patients investigated [10/30 (33%) ImmunoCAP versus 17/30 (57%) Acustar, P< 0.05]. Table 1. Discr… Table 2. Discre…

Conclusions: In conclusion as reported in the literature our data shows that detection of triple-positivity was better with CliA than EliA assay. As triple-positivity APS patients have an increased risk of thrombotic recurrence, standardization in triple-positivity detection is urgently warranted.

TABLE 1 Discrepancies in solid phase immunoassay detection between ImmunoCAP EliA and ACL Acustar. (% of positive patients)

Clincal Characteristics of LA positive patients	Number	aCL IgG ImmunoCAP	aCL IgM ImmunoCAP	aB2GpI IgG ImmunoCAP	aB2GpI IgM ImmunoCAP	aCL IgG Acustar	aCL IgM Acustar	aB2GpI IgG Acustar	aB2GpI IgM Acustar
APS thrombosis	13	5 (38%)	6 (46%)	5 (38%)	6 (61%)	10 (76%)	9 (81%)	11 (85%)	8 (61%)
APSpregnancy morbidity	1	1 (100%)	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)	1 (100%)	1 (100%)
Non APS thrombosis	5	0	0	0	1 (20%)	0	0	0	0
Autoimmune Disease	11	1 (9%)	2 (18%)	0	5 (45%)	2 (18%)	2 (18%)	2 (18%)	2 (18%)
Total	30	7 (23%)	9 (30%)	6 (20%)	12 (40%)	13 (43%)	13 (43%)	14 (46%	11 (36%=

TABLE 2 Discrepancies in triple positivity detection between ImmunoCAP EliA and ACL Acustar. Number of triple-positives in (dis) agreement. (% of posi)

Clincal Characteristics of LA positive patients	Number	Triple positivity in agreement	Triple positivity ImmunoCAP alone	Triple positivity Acustar alone
APS thrombosis	13	9 (69%)	0	4 (30%)
APS pregnancy morbidity	1	1 (100%)	0	0
Non APS thrombosis	5	0	0	0
Autoimmune Disease	11	0	0	3 (27%)
Total	30	10 (33%)	0	7 (23%)

PB1933 | Contribution of Beta2 Glycoprotein1 IgA (B2GP1 IgA) Antibodies Research in Diagnosis of Seronegative Antiphospholipid Syndrome in Cerebral Thrombosis: A Case Series of 21 Patients

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Background: Seronegative antiphospholipid syndrome (APS SN) is a term used to describe patients who have clinical manifestations higly suggestive of antiphospholipid syndrome (APS) but with persistently negative criteria antiphospholipid antibodies (aPL). Several considerations can be explain APS-SN.

Recently, new antigenic targets for aPL have been investigated and the value of isolated B2GP1 IgA antibodies in APS diagnosis is controversy.

Aims: This study aimed to report the pathogenic significance of B2GP1 IgA testing in patients with cerebral thrombotic manifestations (arterial and venous) and persistently negative thrombophilia testing including criteria aPL assays (anticardiolipin, aβ2GPI, and Lupus Anticoagulant).

Methods: It is about a 3 years retrospective study (2017 to 2019) include 21 patients adressed for thrombophilia testing. Stroke was found in 12 patients and cerebral venous thrombosis in 9 patients.

Thrombophilia testing include proteins S, C and antithrombin functional tests, the activated protein C resistance (APCR) for factor V Leiden and criteria antiphospholipid antibodies: Lupus Anticoagulant and aCL, aβ2GPI IgM/IgG ELISA. All thrombophilia testing is realised according international recommendations.

Four non-criteria antiphospholipid antibodies was determined: anti B2GP1 IgA, anticardiolipin IgA et antiprothrombin-phosphatidylserin IgM and IgG ELISA.

Results: Results shows that thrombophilia testing was negative for all patients and 100% of them (21/21) had IgA anti-β2GPI without other aPL, criteria and other non criteria (anticardiolipin IgA, anti prothrombin phosphatidyl serine IgM IgG).

Conclusions: Beta2Glycorpotein IgA research could explain cerebral thrombosis for these patients. Over the past few years much attention has been focused on the diagnostic value of IgA isotype. Studies has previously report a link between anti-β2-GPI antibodies to cerebral infarction.

No consensus exists for non-criteria aPL but we have adopted a sequential approach that improves seronegative-APS diagnosis.

PB1935 | Thrombosis and Antiphospholipid Antibodies

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Background: Presence of APA Is a Biological Marker for Antiphospholipid Syndrome (APS).

Aims: To evaluate the correlation between APA and thrombosis in patients with CVI (cerebrovascular infarct), AMI (acute myocardial infarct), and DVT (deep venous thrombosis) and pregnancy

Methods: It is a retrospective study on patients from the Outpatient Department. We used micro ELISA kit to compare with commercial micro ELISA kits (QUANTA Lite TM b_2GP I).

TABLE 1 Test Results. Correlation with routine tests

	N	aPTT >1.25	INR>1.5	INR 1.5-3	INR>3
SCT- dRVVT-	1575	135	88	78	10
SCT+ dRVVT-	99	38	2	2	0
SCT- dRVVT+	220	22	100	69	31
SCT+ dRVVT+	277	152	33	21	12
TOTAL	2171	347	223	170	53

Results: We made a clinical investigation for three classes of immunoglobulin - b_2GP I antibodies of 1666 patients in four groups with different diagnoses. 436 were diagnosed with CVI, 464 with AMI, 246 with DVT and 520 were pregnant women. All patients have augmented concentration of all three classes of immunoglobulin and there is high correlation of APA and clinical manifestation of the different diseases. At highest risk were patients with DVT, because 30% had augmented IgG (59, 2 IU) and IgA (5, 63 IU) antibodies. The largest portion of them (32%) had augmented IgM (4, 47 IU).

From 436 patients with CVI - 19% had augmented concentration of IgG (40.91IU), but mostly augmented concentration were antibody from IgG classes.

From 464 patients with AIM -17% were with high concentration of IgM (3.56 IU) immunoglobulin classes the value of other classes of immunoglobulin were less.

From 520 pregnant women with pathological pregnancy, (29%) have augmented concentration of IgG (35, 76 IU). Concentration of IgG is the biggest.

Conclusions: CVI and pathological pregnant women were augmented concentration of IgG class,in patients with AMI and DVT were augmented concentration of IgM class Augmented concentration of all APA classes is in correlation with thrombosis, or a very elevated risk for thrombosis. Protocols or algorithms are very importante to follow up and treat.

PB1936 | Lupus Anticoagulant Testing in Patients with Prolonged INR, Mixing Test Results

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Background: Lupus Anticoagulant (LA) is a risk factor for arterial and venous thromboembolisms and obstetrics complications. Guidelines recommend using two techniques for diagnose it. In patients receiving oral anticoagulants the results could be difficult to interpret or even not valorable.

Aims: To describe the results obtained in our laboratory in a large series of patients comparing the results of diluted Russell Viper Venom Time (dRVVT) and Silica Clotting Time (SCT), to analize the influence of prolonged INR, and the results of dRVVTT and SCT mixing test in patients with INR>1.5.

Methods: In our laboratory we receive samples from three hospitals and several general practice centers. Some of the patients are receiving oral anticoagulants (Vitamin K inhibitors) and their INR sometimes is >1.5. We use the ACL-Top 700 analyzers (Werfen), dVVRTScreen/dVVRT Confirm, and Silica Clotting Time reagents. If one of the LA tests gives a Normalized Ratio (NR) >1.2 the result is considered positive. In some cases, when the INR of the patient is >1.5, we perform mixing tests 1:1 (dVVRT and SCT) with normal pooled plasma.

Results: We performed a total 2171 lupus anticoagulant test in our laboratory on 2019. Results are sumarized in table_01. Of 223 samples with INR> 1.5, 35 were positive for SCT, 133 were positive for dRVVT, 33 were positive for both and in 88 the results were negative. After performing mixing tests in 110 dRVVT positive samples 79 gave negative results (NR < 1.2) and in 31 samples positive results were confirmed. After mixing test in 30 positive SCT samples, 21 of them tested positive. 174 of 177 samples with previous negative results remained negative after mixing tests.

Conclusions: The dRVVT test is most frequently affected than SCT in anticoagulated patients with elevated INR.

Mixing tests can help distinguish between genuine positive results and interference by antivitamin K anticoagulants.

PB1937 | Recurrence of Thrombotic Events in Antiphospholipid Syndrome Patients Receiving Prolonged Vitamin K Antagonists Therapy

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Background: Antiphospholipid syndrome (APS) is an autoimmune disease characterized by thrombotic events, pregnancy complications and the presence of persistently elevated levels of antiphospholipid antibodies (aPL). It is recommended that aPL-positive patients with previous venous or arterial thrombosis receive anticoagulant therapy with vitamin K antagonists (VKA) at a target INR of 2.0 to 3.0. Despite adequate anticoagulation, recurrent thrombosis is a well-recognized complication of APS. The optimum intensity of anticoagulation in these patients remains debatable.

Aims: To determine how often recurrence of thrombotic events happen in an APS patient population receiving prolonged anticoagulation with VKA for secondary thrombosis prevention.

Methods: We performed a retrospective chart review of all diagnosed APS patients treated with VKA that currently monitor their INR levels in our institution. Patients who were included reunited the following criteria:

1. They fulfill the revised Sapporo classification criteria;
2. Are receiving prolonged AVK therapy due to previous venous or arterial thrombosis. We investigated their history of thrombotic events and whether those events happened while receiving VKA therapy.

Results: Eighty-three patients were included with median age of 51 years old. Twenty-eight (33.7%) were men and fifty-five (66.3%) were women. Six patients (7.2%) experienced at least one thrombotic event while receiving prolonged VKA therapy - three deep vein thrombosis, two ischemic strokes and one thrombosis of mechanical tricuspid valve. Five of those patients had an INR within the therapeutic range (2-3) at the time thrombosis developed.

Conclusions: The relatively low incidence of recurrent events in this population doesn't allow us to support a switch from the recommended moderate-intensity to high-intensity anticoagulation (INR of 3.0 to 4.0). Still, risk-stratification models are needed to identify APS patients with thrombotic events who would benefit from more aggressive antithrombotic strategies. Clear recommendations on how to manage thrombotic events despite adequate treatment are also needed.

PB1938 | Weakly Positive Lupus Anticoagulants Are also Associated with High Event Rate

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Background: Our institution uses dilute Russell viper venom test (dRVVT) and silica clotting time (SCT) (Instrumentation Laboratory, Bedford, MA) for lupus anticoagulant (LA) testing. We validated our diagnostic normalized ratio (NR) cutoffs at >1.2 and >1.24 for dRVVT and SCT, respectively, as per the package inserts. There was a concern for false positivity for weakly positive LA.

Aims: Investigate weakly positive LA to assess the clinical validity of these cutoffs.

Methods: We performed a retrospective review of LA results (2014-2019). Patients with a weak positive LA (SCT NR of 1.25-1.5 and dRVVT NR of 1.21-1.5) and concurrent anti-cardiolipin antibody (ACA) and anti-beta-2-glycoprotein 1 (B2GP1) antibody testing as well as those with repeat LA testing were included. We also collected pertinent clinical data, if available, which included the following: presence of provoked/unprovoked thrombosis, preeclampsia/pregnancy loss, oral contraceptive use and oral anticoagulation use during testing.

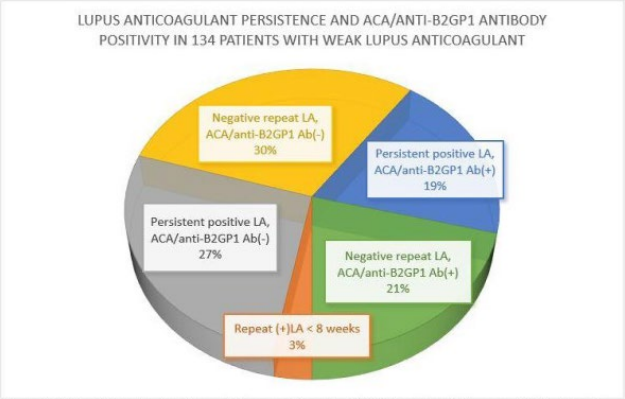

Figure 1: Of the 134 patients with weak lupus anticoagulant (LA), there is a fairly even distribution of those with and without persistent LA and with and without presence of ACA/anti-B2GP1 antibodies. LA, lupus anticoagulant, ACA, anti-cardiolipin antibody, B2GP1, beta-2-glycoprotein 1

FIGURE 1

TABLE 1

	DRVVT 1.21-1.35	DRVVT 1.36-1.5	SCT 1.25-1.35	SCT 1.36-1.5
TOTAL PATIENTS (out of 91 with known clinical history)	41	27	25	27
REPEAT LA NEG	17/41 (41%)	10/27 (37%)	14/25 (56%)	11/27 (41%)
REPEAT LA NEG + AB^ NEG	9/41 (22%)	7/27 (26%)	8/25 (32%)	5/27 (19%)
REPEAT LA NEG+AB^ NEG + NO EVENT†	3/41 (7%)	0/27 (0%)	6/25 (24%)*	0/27 (0%)*
ANY EVENT† PRESENT	29/41 (71%)	13/27 (48%)	13/25 (52%)	16/27 (59%)
UNPROVOKED EVENT† PRESENT	23/41 (56%)	10/27 (37%)	9/25 (36%)	13/27 (48%)

Table 1: Lupus anticoagulant (LA) normalized ratio (NR) for two groups 1.25/1.21<1.35 and 1.35<1.5, both LA groups were associated with similar rates of repeat negative LA and negative ACA/anti-B2GP1 antibodies. Additionally, there was a high event rate in all LA NR groups, and no significant difference was detected by Fisher Exact test (except for one category indicated in table).
*Significant difference between SCT NR groups using Fisher Exact test (p=0.023), remaining were p>0.05.
^ Anti-cardiolipin antibody or anti-beta-2-glycoprotein 1 antibody positive
†Clot or preeclampsia/pregnancy loss

Results: Our positive LA rate was 19.4% (741 out of 3,813 samples on 3,435 patients); 134 of these patients had weakly positive LA results. Of these patients, 26/134 (19%) had both persistent LA and positive ACA/anti-B2GP1 antibodies, whereas 40/134 (30%) were negative for ACA/anti-B2GP1 antibodies and negative on repeat LA (Figure 1). 91/134 had available clinical data to analyze and, of these 91 patients, there was a high event rate in patients with weakly positive LA NR both above and below 1.35 NR (Table 1).

Conclusions: Our data shows that there is a high rate of LA persistence and ACA/anti-B2GP1 antibody positivity within the weak positive LA group (positive NR up to 1.5). Additionally, many of our patients with weak LA had clinical events, thus, suggesting that even weakly positive LA may confer a significant risk of thrombosis or pregnancy loss.

TABLE 1 CD46 expression on lymphocytes and neutrophils of two healthy donors (C1 and C2), processed the day of venipuncture (T0) and 24h after (T24)

	Lymphocytes			Neutrophils		
Sample control	Isotype MFI	CD46 MFI	Ratio CD46/isotype	Isotype MFI	CD46 MFI	Ratio CD46/isotype
C1 T_0	**137**	8253	**60**	256	12756	50
C1 T_{24}	**169**	7581	**47**	338	9058	27
C2 T_0	83	5098	**61**	247	7927	32
C2 T_{24}	70	6107	87	260	10185	39

TABLE 2 Differential expression of CD46 on lymphocytes subpopulations measured in three healthy donors (C1, C2 and C3)

	C1		C2		C3	
Lymphocytes Subpopulations	CD46 MFI	% (cells/µL)	CD46 MFI	% (cells/µL)	CD46 MFI	% (cells/µL)
T lymphocytes (CD3+)	5897	48.2 (723)	6706	65.5 (1310)	10249	80.2 (2000)
Helper T cells (CD3+CD4+CD8-)	11718	66.2 (479)	11376	44.0 (576)	12075	63.0 (1260)
Cytotoxic T cells (CD3+CD8+CD4-)	9496	26.3 (190)	7293	43.9 (575)	8322	34.9 (698)
B lymphocytes (CD19+CD3-)	3244	32.2 (480)	3786	24.7 (494)	4175	14.1 (353)
NK lymphocytes (CD3-CD4-CD8-CD19-)	3003	17.6 (264)	3454	8.6 (172)	3800	2.9 (73)

HUS

PB1939 | Differential Expression of CD46 in Leukocytes by Flow Cytometry: Methodological Aspects

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Background: Ubiquitously expressed CD46 (MCP) is a transmembrane protein that acts as negative regulator preventing host cells injury from complement activation. CD46 alterations are associated to atypical Hemolytic Uremic Syndrome (aHUS). Literature data about CD46 screening for diagnosis by flow cytometry are scarce.
Aims: To establish a protocol of CD46 expression on lymphocytes (L) and neutrophils (N) for clinical test.
Methods: Whole blood (EDTA) from healthy donors (control group, CG) was collected and processed on the same day (T_0, n=23) and at 24h (T_{24}, n=17) with CD46-PE, CD45-PerCP, isotype control-PE markers. Staining was measured through median fluorescence intensity (MFI); reference intervals (RI) were calculated using ratio CD46/isotype. Lymphocyte subpopulations were identified using CD3-PerCp/Cy5.5, CD4-FITC, CD8-APC/H7, CD19APC/H7 markers.
Results: Neutrophils presented more CD46 homogeneous staining and greater cell death after 24h, compared with lymphocytes. Median of ratio and 5th-95th percentiles were calculated in neutrophils (T_0: 42; 32-54/T_{24}: 37; 24-62) and lymphocytes (T_0: 124; 60-168/T_{24}: 99; 47-175). No significant difference was observed between T_0 and T_{24} (p_L=0.49/p_N=0.29). Lymphocytes from two CG samples (C1, C2) showed ratio values in RI's lower limit *(table 1)* and presented distinct cell populations with differential CD46 expression *(table 2)*. In C1, isotype unspecific staining was observed on lymphocytes only *(table 1)* compared to CG (median T_0:66; median T_{24}:70). Lymphocyte subpopulations analysis in C1, C2 and C3 (representative sample from CG), demonstrated that CD46 expression was higher in T cells compared to B and NK cells. Higher percentages of B and NK cells in C1 and C2 could explain the dual expression.
Conclusions: Our results highlighted that CD46 staining on lymphocytes from CG samples showed higher variability, which might depend on the subject's lymphocyte phenotype. Hence neutrophils would be the population of choice despite showing higher cell death rate *in vitro*. In both populations, screening can be performed 24h following the venipuncture.

PB1942 | Personalized Dosing of Eculizumab Using C5 Functional Activity and Eculizumab Level in Complement-mediated Thrombotic Microangiopathy: A Safe and Cost-saving Approach

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Background: Complement-mediated thrombotic microangiopathy (aHUS) is treated with the C5-complement inhibitor eculizumab (ECU). For adults, the standard ECU administration schedule is four-900mg weekly infusions followed by 1200mg every two weeks. *In-vivo* studies have demonstrated complete complement blockade with ECU serum concentrations above 50 μg/mL for aHUS. We have been monitoring ECU trough levels (ECUT) and C5 functional activity (C5FA) for patients with aHUS. For those with ECUT > 100 μg/mL, frequency of ECU 1200mg doses has been decreased.

Aims: Review ECUT obtained according to institution ECU administration practices and perform cost-savings analysis for personalized ECU administration.

Methods: Laboratory database was queried for patients with ECUT and C5FA since the introduction of these assays in 2018. Inclusion criteria included patients with a clinical diagnosis of aHUS receiving medical care at Mayo Clinic. Only patients with clinically confirmed ECUT were included.

Results: 9 patients met inclusion criteria and ECUT ranged from 46-518 μg/mL(Table 1). There was no uniform time when ECUT were obtained in relation to ECU initiation due to variability of patient referral to our institution. ECUT was > 100 μg/mL in 8/9 (89%) patients with the standard administration schedule. At the time of last ECU follow-up (147 (64-674) days, n=6), ECU infusions were prolonged to every three, four, and five weeks for three, one, and two patients, respectively. Nobody experienced a disease relapse with the modified dosing interval. Altering administration from every two to three weeks yields a savings of $110,244/patient every six months (Table 2).

Conclusions: In this study, the standard ECU administration schedule was not necessary for most patients. Use of ECUT over C5FA alone, allows for a more nuanced tailoring approach. Although larger standardized cohorts are necessary to confirm these findings, our preliminary data suggests that personalized dosing of eculizumab using ECUT and C5FA is safe and provides significant cost-saving.

TABLE 1 Demographics and Eculizumab trough levels

Case	Sex	Age at Initiation of ECU (years)	Weight (kg)/BMI (kg/m^2)	ECU trough (μg/mL)	C5 functional activity (U/ml) (RI: 29-53 U/ml)	Time to first ECU trough from initiation of ECU (days)	ECU Follow-up time (First ECU trough to last dose of ECU) days**	Recommended ECU Frequency at last ECU Follow-up	Total Follow-up time (First ECU trough to last clinical visit) days	Using ECU after last follow-up
1	F	65	83.1/22.9	221	17	90	64	3 weeks	64	Yes
2	F	64	58.4/24.4	412	<5	40	674	5 weeks	674	Yes
3	F	44	114/37.0	191	<5	282	504	3 weeks	504	Yes
4	F	72	55.7/22.0	187	6	68	98	3 weeks	98	Yes
5	M	20	66/24.2	464	<5	108	NA	Discontinue	42	No
6	F	22	55.3/20.7	453	<5	152	153	5 weeks	181	No
7†	M	68	83/25.5	441	<6	620	NA	NA	NA	NA
8	F	26	127/45.3	46	63	339	NA	Discontinue	275	No
9	F	30	66/21.8	518	<5	45	142	4 weeks	254	No

*Our practice is to consider decreasing eculizumab frequency (by weekly intervals) if C5 is < reference interval and eculizumab levels >100 μg/mL.
**We consider discontinuation of eculizumab in patients having received treatment for at least six months with stable renal function for ≥ 3 months, and no evidence of microangiopathic hemolytic anemia and thrombocytopenia. The decision to discontinue eculizumab is made based off of clinical stability and risk stratification for recurrence based on presence and type of complement mutations.
† Patient had no clinical follow-up at our institution after initial consultation
Ecu, Eculizumab ; NA, not applicable

TABLE 2 Cost-savings analysis of tailored Eculizumab administration

Table 2A: 6 month estimate of ECU cost*
Cost of ECU X (# of every 2 week interval doses in 6 months)- [(cost of ECU + cost of ECU monitoring assays)X (# of every 3 week interval doses in 6 months)]

	Cost of ECU (1200 mg)**	Cost of ECU and C5 functional activity assays	# of doses over six months	Total Cost
Every 2 week maintenance dosing	$27,657	NA	12	$331,890
Every 3 week maintenance dosing	$27,657	$48.26**	8	$221,646
Total savings with every 3 week schedule/ 6 months	$110,244			

Table 2B: Estimated Individual Patient Cost- Savings

Case	Recommended ECU Administration Schedule Changes	# of ECU Doses Spared Compared to the Traditional every 2 Week Maintenance Schedule	Approximate Cost saving	Long term plan
1	ECU every 3 weeks for 3 months	2	$55,122	Tentative plan to discontinue ECU 7 months after initiation
2	ECU every 3--5 weeks indefinitely	8-12 doses /12 months	$220,488-$331,311 per year	Indefinite because of prior relapse when ECU was discontinued
3	ECU every 2-3 weeks indefinitely	Up to 8 doses/ 12 months	Up to $220,488 per year	Indefinite because of prior relapse when ECU was discontinued
4	ECU every 3 weeks for 4 months	3 doses	$82,731	Tentative plan to discontinue ECU 7 months after initiation
5	NA	NA	NA	At time of initial ECU trough ECU was discontinued
6	ECU every 3-4 week for 3 months; ECU every 5 weeks for 3 months	7 doses/6 months	$137,949	Discontinued ECU 11 months after initiation
7	ECU every 3 weeks with recommendation to continue to modify schedule based on ECU trough levels	At least 8 doses/12 months if ECU is continued indefinitely	$220,488 per year	No follow-up since initial consultation. Forecasted savings is based off of initial recommendation at time of consultation.
8	Discontinue	NA	NA†	Discontinued ECU due to sub-therapeutic ECU level
9	ECU every 3-4 weeks for 4 months	4 doses	$110,436	Discontinued ECU 7 months after initiation

*Infusion chair time expenses were not included in cost-savings analysis
**Cost represents estimated Centers for Medicare and Medicaid (CMS) reimbursement rate
† No cost-saving for this patient but highlights the importance of drug level monitoring because this patient was receiving sub-therapeutic doses for an unknown period of time; therefore uncertain the clinical benefit of the ECU doses received

ECU, eculizumab ; NA, not applicable

NON HUS/TTP MICROANGIOPATHIES

PB1943 | Fulminant Pulmonary Tumor Thrombotic Microangiopathy (PTTM) in a Young Man: Clinical and Immunoistochemical Characterization of a Rare Complication of Gastric Signet Ring Cell Carcinoma

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Background: PTTM is a cancer-related complication with pathogenesis not completely understood. Tumor microemboli are associated with fibrocellular proliferation in pulmonary microvasculature leading to rapid pulmonary hypertension. The antemortem diagnosis is challenging and plasma-exchange is ineffective. Platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) have been hypnotized to have a role in this thrombotic microangiopathy (TMA) and some patients have been successfully treated

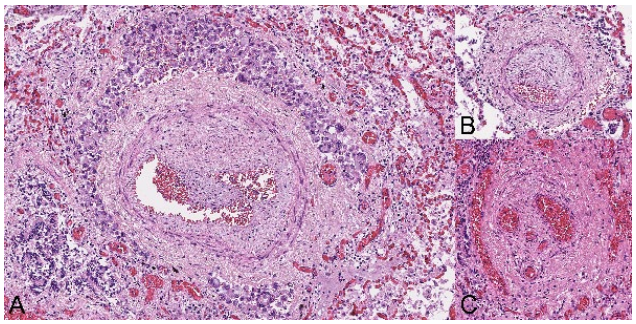

FIGURE 1 Thrombus of adenocarcinoma in lymphatic vessel and an arteriole with intimal proliferation (A). Smaller arterioles with parietal proliferation (B), re

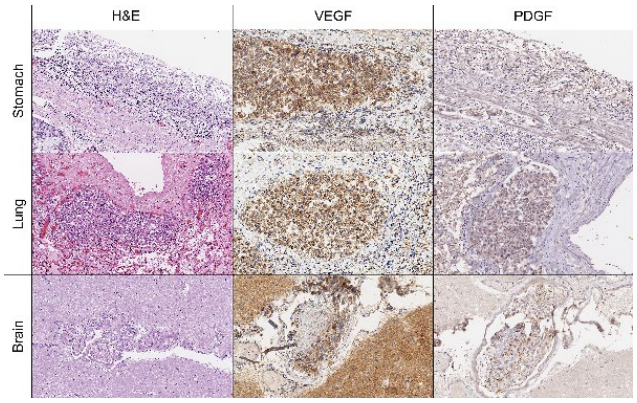

FIGURE 2 Immunohistochemistry. A strong positivity for VEGF is present in primary tumor and metastases to lung and brain. A faint positivity for PDGF is present

with combination therapy of imatinib (PDGF- receptor antagonist) and chemotherapy.

Aims: To describe a well-characterized case of 41 year old male with PTTM and stomach signet-ring cell carcinoma.

Methods: ADAMTS13 activity was measured. Immunohistochemical analyses for PDGF and VEGF were performed on post-mortem samples.

Results: A 41-year-old Colombian man was admitted to Internal Medicin Department at our hospital for lumbar pain and fatigue. Blood analyses showed anemia (hemoglobin 8 g/dL) and thrombocytopenia (131x10^3/uL). After one week, blood investigations showed a worsening of anemia (5.4 g/dL) and thrombocytopenia (28x10^3/uL) with hemolysis. Schistocytes were present, Coombs test was negative. D-dimer was elevated without fibrinogen consumption.

ADAMTS13 activity was 52%, therefore TTP was excluded. The patient was treated with plasmapheresis with no improvement. A rapid deterioration of respiratory exchanges occurred, and the patient was moved to intensive care unit. Echocardiography revealed right ventricle enlargement with PAPs of 65 mmHg. In the subsequent hours, the patient developed acute pulmonary heart leading to death. The bone marrow biopsy showed signet-ring cells of gastrointestinal origin.

Post-mortem examination showed gastric carcinoma with signet-ring cells with metastases. PTTM was confirmed by fibrocellular intimal proliferation of small pulmonary arteries and arterioles. (Fig 1). Tumor cells were positive for VEGF in the stomach, lungs and brain; PDGF showed a lesser degree of expression (Fig 2).

Conclusions: Clinicians should be aware of this cancer-related TMA. Further studies are necessary to characterize PTTM to develop target therapeutical approach.

PB1944 | Hyperferritinemia Promotes a Prothrombotic State by Stimulating VWF Release from the Endothelium and Suppressing ADAMTS-13 Activity

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Background: Hemophagocytic lymphohistiocytosis (HLH) is characterized by pathologic immune activation and uncontrolled inflammation. Autopsies of patients succumbed to HLH had disseminated microvascular thromboses consistent with disseminated intravascular coagulation (DIC) and thrombocytopenia-associated multiple organ failure (TAMOF). Hyperferritinemia is the hallmark of HLH, and mortality is associated with levels of 10,000 to 100,000 ng/ml. It is unclear whether ferritin plays a role in thrombosis. We have previously shown ferritin *in vitro* promotes VWF release from endothelial cells through protein kinase C (PKC) signaling. It is unknown whether ferritin also affects ADAMTS-13function thereby decreasing its ability to cleave large thrombogenic VWF causing microvascular thrombosis.

Aims: To investigate the role of ferritin in promoting microvascular thromboses by (i)identifying whether ferritin in HLH patient plasma can induce VWF release from human umbilical endothelial cells (HUVEC) (ii) determining whether ferritin can suppress ADAMTS-13 activity.

Methods:

(i) We purified ferritin from plasma of HLH patients by size exclusion column. Fractions containing ferritin were used to stimulate HUVEC alone and HUVEC pre-treated with anti-ferritin antibody. Media was tested for VWF by ELISA.

(ii) Plasma from healthy volunteers was incubated with recombinant heavy chain ferritin at 100,000 ng/mL. ADAMTS-13 activity was then measured by ELISA kit (Diapharma).

Results: Compared to unstimulated HUVEC, ferritin fraction from HLH patients significantly induced VWF secretion (3-fold $P < 0.05$). There was a trend towards decreased VWF secretion in HUVEC pretreated with anti-ferritin antibody. Recombinant heavy chain ferritin

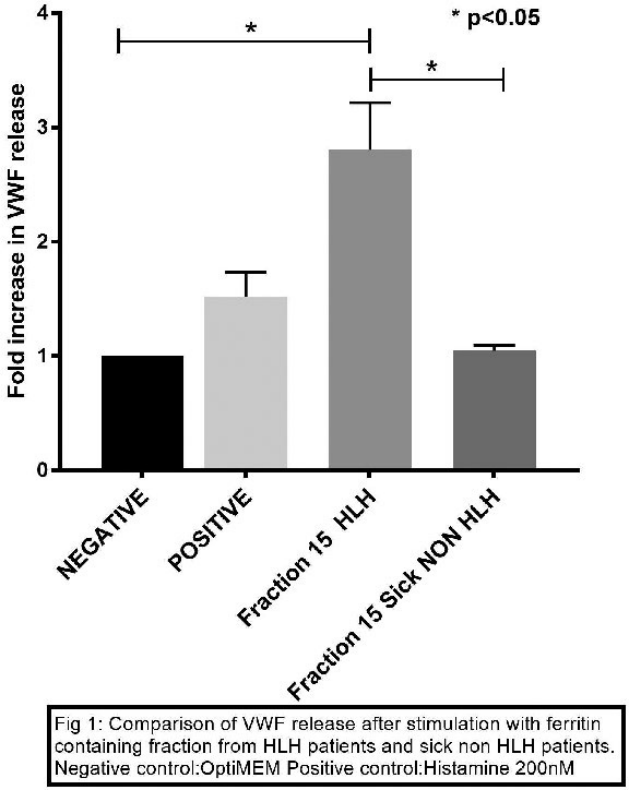

FIGURE 1 Sick vs HLH

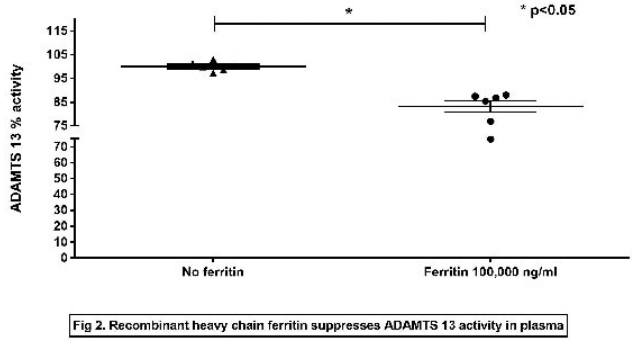

FIGURE 2 Ferritin and ADAMTS13

at 100,000ng/mL significantly suppressed ADAMTS-13 activity in healthy plasma (100% ± 0.8 vs 83% ± 2.4 [mean ± standard error of the mean]), P =0.001.

Conclusions: Ferritin from HLH patient plasma can induce VWF release from endothelial cells, and recombinant ferritin can suppress ADAMTS-13 activity. These findings support the notion that hyperferritinemia may be more than a marker of inflammation, but may also play a role in microvascular thromboses seen in patients with HLH.

PB1945 | Complement Pathway and Endothelial Dysfunction in Severe Preeclampsia

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Background: Severe preeclampsia is a complication of pregnancy characterized by high blood pressure and damage to kidneys, liver and brain that lead to complications for mother and baby. Complement system could be abnormally activated in preeclampsia and lead to endothelial dysfunction.

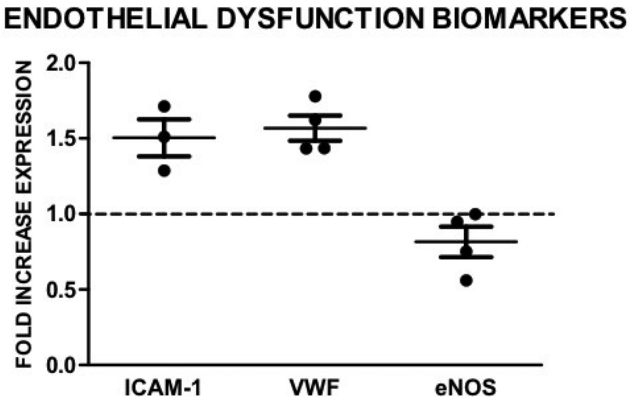

FIGURE 1 Results of the expression of three biomarkers of endothelial dysfunction (ICAM-1, VWF, eNOS) in vitro endothelial cells incubated with serum

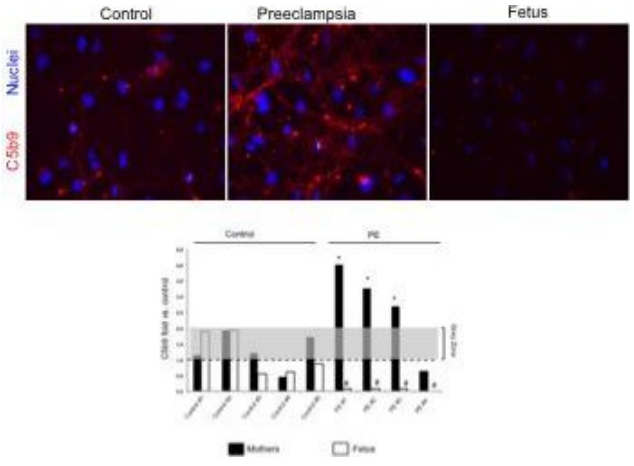

FIGURE 2 Representative microphotographs of the C5b-9 deposit (red) on endothelial cell in vitro (blue-labeled nuclei)

Aims: To evaluate *in vitro* the endothelial function in women with severe preeclampsia (n=9) and assess the functionality of the complement system in both maternal (n=4) and fetal samples (n=4) compared to control mothers and fetuses matched by gestational age.

Methods: Endothelial dysfunction markers (ICAM-1 and vWF) were evaluated in endothelial cells (ECs) in culture exposed to sera from severe preeclampsia patients. Complement activation was assessed by exposing EC to patient's plasma samples mixed with control serum (1:1). C5b9 deposits on EC were analyzed through immunofluorescence and expressed as fold increase vs. control samples.

Results: Expression of endothelial markers of dysfunction was increased in ECs exposed to severe preeclampsia patients serum (figure 1) and that an anomalous enhanced activity of the complement pathway is present in these patients (figure 2). Moreover, significant reduced activity of the terminal phase of the complement pathway in the fetal plasma samples from severe preeclampsia pregnancies when compared to healthy ones.

Conclusions: Severe preeclampsia is associated with endothelial dysfunction and an abnormal activation of the complement pathway. Given the high morbidity and mortality associated with this complication, any therapeutic strategy focused to prevent maternal complications and prolong pregnancy would have a clear benefit for the health of mothers and fetuses. Our present results could be useful in the search for therapeutic targets.

PB1946 | Factor VIII/Protein C and Not ADAMTS13/VWF:Ag Ratio Is a Prognostic Risk Factor for Patients with Cirrhosis and Low MELD Score

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Background: Hemostasis can be mechanism of liver damage in cirrhosis. Low ADAMTS13/Von Willebrand Factor antigen (VWF:Ag) ratio and high factor VIII/protein C (FVIII/PC) mark platelet hyperaggregation and hypercoagulation, respectively.

Aims: Our study aimed at exploring the association of such parameters with the severity of liver disease measured by the Child-Pugh, MELD, MELD-Na scores and their prognostic role on clinical outcome.

Methods: We investigated 123 cirrhotic patients (age 62±12, 58% in-hospital, Child-Pugh:8.2±2.2, MELD:15.6±7.7, MELD-Na:16.3±6.6). Main etiologies were virus/alcohol (40/60%). Hemostatic parameters measured at inclusion were VWF:Ag, ADAMTS13 activity, FVIII, FII, antithrombin, PC and were correlated with the severity of liver disease (Pearson coefficient). Liver decompensations requiring hospitalization, death or transplantation were retrospectively collected and presented as cumulative risk at 12 months from baseline evaluation. The prognostic role of the hemostatic parameters was tested by logistic regression and expressed as risk of adverse outcome predicted by the model.

Results: A pro-hemostatic imbalance was resumed by ADAMTS13/VWF:Ag of 0.21+/-0.14 and FVIII/PC of 6.0+/-6.5. All the hemostatic parameters correlated with cirrhosis severity ($P < 0.05$).

At 12 months: 35 patients (28%) experienced a clinical decompensation, 23 (19%) died, 16 (13%) were transplanted. MELD-Na had the highest prognostic accuracy (AUC=0.809), compared to MELD (AUC=0.774) and Child-Pugh (AUC=0.771). FVIII/PC was the only hemostatic variable associated with the composite clinical end-point (OR:1.215;95%-CI:1.05-1.38; P =0.006).

A prognostic model including MELD-Na and FVIII/PC led to a slight increase of the AUC vs MELD-Na alone (AUC:0.815 vs 0.809). A FVIII/PC≥2.78, the lowest tertile in our series, marked a high predicted-risk of clinical outcome in the category of MELD-Na< 15 (45% vs 27%) compared to MELD-Na≥15 (83% vs 75%).

Conclusions: Markers of primary and secondary hemostasis correlate with common indexes of liver disease severity. FVIII/PC ratio may have a relevant prognostic role in patients with MELD-Na< 15.

PB1947 | An Unusual Case of Cerebral Cavernous Malformation Complicated with Consumptive Coagulopathy and Heightened Fibrinolysis

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Background: Intracranial hemorrhage in cerebral cavernous malformation (CCM) are well known. CCM may cause systemic abnormal bleeding, its pathogenesis was rarely reported. Antiangiogenetic agents were tried with variable therapeutic effects.

Aims: To study the pathogenesis of hemostatic defects in an unusual case of CCM experiencing systemic bleeding, and to present remarkable therapeutic effects of combined use of antiangiogenetic agents.

Methods: Hematological, biochemical and image studies were done routinely. Thrombin-antithrombin complex (TAT), and plasmin- antiplasmin complex(PAP)were detected employing enzyme immnoassay technique.

Results: A 30 years-old Taiwanese lady was doing well until February,2019 when she began to experience soreness and swelling of multiple parts of the body, and many small dark-red nodules

TABLE 1 Laboratory data of the patient

	Normal Range	Before use of Bevacizumab	2.5 months after use of Bevacizumab
Hb(gm/dl)	12.0-15.0	6.4	10.1
Reticulocyte(%)	0.4-2.2	10.5	3.1
Platelete(/ul)	157-377*10 3	46	139
Fibrinogen(mg/dl)	194-421	91.2	243.9
Factor V:C (IU/dl)	78-151	40.4	50.6
haptoglobin(mg/dl)	30-200	<8.1	<8.1
D-dimer(ng/ml)	60-325(FEU)	>10000	>10000
LDH(U/L)	140-271	657	578
TAT(ng/ml); PAP(ng/ml)	0.77-5.5; 13.9-241.2	14.9; 10270	Not yet tested; Not yet tested

appearing over trunk, extreimities and tongue surface. Extensive petechiae and purpura developed at her left thigh and right hand in March,2019.She had no neurologic disorders. Laboratory examinations revealed decreased levels of hemoglobin,platelet, fibrinogen,haptoglobin, and factor V:C, and increased levels of reticulocyte, indirect bilirubin(3.5mg/dl), LDH, D-dimer,TAT,and PAP(Table1). Coomb's tests were negative,euglobulin lysis time was >2 hours. These data were suggestive of consumptive coagulopathy and heightended fibrinolysis. Magnetic resonance image of the brain and soft tissues showed typical pictures of CCM(Fig 1). Genetic analysis identified heterozygous mutations of *CCM I*, IVS6+59 A to G,IVS7+65 G to C, IVS10+63 C to G and P.Val 660 Val in the patient and her father.

Rosuvastatin, sirolimus and transamin were tried since April 2019, Sirolimus was switched to everolimus since August 2019. These antiangiogenic and antifibrinolytic agents were used in vain. Bevacizumab was added in November 2019. Patient's hemostatic defects, hemolytic status and soft tissue swellings got much improved(Table 1)

Conclusions: We present an unusual familial case of CCM complicated with consumptive coagulopathy and heightened fibrinolysis as well as microangiopathic hemolysis causing abnormal bleedings and anemia, which were scarcely reported before. Bevacizumab combined with other antiangiogenetic agents seems effective.

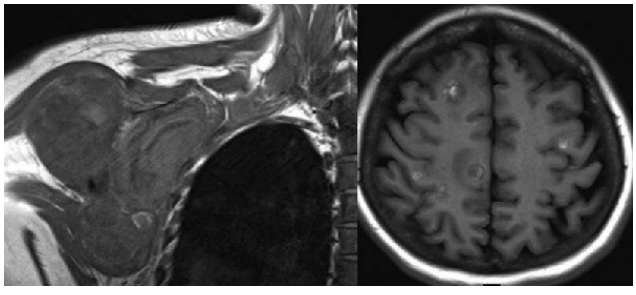

FIGURE 1 T1-weighted images of right shoulder and brain show multiple blood content lesions suggestive of multiple cavernous malformations

VASCULAR BIOLOGY

BLOOD CELLS AND VESSEL WALL

PB1948 | The Red Laser- Mediated Photobiomodulation Therapy, Accompanied by Aspirin Administration, Improve Endothelial Dysfunction after Balloon Angioplasty in the Rabbit Model of Carotid Artery Injury

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Background: The healthy endothelial cells are essential for maintenance of vascular homeostasis involving antioxidant, anti- inflammatory, pro- fibrinolytic, anti- adhesive or anticoagulant effects. As is well recognized, the majority of atherosclerosis start with a vascular injury, resulting in endothelial dysfunction.

Aims: In this study, we aimed to investigate the effect of the red laser- mediated photobiomodulation therapy, accompanied by aspirin administration on endothelial function in the rabbit model of carotid artery injury.

Methods: Briefly, New Zealand white rabbits underwent balloon dilatation injury at the right common carotid arteries. After 2 days, common carotid arteries of the treatment group at the lesion region, treated using extracorporeal low- level red laser (λ= 635 nm, E=100 J/cm2)- mediated photobiomodulation therapy accompanied by aspirin (10 mg/Kg) administration. In order to evaluate endothelial- dependent relaxation, acetylcholine- mediated dilation (AMD) was measured during the infusion of acetylcholine at a rate of 0.5 g/kg/ min and endothelial independent relaxation was evaluated by measuring nitroglycerin mediated dilation (NMD) during the infusion of nitroglycerin at a rate of 5 g/kg/min.

Results: Results from B- mode ultrasound images showed significant differences in AMD between the treated and the non- treated rabbits (P>0.05), whereas there were no significant differences in NMD between the treatment and normal groups (P>0.05). No microscopic

intimal lesions were seen in the normal and treated rabbits, but intimal thickening was observed in the histological studies in the non-treated rabbits.

Conclusions: Low- level red laser- mediated photobiomodulation therapy accompanied by aspirin administration can cause to the improve endothelial dysfunction via increasing endothelial nitric oxide (NO) synthase. This protocol may be a potential treatment to endothelial dysfunction after balloon angioplasty.

PB1949 | Neointimal Hyperplasia Regression using Lipid- Based Encapsulated Methotrexate Nanoparticles- Mediated Catheter-Based Low- Level Blue Laser Photodynamic Therapy

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Background: Neointimal hyperplasia is an important clinical entity in vascular surgery because it limits the long- term effectiveness of surgical and endovascular interventions. Neointimal hyperplasia is usually defined in an artery as thickening of the intimal layer after an injury such as angioplasty, stenting or surgical repair. Neointimal hyperplasia is also used to describe the thickening of venous and prosthetic bypass grafts that leads to reduced lumen diameter and flow and, ultimately, graft occlusion and thrombosis.

Aims: In this study, we aimed to investigate the effect of the low-level blue laser- mediated photodynamic therapy on neointimal hyperplasia reduction accompanied by lipid- based encapsulated methotrexate nanoparticles administration in the rabbit model of the carotid artery neointimal hyperplasia.

Methods: Endothelial denaturation of the right common carotid artery was performed by ballooning in New Zealand white rabbits. After 4 weeks, histopathology results showed the formation of the neointimal hyperplasia in all of the rabbits' arteries. Then treatment group underwent catheter- based low- level blue laser (λ= 417 nm, E= 150 J/cm2)- mediated photodynamic therapy accompanied by lipid- based encapsulated methotrexate (10 mg/Kg) nanoparticles administration.

Results: Results from histopathology, B- mode and color Doppler ultrasonography showed a significant reduction in the mean value for blood mean velocity, smooth muscle cells density in the neointima layer, wall mean thickness and percentage of luminal cross- sectional area of stenosis and significant increase in the mean value for blood volume flow at the stenotic region in the treatment group compared with the other groups ($P>0.05$).

Conclusions: Enhanced anti- inflammatory effect of methotrexate - induced by catheter- based low- level blue laser photodynamic therapy, can cause to the smooth muscle cells density reduction and significantly dilate the luminal cross- sectional area of stenosis. This protocol may be a potential treatment to neointimal hyperplasia.

PB1950 | Neutrophils and Neutrophil Extracellular Traps (NETs) Can Promote Clotting and Impact Clot Structure in a Distinctive Manner

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Background: Neutrophils release mediators (e.g. matrix metalloproteinases and serine proteases) which can influence thrombus formation. Neutrophil Extracellular Traps (NETs), which are extruded from neutrophils, have been shown to provide a "scaffold" for thrombosis and to increase the resistance of clots to fibrinolysis and thrombolysis. However, the interactions between neutrophils or NETs and fibrin(ogen) in clots, as well as the mechanisms behind these interactions are not yet fully understood.

Aims: To investigate the role of neutrophils/NETs in blood coagulation, fibrin formation, clot stability and clot mechanical properties, in order to decipher how neutrophils/NETs interact with the fibrin network.

Methods: Human neutrophils were isolated from whole blood by the standard density gradient centrifugation method. Neutrophils were stimulated by Phorbol 12-myristate 13-acetate to generate NETs. Turbidity measurements were used to measure the kinetics of clot formation. Confocal microscopy was used to investigate the effects of neutrophils/NETs on overall clot network structure. Permeation experiments were carried out to investigate clot porosity.

Results: Neutrophils and NETs promoted clot formation in pooled normal plasma without the addition of other triggers of coagulation. Both also delayed clot lysis. The procoagulant effect of neutrophils and NETs were also observed in FXII- and FVII-deficient plasma. However, the procoagulant effect of neutrophils was not observed, while NETs still induced clotting, in FXI-deficient plasma. NETs increased the density of clots, particularly in the areas immediately surrounding the NETs, but neutrophils did not. Neutrophil-induced clots were weak and showed large pores. NETs did not significantly affect the average pore size of clots.

Conclusions: NETs accelerate clotting and contribute to the formation of a denser clot architecture. These effects are not mediated through FVII, FXII or FXI but likely involve other components present in the plasma. Neutrophils, or mediators they release, induce clotting via FXI.

PB1952 | Morphological and Functional Alterations of Platelets from Transgenic APP23 Mice at Early Alzheimer Disease State

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Background: Deposits of Aβ in brain parenchyma and cerebral vessels are the pathological hallmarks of Alzheimer's disease (AD). Recently, we demonstrated that aged Alzheimer transgenic mice (APP23), which develop amyloid-β deposits in the brain parenchyma and cerebral vessels, have pre-activated platelets in blood and enhanced integrin activation upon agonist stimulation. Moreover, platelets adhere to vascular amyloid-β deposits and cause vessel occlusion in aged APP23 mice. A comprehensive analysis of these AD transgenic mice revealed that activated platelets directly contribute to vascular amyloid plaques by promoting the formation of Aβ aggregates.

Aims: Investigation of initial changes of platelets from middle-aged transgenic APP23 mice (at the age of 7 to 10 month), which already have amyloid pathology in the brain parenchyma, but still no vascular amyloid deposits.

Methods: FACS analysis and *in vitro* platelet function.

Results: Middle-aged transgenic APP23 mice had unaltered platelet count, platelet size and glycoprotein expression comparable to control mice. However, transmission electron microscopy analysis showed a significantly increased number of dense granules. The number of α-granules was increased only by trend using platelets from middle-aged APP23 mice. Secretion of α- and dense-granules was increased selectively upon stimulation with CRP that stimulates the major platelet collagen receptor glycoprotein (GP) VI. Additionally, we observed enhanced CRP-triggered aggregation of platelets from middle aged APP23. Flow chamber experiments revealed increased thrombus formation on collagen at high shear rates (1.700 sec-1) ex vivo but unaltered formation of thrombi under moderate shear of 1000 s-1. However, the binding of vWF to platelets was unaltered in APP23 transgenic platelets.

Conclusions: APP23 mice show morphological and functional alterations of platelets before vascular amyloid plaques develop in these mice indicating that platelet changes in aged APP23 mice are not only the result of vascular Aβ deposits. Thus, anti-platelet therapy of AD has to start as early as possible.

PB1953 | CTL-2 is a VWF Receptor Involved in Neutrophil Activation

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Background: Genome wide association studies linked the *SLC44A2* rs2288904 (461G>A) polymorphism with venous thrombosis (VT) in humans. It leads to HNA-3a (human neutrophil antigen-3a) or HNA-3b epitope expression by the choline transporter like protein-2 (CTL-2). HNA-3b is associated with a 30% decreased risk of VT. CTL-2 is a ubiquitous transmembrane protein identified as a receptor for Von Willebrand factor (VWF).

Aims: In order to explain the link between CTL-2 and VT development we wanted to determine how CTL-2 expressing either HNA-3a or HNA-3b on neutrophils could modulate their adhesion and activation on VWF under flow.

Methods: Neutrophils homozygous for the HNA-3a- or the HNA-3b-coding allele purified from healthy blood donors were perfused in flow chambers at venous shear rates ($100s^{-1}$). Different matrices including VWF, and different blocking antibodies were tested. Neutrophil activation under VWF and LPS challenge was evaluated at various cell concentrations in different flow conditions. Neutrophil mobilization was also measured by intravital microscopy in venules from *SLC44A2*-KO and WT mice after histamine-induced endothelial degranulation and anti-Ly6G staining.

Results: HNA-3a expression was required for CTL-2-mediated neutrophil adhesion to VWF at $100s^{-1}$. This adhesion could occur independently of calcium mobilization and beta-2 integrin engagement. We observed that adhesion of HNA-3a-expressing neutrophils was enhanced when they were preactivated with LPS. Moreover, specific shear conditions could act as a “second hit” on those, increasing their activation and inducing the formation of neutrophil extracellular trap aggregates. In vivo, mice lacking CTL-2 showed a massive reduction in neutrophil rolling in inflamed mesenteric venules.

Conclusions: Our results show that CTL-2/HNA-3a is important in the adhesion and activation process of neutrophils in veins under inflammation and when submitted to specific shears conditions. Neutrophils expressing CTL-2/HNA-3b not being associated with these observations, these results could thus explain the association between the HNA-3b epitope and the reduced risk for VT in humans.

PB1955 | Tripeptides Affected Hemostas in Various Pathologies

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Background: For modern medicine and pharmacology, the most important are the problems of finding new drugs that are safe and without side effects. The use of drugs based on regulatory peptides can significantly help in solving these problems. However, until recently, few such peptides have been found.

Aims: To study the effect of small regulatory peptides, which are fragments of many natures of compounds, such as collagen, neurohormones, vasopressin, oxytocin, luliberin on platelet aggregation. Data on the effect of tripeptides on aggregation are presented on immobilization stress models and on the development of experimental inflammation.

Methods: Experiments were carried out on white mongrel male rats weighing 180-200 g body. A pro-inflammatory agent was used to simulate experimental peritonitis - 40% sodium thioglyaolate solution 4g / kg, administered intraperitoneally. We are using peptide Pro-Gly-Pro (PGP) fragment of collagen in dose 3.7 mkg/kg intramuscularly for prophylactic and treatment inflammation. Platelet aggregation (PA) was recorded on a aggregometer. Inductor aggregation - ADP -10 mM. In the next series of experiments, animals were subjected to immobilization stress for 60 minutes and determined the effect of C-terminal tripeptides of neurohypophysial hormones on PA. All experiments were performed according to the documents of the European Science Foundation.

Results: The development of inflammation causes an increase in PA with a maximum effect 2 hours after administration of thioglycolate. Prophylactic peptide administration significantly reduced PA, whereas peptide did not show any statistically significant results on the background of an already developed inflammation. C-terminal tripeptides of neurohypophysial hormones Pro-Arg-Gly and Pro-Leu-Gly also had a protective effect on PA against immobilization preventing the development of a prothrombotic stress effect.

Conclusions: Thus, we can note that the tripeptide of various origin can have a prophylactic protective effect, reducing PA in inflammation and immobilization stress.

PB1956 | Expression Profiles of the Internal Jugular and Saphenous Veins: Focus on Hemostasis Genes

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Background: The ample heterogeneity of veins, related to their specific role and position should modulate the transcriptional profile of anticoagulants and procoagulant genes which contribute to the "in situ" hemostasis balance, and could modulate the ability of the individual vascular bed to counteract prothrombotic stimuli. The internal jugular vein (IJV) has a major role in cerebral venous return towards the heart, and differs from saphenous vein (SV) for morphological and hemodynamic characteristics.

Aims: To compare the IJV and SV transcriptomes, which could participate in venous bed specificity and vulnerability to thrombus formation.

Methods: Microarray-based transcriptome analysis in wall and valve specimens from IJV and SV collected during surgical reconstruction of IJV by patch angioplasty in multiple sclerosis patients with impaired brain outflow. Multiplex antigenic assay in paired jugular and peripheral plasma samples.

Results: 3375 differentially expressed transcripts in walls defined distinct venous expression profiles. The "complement and coagulation cascade" emerged among the enriched pathways. In IJV, upregulation of genes for coagulation inhibitors (TFPI, PROS1), activated protein C pathway receptors (THBD, PROCR), fibrinolysis activators (PLAT, PLAUR), and downregulation of the fibrinolysis inhibitor (SERPINE1) and of contact/amplification pathway genes (F11, F12), would be compatible with a thromboprotective profile in respect to SV. Further, in SV valve the prothrombinase complex genes (F5, F2) were up-regulated and the VWF showed the highest expression. Differential expression of several VWF regulators (ABO, ST3GAL4, SCARA5, CLEC4M) was also observed. Among other differentially expressed hemostasis-related genes, heparanase (HPSE)/heparanase inhibitor (HPSE2) were up-/down-regulated in IJV, which might support procoagulant features and disease conditions. For several proteins, encoded by differentially expressed genes, the jugular plasma levels were lower and highly correlated with peripheral levels.

Conclusions: The IJV and SV rely on differential expression of many hemostasis and hemostasis-related genes to balance local hemostasis, potentially related to differences in vulnerability to thrombosis.

PB1957 | The Induction Strategies Administered in the Treatment of Multiple Myeloma Exhibit a Deleterious Effect on the Endothelium

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Background: The induction treatment for multiple myeloma (MM) patients has changed drastically in the last few years, including proteasome inhibitors (PI) and immunomodulatory agents. In parallel with these changes, the incidence of engraftment syndrome (ES), where endothelial damage implication cannot be disregarded, has increased.

Aims: Investigate whether the induction strategies (PI, such as bortezomib, together with immunomodulatory drugs, such as thalidomide, and dexamethasone) damage the endothelium, and explore the role of defibrotide (DF) as an endothelial protective agent.

Methods: Human microvascular endothelial cells (EC) in culture were exposed to the compounds (bortezomib -Velcade-, V; thalidomide, T; and dexamethasone, D) separately, or in combination without (VTD) and with DF (VTD+DF). Differences with control (no drug) were measured in markers of: i) inflammation (expression of ICAM-1 and adhesion of leukocytes under flow conditions, at the cell surface, ii) production of VWF, iii) cell permeability (VE-cadherin expression and cell monolayer integrity), and iv) oxidative stress (ROS production and eNOS expression).

Results: The expression of ICAM-1 and VWF were significantly higher in VTD but similar to control in VTD+DF. Separately, bortezomib demonstrated to be the main deleterious agent whereas dexamethasone showed no harmful effect. Leukocyte adhesion showed similar trends. VE-cadherin expression was significantly lower in VTD and slightly normalized in VTD+DF. EC permeability increased only with bortezomib. No significant changes were observed in ROS production and eNOS expression.

Conclusions: Our results demonstrate that bortezomib exerts the most damaging action on the endothelium in our in vitro approach, leading to a proinflammatory profile, increased expression of VWF, potentially modifying the prothrombotic behavior of the extracellular matrix, and compromised vascular integrity. Defibrotide prevents these damaging effects. A better knowledge of the mechanisms of the induction drugs will allow the design of effective protective measures to prevent the engraftment syndrome.

PB1959 | Colonization with the Minimal Microbial Consortium Altered Schaedler Flora Hampers the Formation of Neutrophil Extracellular Traps in Acute Mesenteric Ischemia-Reperfusion Injury

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Background: While the role of neutrophil extracellular traps (NETs) in sepsis is well-defined, the impact of the commensal gut flora and related activation of innate immune signaling for NETosis in the mesenteric microcirculation during mesenteric ischemia-reperfusion (MIR) is entirely unresolved. Under this condition, gut microbes leak into the portal circulation and need to be sequestered by activated neutrophils to prevent systemic dissemination. To investigate the impact of gut microbiota on this issue in a controlled experimental environment, Altered Schaedler Flora (ASF) mice, which have a defined minimal microbial consortium of eight gut bacteria, were studied.

Aims: To explore how the defined ASF affects NET-formation and leukocyte recruitment in acute MIR injury.

Methods: In the MIR model, laparatomy was applied to 6-8 weeks old mice under anesthesia, inducing ischemia in the small intestine by ligating the *A. mesenterica cranialis* with a microclamp. After 1 hour, reperfusion was allowed by removing the clamp. Adherent leukocytes (stained with Acridine Orange) and NETs (stained with SYTOX Orange) were counted *in vivo* in a 0.06 mm^2 area of interest with a high-speed epifluorescence microscope both before the ligation and after reperfusion. Germ-free (GF) C57BL/6J mice, ASF mice and ASF mice treated with Neomycin and Ampicillin (ASF+Abx) were studied. Statistical analyses were performed with the independent samples Student's t-test (age-matched groups).

Results: Post ischemia, GF mice showed significantly increased numbers of adherent leukocytes in mesenteric venules, whereas ASF mice showed a decrease. NET formation was significantly increased in GF, ASF and ASF+Abx mice after reperfusion. ASF mice showed less NETosis compared to GF controls. As a confirm, depletion of the Altered Schaedler Flora by antibiotics restored a trend of elevated NET formation.

Conclusions: The observed reduction in NETing neutrophils in ASF mice relative to GF controls demonstrates a role of the gut microbiota as an environmental pre-conditioning factor in MIR-induced NETosis.

PB1960 | Role of Thrombopoietin in Rescuing Vascular Niche of ITP Patients

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Background: The vascular niche of immune thrombocytopenia (ITP) is proven to be damaged.
It is presented as the impaired functions of migration and tube-formation in bone marrow endothelial progenitor cells (BM-EPCs). Our previous studies shown that thrombopoietin (TPO) has a protective effect on human endothelial cells.
Aims: This study aims to investigate whether TPO has a protective effect in ITP-EPCs, and to explore its potential mechanism.
Methods: BM-EPCs were isolated from ITP patients, and divided into two groups: TPO-treated group and TPO-free (control) group. CCK8 was used to explore the proliferative effect of TPO on BM-EPCs. BM-EPCs was identified by flow cytometry with co-expression of mouse anti-humanCD133, mouse anti-human CD34 and mouse anti-human vascular endothelial growth factor receptor (CD309) monoclonal antibodies. Moreover, the number of EPCs was evaluated with DiL-Ac-LDL uptake and FITC-UEA -I binding assay. The BM-EPCs functions were examed by tube-formation and migration experiments.
Results: The EPCs isolated from ITP patient in TPO-treated group exhibited longer fusiform and elliptical shape after 5 days and cobblestone-like morphology after 7-10 days culture. The result of CCK-8 showed TPO promoted proliferation of EPCs and the optional drug concentration is 100 ng/ml. Much higher co-expression of CD34/CD133/CD309 in TPO-treated group was detected by flow cytometry. The number of DiL-Ac-LDL (red) and FITC-UEA -I (green) double-stained cells in the TPO group was significantly higher than that of control group (n=10, P<0.05). The tube formation (n=10, P < 0.05) and migration capacity (n=8, P < 0.05) were significantly enhanced in TPO-treated group.
Conclusions: Our studies indicates that TPO exerts protective effect of BM-EPCs in ITP patients, which may rescue megakaryopoiesis and reduce hemorrhage in these cases.

PB1961 | The Antioxidant and Antiinflammatory Effect of Synbiotic Beverage from Soybean Water Kerfir on Aortic Tissue of Hyperlipidemic Rats Model

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Background: Hyperlipidemia is the pathological condition that can caused cell injury which characterized by low level of antioxidant and high level of inflammatory activity, including blood vessel. Synbiotic is the combination of prebiotic and probiotic. Soybean is one of the most potential prebiotics which have an antioxidant properties. Water kefir is a fermented milk which contain high amount of lactic acid bacteria.
Aims: The aim of this study was to determine the antioxidant (MDA and SOD) and antiinflammatory (neutrophil count) effect of soybean water kefir on aortic tissue of hyperlipidemic rats model.
Methods: The research used experimental method with post-test only control group design. The sample of the study were 15 male rats (*Rattus norvegicus*) aged 2-3 months and weight 180-250 grams. All groups were acclimatized for one week and fed ad libitum for 8 weeks. Group II and III were given quail egg yolk with dose 5 ml/200 grams of body weight for 4 weeks. While group III was given the soybean water kefir with dose of 5 ml/200 grams of body weight for the next 4 weeks. At the end of the study, rats were terminated. Aortic organ were examined for MDA and SOD level, while blood taken in rats tail were examined under light microscope with Wright staining to know the neutrophil count.
Results: Mean of MDA on each group were consecutively 2.48±0.37, 12.17±0.38, 5.48±0.20 nmol/g. Mean of SOD on each group were consecutively 72.34±0.48, 3.29±0.09, 6.14±0.32%. Mean of neutrophil count were 40.33±0.47, 62.51±0.39, 45.56±0.35%, respectively. The one-way ANOVA showed significant difference in MDA, SOD, and Neutrophil count between these group with P =0.00. Bonferroni post hoc test also showed significant comparison between these group with P =0.00.
Conclusions: The soybean water kefir was significantly increase the antioxidant activity and decrease the inflammatory activity in hyperlipidemic rats model (P < 0.05).

PB1962 | Vessel-On-A-Chip Model Revealing Rapid Offset and Onset Mechanisms of Collagen- and Thrombin-Induced Platelet Activation

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Background: The vascular endothelium has an established antiplatelet and anticoagulant role, acting on flowing blood. To understand the mechanisms and kinetics of these platelet- and coagulation-inhibitory processes *in vitro* endothelial models need to be developed.
Aims: Establish an endothelialised microfluidic model to determine the spatio-temporal mechanisms of vascular control of collagen- and thrombin-induced platelet activation under flow.
Methods: Human umbilical vein endothelial cells (HUVEC) were cultured on collagen and TF in microfluidic channels, and subjected to whole blood perfusion. Platelet activation after incubation with HUVEC under stasis was monitored from Ca^{2+} fluxes using Calcium-6 dye.

Results: Culturing of HUVEC on collagen to (sub)confluency under stasis resulted in cell patches containing a glycocalyx and expressing von Willebrand factor (VWF), as shown by multicolor confocal microscopy. When Calcium-6 labelled platelets were incubated with HUVEC, platelet Ca^{2+} fluxes were suppressed in response to thrombin or collagen receptor agonist CRP-XL, in a time dependent manner ($t_{1/2}$ = 10 minutes). Similarly, an immediate abrogation of platelet Ca^{+2} fluxes by stable prostacyclin pointed to the involvement (at least in part) of cAMP/PKA pathway, as confirmed by VASP phosphorylation profiles.
Subjection of the HUVEC, cultured on collagen with(out) tissue factor, to recalficied whole blood in microfluidics resulted in remarkable changes in collagen/VWF-induced platelet activation and fibrin generation, in comparison to channels not containing HUVEC. Shear-dependent platelet adhesion and activation occurred rapidly, but remained strictly confined to the collagen/TF spots in between HUVEC patches. Fibrin formation was delayed, and again only occurred in between the HUVEC patches. These effects were modified by treatment with thrombin inhibitor or heparinase (glycocalyx degradation).
Conclusions: The developed endothelial model revealed that HUVEC patches rapidly, but only locally, suppressed collagen- and thrombin-induced platelet activation under flow, thus pointing to rapid offset and onset mechanisms. Local control is provided by cAMP elevation and coagulation suppression.

PB1963 | Redox Regulation of Neutrophil Mac-1 Function by Protein Disulphide Isomerase

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Background: The $\alpha_M\beta_2$ integrin (or Mac-1) controls neutrophil mobilization during inflammation by binding to multiple ligands, including ICAM-1, fibrinogen and CD40-L. Thiol oxidoreductase PDI binds to Mac-1 and promotes neutrophil trafficking in animal models, however the mechanism is not understood.
Aims: We hypothesized that PDI regulates neutrophil trafficking by reduction and/or oxidation of disulphide bonds in Mac-1.
Methods: The effect of PDI on the redox state of Mac-1 disulphides was determined by differential cysteine alkylation-coupled mass spectrometry. The binding of ligands to Mac-1 was measured by flow cytometry and plate adhesion using BHK cells expressing wild type or disulphide bond mutant Mac-1 (Cys to Ser substitutions). Neutrophil adhesion to endothelial cells and ligands under shear was measured by microfluidics. Neutrophil trafficking *in vivo* was measured using a tail-wounding model in Tg(lyzC:gfp) zebrafish.
Results: PDI reduced 2 of the 28 disulphide bonds in the β_2 subunit of Mac-1; Cys169-Cys176 and Cys224-Cys264. Under static conditions, ablation of both disulphides Cys169-Cys176 and Cys224-Cys264 resulted in decreased adhesion to fluorescently-labelled fibrinogen,

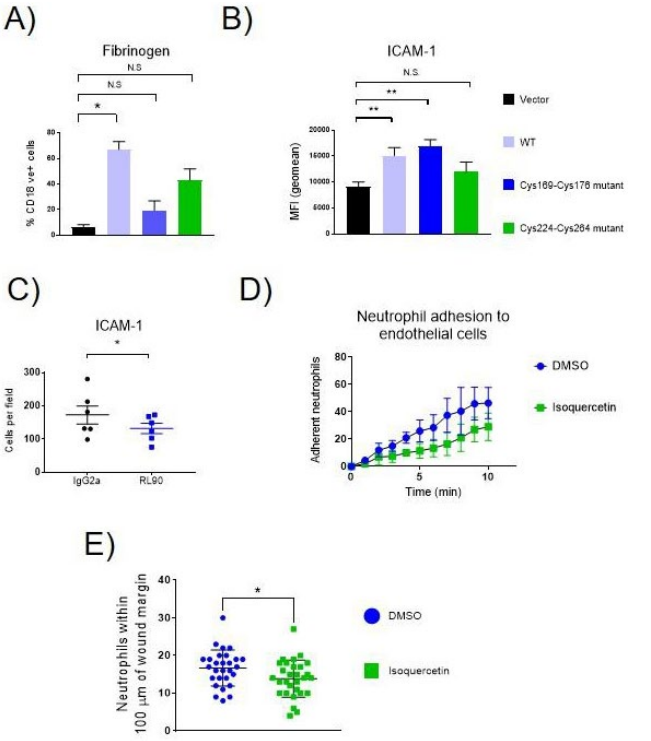

FIGURE 1 The role of PDI and disulphide bonds in Mac-1 ligand binding and neutrophil function *P<0.05, **P<0.01

however only the absence of Cys224-Cys264 decreased adhesion to ICAM-1 detected by flow cytometry with anti-ICAM-1 Ab (Figure 1A, B). PDI was detected at the leading edge and trailing end of mobilizing neutrophils and colocalised with β_2 integrin. PDI inhibitory antibody RL90 decreased adhesion of fMLF-primed neutrophils under shear (0.35 dynes/cm^2) to ICAM-1 but not to fibrinogen (Figure 1C). The PDI small molecule inhibitor isoquercetin decreased adhesion of neutrophils to endothelial cells stimulated with TNF-α (Figure 1D) and neutrophil migration to the site of zebrafish tail injury (Figure 1E).
Conclusions: PDI decreases the affinity of Mac-1 for fibrinogen and ICAM-1 by reducing disulphide bonds in the β_2 subunit. The consecutive reduction-oxidation of Mac-1 disulphide bonds may facilitate cycles of adhesion-de-adhesion to substrate, facilitating neutrophil mobilization. Blocking of PDI function may inhibit neutrophil mobilization to sites of inflammation.

PB1964 | Mechanisms and Functional Consequences of Platelet-derived Extracellular Vesicle and Chemokine Internalisation by Endothelial Cells

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Background: Atherosclerosis is characterized as inflammation of the endothelial vessel wall leading to plaque formation and eventually

causing cardiovascular complications. Platelet-derived extracellular vesicles (PEVs) and chemokines are known to upregulate the inflammatory state, leading to an acceleration of plaque formation. PEVs and chemokines can mediate communication and interactions between endothelial and immune cells, which implies direct interactions of PEVs and even uptake by these cells. However, the molecular mechanisms of adhesion and uptake and their functional consequences of the recipient cells remain unclear.

Aims: The characterisation of the uptake of PEVs and chemokines by endothelial cells and the functional consequences.

Methods: PEVs were isolated by centrifugation from platelet concentrates. Chemokines CCL5 and CXCL4 were expressed and purified from E. coli. PEV internalisation of Alexa-Fluor 488 anti-CD41 labelled PEVs was assessed during incubation of HUVECS for 12 hours and imaged by multicolour fluorescence microscopy. Changes in cytosolic [Ca2+] in Fluo-4-loaded HUVECs were recorded for 10 min, using a Zeiss LSM 510 confocal microscope. Monocyte adhesion was performed with Syto-13-labelled THP-1 cells and perfused over differently treated HUVECs.

Results: HUVEC cells appeared to take-up PEVs, which was increased when F-actin polymerisation was inhibited. Interestingly, PEV uptake was not affected after dynamin endocytosis was inhibited. PEVs or chemokines caused repetitive oscillatory transients in cytosolic Ca^{2+} signalling of HUVECs, in agreement with G-protein coupled receptor signalling. In addition, monocyte adhesion on TNF-α treated HUVECS was increased and further significantly stimulated by addition of PEVs or chemokines.

Conclusions: PEV uptake by endothelial cells is regulated via a cytoskeletal mechanism and might not be controlled by dynamin. Monocyte adhesion and intracellular calcium responses of endothelial cells are increased by PEV and chemokines.

PB1965 | Aortic Ring Angiogenesis Proceeds Normally Despite Minimal Tissue Factor Expression

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Background: Tissue factor (TF) is a pro-coagulant receptor that is thought to promote tumor-associated angiogenesis through MAPK signaling. TF is highly expressed in malignancy and the amount of TF expressed by cancer cells directly correlates with the density of tumor vasculature. Thus it has been suggested that angiogenesis is dependent on TF expression. However, we have reported that TF in pericytes is downregulated during angiogenesis in normal wound healing. TF downregulation is confined to the angiogenesis phase of wound healing and only occurs in pericytes that encircle the angiogenic vessels.

Aims: The goal of this study was to characterize the relationship between TF expression and pericyte function during physiologic angiogenesis using the aortic ring model.

Methods: TF knockout (KO) is embryonic lethal. Therefore, we used low TF-expressing mice. These mice bear two alleles for human TF which are driven by the human TF promoter, resulting in expression of ~1% normal TF levels. Abdominal aortas were harvested from mice aged 4 to 8 weeks, cut into 0.1 cm rings, and cultured in collagen matrices for 21 days. The number of neovessels sprouting from each ring was counted on select days.

Results: Surprisingly, there was no difference in the number of sprouts from WT and low TF-expressing rings at any time point (n=24 rings per group). To further characterize angiogenesis we utilized immunofluorescent staining and confocal microscopy to evaluate pericyte morphology and sprout coverage in 7-day-old cultures. Image J was used to analyze the proportion of the vessel area that stained for the pericyte marker NG2. Results showed no difference in the amount of pericyte coverage or pericyte morphology.

Conclusions: These data suggest that normal aortic ring angiogenesis is maintained despite minimal TF expression. This further suggests that unlike pathologic angiogenesis, the amount of physiologic angiogenesis does not correlate with the amount of expressed TF.

PB1966 | Development of a Novel Mouse Model of Abdominal Aortic Aneurysm with Intraluminal Thrombus

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Background: Abdominal Aortic Aneurysm (AAA) is defined as an irreversible arterial wall dilation with a diameter >50% and characterized by thinning and weakness of the vascular wall. In humans, aneurysms are associated with intramural thrombus and are prone to rupture which result in death. Thrombi from AAA patients are highly enriched in leukocytes and bacteria, *eg. Porphyromonas gingivalis* (*Pg*). This leukocyte-rich thrombus is considered the driving force of vessel wall rupture. Beyond their role in aneurysmal thrombus formation, platelets support leukocyte recruitment and interact with bacteria. This cross-talk is an important feature in thrombo-inflammatory vascular disease.

Aims: To decipher the role of platelets in intraluminal thrombosis in AAA.

Methods: AAA was induced by applying an elastase-soaked filter paper on the infrarenal abdominal aorta of WT mice and mice lacking the GPIbα subunit of the platelet receptor for von Willebrand factor hIL4R/GPIbα mice, the ADP receptor P2Y12, or the thrombin receptor PAR4.

Results: In elastase-treated WT mice, an early recruitment of platelets and leukocytes to the damaged vessel wall is observed

by intravital microscopy. No thrombi were observed by histology. Histological analysis of elastase-treated WT mice injected with Pg, showed large thrombi in the dilated vessel wall in 70% of the mice (9 out of 13 mice). These thrombi were enriched in platelets, leukocytes and neutrophil extracellular traps. Similar to WT mice, intraluminal thrombosis occurred in 67% of hIL4/GPIbα mice (3 out of 5). P2Y12 deficient mice showed an incidence of 50% (2 out of 4) of intraluminal thrombosis. Remarkably, PAR4 null mice have a significant reduced intraluminal incidence thrombosis of 25% (1 out of 4 mice).
Conclusions: We established a novel mouse model of AAA with an intraluminal thrombus. Unlike the GPIbα and PY12 receptors, our data suggest that PAR4 plays a critical role to the intraluminal thrombus formation and platelet activation in *Pg*-injected mice.

PB1967 | Optimization of Disease- and Patient-specific *in vitro* Assays for Endothelial Dysfunction Investigation by Using Endothelial Colony-forming Cells

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Background: The study of endothelial dysfunction (ED) is crucial to identify pathogenetic mechanism(s), define distinct clinicopathological entities and guide patient treatment in several pathological settings. Nevertheless, it is currently hindered by the limited availability of patient-specific primary cells. In this context, endothelial colony-forming cells (ECFCs) represent a valuable tool, as their characterization allows non-invasive functional assessment of the endothelial compartment.
Aims: To introduce the use of ECFCs as a patient-specific primary cell source to analyze ED in

a). thrombogenesis studies under flow conditions, and
b). thrombin generation assay (TGA) on endothelial cells.

Methods: Standard thrombogenesis assays and TGA were modified, using ECFCs as a substrate compared with commercially-available human umbilical venous endothelial cells (HUVECs) as a control. ECFCs were isolated from healthy donor peripheral blood using a protocol previously optimized in our lab. Endothelial function was assessed at passages 4-6, in basal conditions or after activation with TNFa, by analyzing: a) platelet deposition and fibrin formation under flow conditions, assessed in thrombogenesis assay by confocal microscopy; b) thrombin formation assessed by TGA on endothelial cells - a modification of Hamker's method. Expression of the adhesion molecule CD106 (VCAM-1) and the pro-coagulant molecule Tissue Factor (TF) were also assessed, by flow cytometry.
Results: The experimental setting of both assays was optimized on HUVECs and then tested on ECFCs, confirming on both cell types the ability of the assays to discriminate between unstimulated and activated cells. In fact, activated cells were characterized by a) increased platelet deposition and fibrin formation, and b) thrombin generation, compared with unstimulated cells. Activation of TNFa-treated endothelial cells was also confirmed by assessing the upregulation of CD106 and TF.
Conclusions: Our results support the use of ECFCs for patient-specific *in vitro* investigation of ED, thus paving the way for their application in personalized modeling of vascular diseases.

PB1968 | Generation and Isolation of Extracellular Vesicles from Endothelial and Smooth Muscle Cells Grown out of Human Blood

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Background: Smooth muscle cells and endothelial are often sites of pathology and drug action. The potential to grow these cells from blood (termed blood outgrowth endothelial and smooth muscle cells; BOECs and BO-SMCs respectively) without the need for surgery has revolutionised the vascular field. Unlike BOECs, for which there are >100 publications, there are only 12 papers on BOSMCs isolated from humans and no studies that have managed to isolate extracellular vesicles (EVs) from both cell types. EVs are involved in cell communication and can also serve as biomarkers in disease conditions.
Aims: Obtain fluorescently labelled EVs from pre-labelled BOECs and BOSMCs under inflammatory conditions.
Methods: BOECs and BOSMCs were isolated from healthy donors (Ahmetaj-Shala et al, FASEB J, 2020). Cells grown to confluence on a T25 were washed with PBS and gently scraped in fresh PBS. The suspension was centrifuged at 200 x g for 5 minutes. The pellet was resuspended with 0% serum EGM-2 media, labelled with fluorescent CellTracker™ Red CMTPX (10 μM) and incubated for 30 minutes in the dark. Cells were then seeded on a T25 and stimulated with LPS (1 μg/ml) and a control sample was collected. Cells were then incubated for 16 hours, at 37oC, 5% pCO2. The media was collected and, together with the control sample, centrifuged first at 2,000 g for 10 minutes to pellet cells and debris. The supernatant was recovered and centrifuged again at 15,000 g, for 1 hour. The EV-containing pellet was resuspended and analysed through flow cytometry (BD Fortessa).
Results: Both BOECs and BOSMCs were successfully labelled and generated EVs after stimulation with LPS. Consequently, EVs were

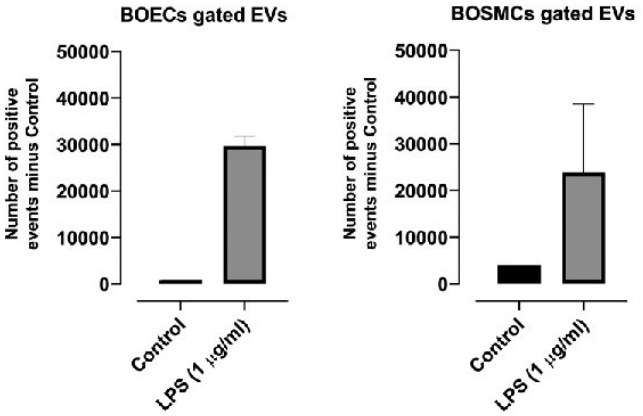

FIGURE 1 LPS stimulation facilitates the generation of extracellular vesicles in BOECs and BOSMCs

also fluorescently labelled and tracked after flow cytometric analysis (Figure 1).

Conclusions: This novel and robust method will allow further exploratory research in order to understand the physiology of BOECs, BOSMCs and their EVs.

PB1969 | The Effects of Hypoxia on Activation of Endothelial Cells

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Background: Venous thrombi are formed in the valve pockets of the deep veins in a microenvironment characterized by vortex flow and severe hypoxia (sustained and intermittent). Hypoxia induces cellular stress and inflammation, and is believed to trigger and contribute to thrombus formation in the valve pocket via mechanisms that are still largely unknown. Intravascular cells, inflammatory mediators and procoagulant extracellular vesicles (EVs) are thought to be involved and to drive the thrombus formation in the valve pocket.

Aims: To shed light on the triggering role of hypoxia in thrombus formation we aim to establish an in vitro model by which the effects of hypoxia on intravascular cells are examined and monitored, as well as the procoagulant potential of EVs that are produced by such cells under sustained and intermittent hypoxia.

Methods: A state-of-the-art hypoxia chamber was used to induce sustained and intermittent hypoxia in cultures of intravascular cells including endothelial cells, platelets and monocytes. The effects on cells were monitored at transcript and protein levels. EVs were isolated from culture supernatants by differential centrifugations, quantified, characterized and their procoagulant potential by determining the procoagulant phospholipid activity (PPL activity).

Results: A hypoxia chamber has been successfully setup to generate significant oxygen deprivation, which was measured and confirmed in culture media. Multicolor flow cytometric analyses of human umbilical vein endothelial cells (HUVECs) displayed the potential of detecting cellular activation following inflammatory stimuli, monitored as elevated expression levels of ICAM-1 and tissue factor (TF). Endothelial EAhy926 cells exposed to short-term hypoxia (down to 1% oxygen) decreased the expression of endothelial protein C receptor (EPCR) compared to cells cultured in normoxic conditions. Modest changes were also detected for the expression of thrombomodulin.

Conclusions: Inflammatory stimuli and exposure to hypoxic conditions rapidly activated endothelial cells (within 4 hours) and modulated key molecules in the coagulation- and inflammation pathways.

PB1970 | Differential Expression of Inflammatory Mediators in Venous Tissue and Serum Suggest Natural Killer (NK) Cell-mediated Responses in Chronic Venous Insufficiency

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Background: Varicose vein (VV) disease affects millions of people causing fatigue, aching pain, itching, and bleeding. In severe cases, patients with VV develop venous leg ulcers (VLU), even in the absence of deep venous thrombosis. VLU are chronic and often refractory in the long term, despite better healing rates with endovenous ablation (EVA). Inflammation may predispose VV patients to develop VLU.

Aims: In this study, we evaluated inflammatory mediator profiles from serum and vein segments from patients with VV.

Methods: Vein segments were obtained from patients undergoing microphlebectomy for VV and were digested enzymatically. Additionally, serum from within refluxing VV was obtained during EVA and percutaneous sclerotherapy. Infrared thermography was utilized to assess skin temperature over refluxing and competent leg veins. Protein-level inflammatory mediators from VV and serum

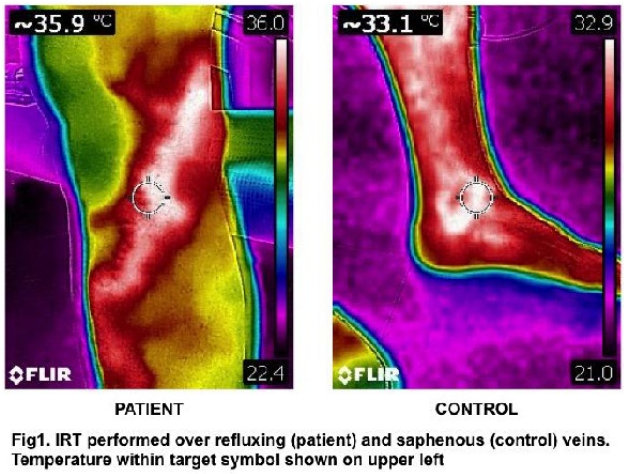

FIGURE 1 Figure demonstrates temperature differences over refluxing and competent leg veins

were analyzed using multiplex analysis, and individual mediators were compared among groups using Students t-test.

Results: Temperatures over refluxing veins were higher by 3 degrees Celsius than those over competent leg veins (P < 0.03).VV vein segments (N=5) expressed significantly lower concentrations of IL-1b (P < 0.02), IP-10 (P < 0.003), MCP-1 (P < 0.0003), MIP-1b (P =0.0003), and TNF-a (P < 0.01) when compared to serum (n=14). In contrast, IL-15 was expressed at significantly higher levels within the vein wall compared to serum (P < 0.0001).

Conclusions: Inflammation may play a critical role in varicose vein disease, prompting advancement to ulceration. Refluxing veins have higher surrounding skin temperature, confirming an inflammatory process occurring at the vein wall. Unlike other inflammatory mediators which were present either at similar or higher levels in serum, IL-15 was present at significantly higher levels within the vein wall than in serum. As a positive modulator of NK cell function and cytotoxicity, IL-15 may represent a local inflammatory response in the vein wall, and may be a target to attenuate progression to VLU in the future.

ENDOTHELIAL CELL SIGNALING

PB1971 | Elevated Plasminogen Activator Inhibitor Type-1 and Soluble Thrombomodulin of Type 2 Diabetes Mellitus Subjects with Increasing Loss of Glycemic Control in Calabar, Southern Nigeria

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Background: Endothelial cell markers such as soluble thrombomodulin, (STH) and plasminogen activator inhibitor type-1 (PAI-1) among many others, are circulating molecules synthesized in various amounts by the endothelium. Assessment of such markers in diabetic subjects on treatment would reveal the extent of hemostatic complications taking place in the given population.

Aims: The study is aimed at providing information on plasminogen activator inhibitor type-1 and soluble thrombomodulin levels of diabetes mellitus type 2 subjects based on glycemic control.

Methods: The subjects were made up of ninety diabetes mellitus type 2 subjects aged 35-75 years attending clinic at University of Calabar Teaching Hospital with ninety age and sex-matched non-diabetic control subjects. Ethical approval was obtained from the Health Research Ethical Committee (HREC) of the hospital. Bio-data and related information were obtained using a well-structured questionnaire. Each participant in the research gave informed consent. Seven milliliters of venous blood were collected from each participant for analyses of glycated hemoglobin by ion exchange resin method. PAI-1 and STH were assayed by enzyme-linked immunosorbent assay method. Data analysis was done using SPSS version 22.0. Student t-test was used to compare means between test and control subjects. Statistical significance was drawn at P ≤ 0.05.

Results: More females than males were affected with type 2 diabetes in the research. HbA1c, PAI-1 and STH were significantly higher than the control subjects (Table 1).

Figure 1 shows increasing mean values of STH and PAI-1 as glycemic control is challenged.

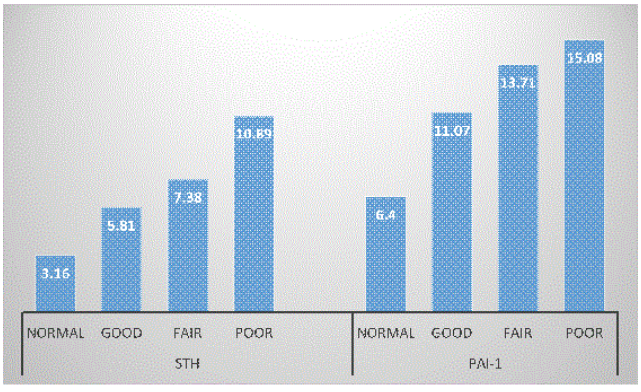

FIGURE 1 Mean values of STH and PAI-1(ng/ml) based on glycemic control

TABLE 1 Endothelial cell markers ad glycemic control of diabetic and non-diabetic subjects

Parameters	Diabetic Subjects n=90 (100%)	Control Subjects n=90 (100%)	P-value (P≤0.05)
Males	23 (25.6)	23 (25.6)	
Females	67 (74.4)	67 (74.4)	
PAI-1 (ng/ml)	13.14±4.12	4.86±1.54	0.000
STH (ng/ml)	8.59±3.21	0.65±0.27	0.000
HbA1c (%)	7.28±1.66	5.50±0.86	0.000
Normal glycemic control (<6.0%)	14 (15.6)	90 (100)	
Good glycemic control (6.0-6.8%)	7 (7.8)	0	
Fair glycemic control (6.8-7.65%)	18 (20.0)	0	
Poor glycemic control (>7.65%)	51 (56.7)	0	

Conclusions: This research observed marked endothelial dysfunction among diabetics in the study locality as evidenced by significantly increased STH and PAI-1 levels based on the degree of glycemic control. For better management of diabetic subjects in this locality, it may be necessary to incorporate PAI-1 and STH as first line tests.

PB1972 | Snake Venom Cysteine-Rich Secretory Proteins (SVCRISPS) from North American Vipers: Mechanism of Action on Blood and Lymphatic Endothelial Cells

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Background: Snake venom Cysteine-Rich Secretory Proteins (svCRiSPs) are important components in the venom of many species of snakes including Viperidae and Elapidae. Although the widespread distribution of svCRiSPs in snake venoms, little is known of the contribution that they make to the local pathophysiology of snakebite.
Aims: This work aimed to investigate the role of svCRiSPs from the most medically significant species of North American vipers (*Crotalus atrox*, *Crotalus adamanteus*, *Crotalus scutulatus scutulatus*, and *Crotalus horridus*), focusing on the cellular and molecular mechanisms underlying vascular biology in snakebite.
Methods: We evaluated the biological activity of svCRiSPs (Css-CRiSP, Catrox-CRiSP, Cada-CRiSP, and Chor-CRiSP) by using both *in vitro* assays on human dermal lymphatic endothelial cells (HDLECs) and human dermal blood endothelial cells (HDBECs) permeability and *in vivo* Miles assay of vascular permeability. To elucidate the main pathway underlying the endothelial permeability induced by svCRiSP, we initially screened the changes in protein expression and phosphorylation in HDLECs and HDBECs 30 min after treatment with Css-CRiSP using reverse phase protein arrays (RPPA). The supernatant of the cell culture medium was also collected and measured the nitric oxide (NO) production using the Griess reagent.
Results: Css-CRiSP is the most potent cause of acutely increase vascular permeability compared to other crotaline CRiSPs. We observed that protease activating receptor (PAR) and several signaling molecules involved in cell mobility, immune response, and MAPK pathway, were elevated to varying degrees in HDBECs and HDLECs. Additionally, Css-CRiSP induced NO release in both HDBECs and HDLECs.
Conclusions: These preliminary observations suggest that Css-CRiSP induce the increased endothelial monolayer permeability in HDBECs and HDLECs via different mechanisms. Knowledge gained from these studies provides insights into the molecular mechanisms that underlie the effects of svCRiSPs on vascular function and contributes to a new level of understanding of the pathophysiology of snakebite.

PB1974 | Trimethylamine N-oxide (TMAO) Disrupts Haemostasis Balance in Human Vascular Endothelial Cells

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Background: The gut microbial metabolite, Trimethylamine N-oxide (TMAO), was previously reported to induce platelet hypersensitivity, which leads to thrombotic risk. However, the molecular mechanism underlying the effects of TMAO on endothelial cells (EC), which is the primary vessel wall contact with the lumen, remains unclear. Importantly, accumulating data suggest that TMAO linked to cardiovascular and metabolic diseases through the molecular mechanism remains elusive.
Aims: To investigate the effect of TMAO on haemostasis balance in human EC and mice.
Methods: To test the PCA of TMAO in HUVEC and HAEC, we performed a one-stage clotting assay. TF blocking antibody and signaling inhibitors were used to test the role of TF in TMAO-induced PCA. Quantitative PCR was performed to measure the TF, TM, IL6, TFPI, and IL1b expressions at mRNA levels. To test the PCA and thrombotic risk by TMAO in mice, we challenged the mice with TMAO (8 mg/kg; 3h) and measured the TAT and D-dimer levels by ELISA. FeCl3-induced carotid artery thrombosis model, as well as in vitro platelet aggregation assays were performed to understand the effect of TMAO on platelets.
Results: TMAO induced TF expression in EC at mRNA and protein levels, dose-dependently. TF blocking experiment confirmed that the increased PCA by TMAO is TF-dependent. Also, MAPK signaling inhibitors abolished TMAO-induced TF expression. However, TMAO challenged mice failed to develop systemic activation of coagulation (TAT and D-dimer) as well as $FeCl_3$-induced carotid artery thrombosis model. In line with these findings, TMAO failed to aggregate mouse platelet *in vitro*, suggesting TMAO has no prothrombotic effect in mice.
Conclusions: Our results indicated that TMAO triggers TF-dependent PCA and disrupts the TM expression in human EC. However, in vivo activation of coagulation did not alter by elevated levels of circulating TMAO in mice.

PB1975 | The Survival of Endothelial Cells in the Conditions of Nutrient Deficiency

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Background: The endothelium serves an integral role in the hemostatic system. Endothelial cells are adapt their phenotype according to nature of local environment. Various component of thrombus can cause damage to endothelium in tissue culture and local environment can create deficient conditions. Since endothelial cells are important component of blood vessels, they can be triggered to induce angiogenesis upon stimulation.

Aims: The aim of the present work was to study biological effects of VEGF on endothelial cells in the conditions of unfed culture.

Methods: We used cultured aortic endothelial cell line (MAEC). Cells were incubated in condition of unfed culture. Cytotoxic/proliferative effect was determined using cytofluorymetric analysis. The level of glucose and NO in incubation medium were determined using a standard set.

Results: VEGF stimulates cell proliferation and increases number of cells in 1.6 and 2 times on the fifth day compared with control and anti-VEGF, respectively. The number of dead cells is the lowest under of influence VEGF. VEGF increased glucose consumption by cells 1.5 and 1.4 times compared with control and anti-VEGF, respectively, on the first day of incubation. However, on other days the number of cells was increased significantly, while consumption of glucose by cells was reduced. VEGF stimulates NO-production 2.3 times on the first day and 3.5 times on the second day compared with the control. Activating effect of anti-VEGF relative to the production of nitric oxide on the 4th-5th day of incubation is shown. Nitric oxide demonstrated multidirectional action, because the increase of its concentration under the influence of VEGF significantly stimulated the proliferation of endothelial cells, while its increase under the influence of anti-VEGF leads to increasing the number of apoptotic cells.

Conclusions: This study detected a relationship of proliferation, cell survival, glucose uptake and production of nitric oxide by endothelial cells in conditions of unfed culture.

PB1976 | TRPV4 Channel Activation Induces the Transition of Venous and Arterial Endothelial Cells Toward a Pro-Inflammatory Phenotype

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Background: The Transient Receptor Potential Channel 4 (TRPV4) of endothelial cells contributes to many important functions including regulation of Ca^{2+} homeostasis, cell volume, endothelial barrier permeability and smooth muscle tone.

Aims: The aim of this work is to study TRPV4 role in the transition of endothelial cells toward a pro-inflammatory phenotype.

Methods: We characterize effects of pharmacological activation of TRPV4 channels on both arterial and venous endothelial cells and their protection using TRPV4 antagonists.

Results: We show that activation of TRPV4 channels with GSK1016790A, a potent TRPV4 agonist, triggers robust and sustained Ca^{2+} influx, which is blocked by both TRPV4 antagonists HC-067047 and RN9893. TRPV4 activation triggered actin cytoskeleton and adherent junction (VE-Cadherin) rearrangement in both arterial and venous endothelial cells and lead to rapid decreases of trans-endothelial electrical resistance. In addition to its effect on endothelial barrier integrity, TRPV4 activation selectively increases ICAM-1 surface expression in arterial and venous endothelial cells, due to the stimulation of ICAM-1 gene expression thanks to the NfκB transcription factor. TRPV4 channel activation also induced apoptosis of venous endothelial cells, while TRPV4 antagonists slowed down apoptosis of endothelial cells, even in the absence of TRPV4 activation.

Conclusions: As Altered barrier integrity, increased adhesion molecule expression and apoptosis are hallmarks of the pro-inflammatory state of endothelial cells, our results indicate that that TRPV4 channel and the resulting calcium influx can induce the transition of both venous and arterial endothelial toward a pro-inflammatory phenotype and suggest that pharmacological inhibition of TRPV4 could protect endothelium in pathology involving dysfunctional endothelial inflammation.

PB1977 | MADD Regulates von Willebrand Factor Trafficking in Endothelial Cells via Activation of Weibel-Palade Body-Associated Rab GTPases

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Background: Von Willebrand factor (VWF) is a multimeric protein that plays a crucial role in hemostasis. Endothelial cells synthesize and process VWF and store mature VWF multimers in specialized organelles called Weibel-Palade bodies (WPBs). In steady-state VWF from WPBs is continuously secreted into the vascular lumen through the basal pathway to maintain circulating VWF plasma levels. Upon vascular injury rapid stimulus-induced WPB exocytosis occurs via the regulated pathway to enable formation of VWF strings, which promote platelet adhesion at the damaged site. Rab GTPases (Rabs) belong to a family of proteins involved in vesicle trafficking. The secretory Rabs Rab3B, Rab3D, and Rab27A have been implicated in WPB trafficking, but how these Rabs are activated and recruited to WPBs remains unknown. Rabs require a guanine exchange factor (GEF) for their activation and recruitment to specific membranes. Previously, MAP-kinase activating death domain (MADD) has been identified as a GEF for Rab27A and Rab3 isoforms in melanocytes and neuroendocrine cells, respectively.
Aims: We investigated if and how MADD is involved in the regulation of VWF secretion.
Methods: We performed shRNA-based knockdown in primary endothelial cells and determined intracellular localization of WPBs and Rabs by immunofluorescence microscopy. Rab activity was assessed using GST-tagged Rab-specific effectors as pulldown bait and readout by Western blotting. VWF secretion was measured by ELISA.
Results: MADD knockdown resulted in loss of WPBs at the cell periphery resembling the accumulation of WPBs in the perinuclear region previously observed in Rab27A depleted cells. Recruitment of Rab27A and Rab3D to WPBs was decreased upon MADD knockdown, suggesting that MADD activates these Rabs. Indeed, Rab activity assays showed reduced active Rab27A, Rab3D, and Rab3B upon MADD silencing. MADD depleted cells exhibited decreased histamine-evoked VWF release, but increased basal release.
Conclusions: MADD acts as a master regulator in VWF secretion through activation and recruitment of secretory Rabs to WPBs.

PB1979 | Novel Anti-Inflammatory Network: The Phosphatase Domain of Soluble Epoxide Hydrolase Mediates the regulation of β2 Adrenergic Receptor-IL-6 Signaling and Vascular Endothelial Activation

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Background: Mammalian soluble epoxide hydrolase (sEH) is a homodimeric protein with two distinct activities in each monomer, C-terminal epoxide hydrolase (C-EH) and N-terminal lipid phosphate phosphatase (N-phos). Using inhibitors of each of the two activities, we demonstrated an essential role of N-phos in the regulation of vascular endothelial activation. Furthermore, we recently found another role of N-phos to induce the physiological defense system based on the gene expression analyses in the liver of sEH KO and sEH inhibitor-treated mice.
Aims: This study was undertaken to comprehensively understand the two N-phos-mediated anti-inflammatory mechanisms.
Methods: Gene expression was evaluated in inhibitor-treated wild type and sEH-deficient mice by microarray and rt-qPCR. Plasma IL-6 level was measured using AlphaLISA (PerkinElmer). VEC activation was induced by LPS in the presence of inhibitors or lipid phosphates either treated or untreated with sEH.
Results: Inhibitors selective to N-phos and N-phos/C-EH dual inhibitors, but not a C-EH-specific inhibitor, suppressed LPS-induced CAM expression in VECs. Highly polar lipid phosphates from liver also suppressed the CAM expression, and the treatment of the fraction with sEH diminished the activity, suggesting a key role of N-phos in the regulation vascular inflammation. When in jected to wild type mouse, the N-phos inhibitor increased plasma IL-6 levels, which led to the induction of acute phase response genes that might contribute to the physiological defense system. The induction of IL-6 was suppressed by a β2-adrenergic (β2AR) blocker. Collectively, there might be an N-phos-sensitive intracellular signaling molecule that regulate the expression of CAM and IL-6 depending on the cell types and triggering stimuli.
Conclusions: A novel mechanism mediated by N-phos contributes to the regulation of inflammation and the physiological defense system.

PB1980 | Epigenetic Regulation of Endothelial Dysfunction in Venous Thromboembolism

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Background: Venous thromboembolism (VTE), which encompasses pulmonary embolism and deep vein thrombosis, is a frequent disease that is associated with vein wall fibrosis. Endothelial cells undergo phenotypical changes named the endothelial to mesenchymal transition (EndMT), characterized by the loss of endothelial markers

and the acquisition of mesenchymal markers leading to fibrosis. Transforming growth factor (TGFβ) is the most potent inducer of EndMT. A recent study showed that in chronic thromboembolic pulmonary hypertension, TGFβ induced EndMT and impaired thrombus resolution. In addition, epigenetic mechanisms regulate EndMT. However, the molecular mechanisms implicated in TGFβ signalling in the context of venous thromboembolism are unknown. We hypothesized that epigenetic processes regulate the TGFβ signaling pathway in endothelial cells promoting EndMT and recurrent thrombosis.

Aims: The aim of this study was to test if EndMT is regulated by epigenetic mechanisms in recurrent thrombosis.

Methods: Endothelial cells were treated with TGFβ, thrombin or both during 72 hours. To study the role of histone deacetylase (HDAC) in EndMT, endothelial cells were also incubated in presence of vorinostat, an HDAC inhibitor. Real time PCR and western-blot were performed to analyze endothelial and mesenchymal marker expression.

Results: Expression of VE-cadherin was not modified by TGFβ and thrombin treatment. Calponin expression was increased by TGFβ and thrombin even in the presence of vorinostat. Interestingly, α-smooth muscle actin and collagen expression was increased by thrombin and TGFβ and inhibited by vorinostat treatment.

Conclusions: We found that treatment of endothelial cells with TGFβ and thrombin is associated with EndMT. Our preliminary data suggest that HDACs contribute to EndMT. HDAC may repress the expression of antifibrotic factors in endothelial cells during venous thrombosis. The discovery of biomarkers or new therapeutic targets would be instrumental in guiding decisions of treatment for patients with a high risk of recurrent VTE.

PB1982 | Endothelial Protein C Receptor Signaling via Protease-activated Receptor 1 Promotes Neovascularization after Ischemia in Mice

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Background: Thrombomodulin and the endothelial protein C receptor (EPCR) participate in anticoagulation by binding thrombin and supporting protein C activation. Coagulation control is crucial for the prevention of major ischemic events, but little is known about coagulation protease signaling during angiogenesis following ischemia.

Aims: To study the importance of the endothelial thrombomodulin-EPCR system in ischemia-induced new vessel formation.

Methods: Unilateral hindlimb ischemia was induced in mice with deletions or targeted mutations of thrombomodulin, EPCR or protease activated receptors (PARs), and reperfusion of distal hindlimbs was monitored using Laser Doppler perfusion imaging (LDPI). Neovascularization in gastrocnemius muscles was analyzed using confocal microscopy and flow cytometry. The aortic ring assay was used to study angiogenic sprouting ex vivo. Next Generation Sequencing of primary mouse endothelial cells was performed to determine the molecular mediators involved in proangiogenic EPCR signaling.

Results: Hindlimb reperfusion and angiogenesis were significantly increased in hypercoagulable mice with a thrombomodulin point mutation on a mixed genetic background associated with elevated EPCR expression indicating a role for EPCR in neovascularization. Analysis of mice on a homogenous genetic background showed that deletion of EPCR in Tie2.Cre-positive cells or of the endothelial thrombin receptor PAR1, but not of PAR2 or PAR4, significantly diminished reperfusion following hindlimb ischemia. Revascularization was significantly impaired in mice with a targeted mutation (PAR1 R46Q) preventing PAR1 activation by activated protein C (aPC) and only marginally in those with disruption of the canonical thrombin cleavage site (PAR1 R41Q). Aortic ring assay in combination with TR41 and TR47 peptides confirmed PAR1-dependent angiogenic response upon Arg-46 cleavage by aPC. NGS revealed upregulation of pathways involved in the regulation of eNOS signaling.

Conclusions: In addition to prior studies implicating thrombin signaling as major driver for pathological angiogenesis, our data indicate that neovascularization after peripheral ischemia is controlled by EPCR–aPC–PAR1 signaling in endothelial cells or progenitor populations.

PB1983 | The R398w Gata-2 Variant Negatively Regulates E-Nos Activity: Possible Role in GATA2 Deficiency-associated Thrombosis

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Background: GATA2 is a transcription factor with key roles in hematopoiesis. GATA2 gene mutations have been associated with a variety of inherited and acquired disorders, including myelodysplastic syndromes. Among the spectrum of GATA2 deficiency-associated disease venous thrombosis has been observed in 25% of patients but with unknown mechanisms. In airway epitelium endothelial nitric oxide syntase (eNOS) was reported to be regulated by GATA2.

Aims: To assess the impact of a GATA2 variant on eNOS expression and neoangiogenesis. To evaluate the impact of GATA2 silencing on eNOS in endothelial cells.

Methods: Blood outgrowth endothelial cells (BOECs) and platelets were isolated from peripheral blood of proband and carriers with familial GATA2 heterozygous R398W mutation and healthy controls. One carrier had suffered unprovoked venous thrombosis. GATA2 and eNOS expression were evaluated by RT-PCR and Western Blotting, eNOS activity by flow-cytometry using DAF-2DA, NOx and (H^3)L-arginine conversion, neoangiogenesis in Matrigel-coated plates.

Results: Platelets from GATA2 R398W-variant carriers showed impaired NO-production (1.02±0.34vs2.01±0.32, $P < 0.05$). BOECs also showed a significant reduction of eNOS mRNA and protein and of eNOS activity compared with controls (H^3L-arg-to-H^3L-citrulline conversion: 90±5.2 vs 137.57±3.5 pmol/10^8cells, $P < 0.05$). Moreover, BOECs showed defective neoangiogenesis (vessel number:19±2 vs 126±3, branching points: 13±3 vs 71±4, length: 7.35±0.3mm vs 16.84±0.2mm, $P < 0.05$) which was completely restored by treatment with the NO-donor SNAP. GATA2-gene silencing with hs.Ri.GATA2siRNA in control BOECs significantly reduced eNOS mRNA and protein expression (-70%) compared to scramble siRNA and reduced angiogenesis (vessel number: 27±2vs154±3, branching points: 22±4 vs 139±2, length: 4±0.23mm vs 17±0.74mm, $P < 0.05$), totally restored by SNAP.

Conclusions: Our results have a two fold implication: they unravel for the first time a thrombogenic mechanism of GATA2 mutations; they definitely establish the regulation of eNOS by GATA2 in endothelial cells and show that control of endothelial neoangiogenesis by the eNOS/NO axis depends on GATA2 regulation.

PB1984 | Astrocytes Are Central Contributors in Restoration of the Blood Brain Barrier via Transcriptional Upregulation of Key Gap Junction Proteins between Endothelial Cells

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Background: The Blood Brain Barrier (BBB) is the major Neurovascular unit in the brain comprising of endothelial cells, astrocytes, and pericytes. Traumatic Brain Injury is characterized by loss of BBB integrity leading to increased barrier permeability, neuroinflammation, and poor clinical outcomes. Specific mediators released following injury lead to BBB gap junction degradation.

Aims: We hypothesize that astrocytes play a central role in restoration and maintenance of BBB integrity following trauma by upregulating expression of proteins that form gap junctions between endothelial cells and that this upregulation is delayed through a crosstalk feedback loop between astrocytes and endothelial cells.

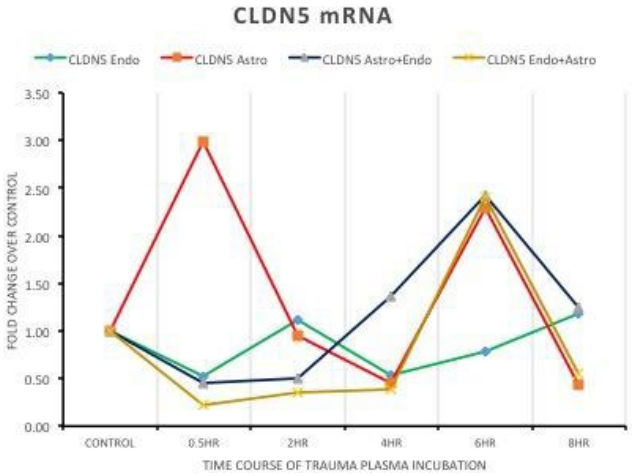

FIGURE 1 Claudin-5 mRNA Expression

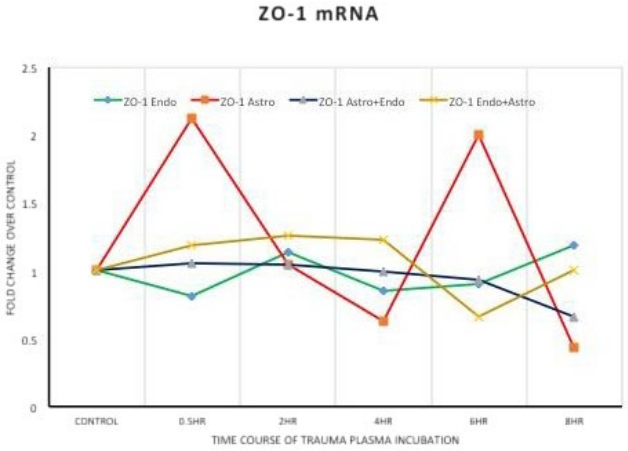

FIGURE 2 ZO-1 mRNA Expression

Methods: Monocultures and co-cultures of HCMECs and astrocytes were incubated with trauma plasma and RNA was extracted at the end of an 8hr time course experiment. Expression of CLDN5, ZO-1 and GAPDH was assessed by RT-qPCR using Taqman chemistry and pre-validated primer probes sets.

Results: The co-cultures have a delayed expression of CLDN5 compared to the immediate upregulation in expression seen in astrocyte monocultures at 0.5hr. The CLDN5 co-cultures and astrocyte monoculture showed a robust spike in expression at 6hr, in contrast to ZO-1 co-cultures which showed no increased response above baseline. The astrocyte monoculture however, did present with a spike above baseline for ZO-1 expression at 6hr.

Conclusions: Comparison of expression data between the monoculture and co-cultures suggest that astrocytes are key players in restoration of BBB integrity after trauma via transcriptional upregulation of gap junction proteins such as CLDN5 and ZO-1. In co-culture, there appears to be mechanisms that prevent astrocytes from upregulating gap junction protein expression to restore BBB robustly for CLDN5. Also in co-culture, ZO-1 expression from astrocytes is muted compared to astrocyte monoculture. Future work will focus

on mechanistic studies to delineate signaling pathways and transcriptional responses impacted by cross talk between endothelial cells and astrocytes affecting BBB integrity.

INFLAMMATION AND SEPSIS

PB1985 | The Anti-Inflammatory Effects Of 3′-Hydroxygenkwanin from Aquilaria sinensis in Human Neutrophils

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Background: Neutrophils play an important antimicrobial role in the immune system. However, inappropriate neutrophil functions and hyperactivity lead to pathological inflammation and tissue damage.
Aims: This study investigates anti-inflammatory effect of the underlying mechanism of 3′-hydroxygenkwanin (ASSB-21) from *Aquilaria sinensis* in human neutrophils.
Methods: Signaling pathways regulated by ASSB-21 to oppose fMLP-induced respiratory burst, surface marker, migration were evaluated by immunoblotting analysis and flow cytometry.
Results: Neutrophils were activated with *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) and phorbol 12-myristate 13 acetate (PMA) in this study. Briefly, ASSB-21 inhibited fMLP-induced reactive oxygen species (ROS) production (IC_{50}: 6.03±1.03 μM), cathepsin G released (IC_{50}: 27.51±6.80 μM) and neutrophil chemotaxis in a concentration dependent manner without affecting these induced by PMA. ASSB-21 (5-20 μM) inhibited fMLP-induced Akt phosphorylation. ASSB-21 (10-20 μM) inhibited fMLP-induced PIP3 levels indicated that ASSB-21 affected the activity of PI3K. In addition, ASSB-21 (20-50 μM) did not scavenge oxygen free radical in the xanthine/xanthine oxidase system. Theneutrophil viability and toxicity did not affect by ASSB-21 (20-50 μM). Furthermore, ASSB-21 (20 μM) inhibited PMA-induced neutrophil extracellular traps (NETs) formation. ASSB-21 (5-20 μM) inhibited PMA-induced Akt phosphorylation. However, ASSB-21 (20 μM) did not inhibit fMLP-induced and PMA-induced CD11b expression, intracellular calcium mobilization, phosphorylation of ERK1/2 and p38.
Conclusions: According to this data, ASSB-21 inhibited fMLP-induced neutrophils ROS production, cathepsin G released and chemotaxis by inhibiting PI3K pathway.

PB1986 | The Tissue Factor Surface Expression of Monocyte Subsets during Sepsis

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Background: Monocytic surface expression of tissue factor (TF) may contribute to sepsis-associated coagulopathy. Monocytes are divided into three subsets that differ in functions: classical, intermediate, non-classical.
Aims: To investigate TF expression between monocytic subsets during sepsis.
Methods: Participants were recruited from four UK intensive care units (ICU). Blood samples were taken from two cohorts, those critically ill with a diagnosis of sepsis (sepsis cohort, n=18) and those who were critically ill without sepsis (critically ill cohort, n=10). Individuals with sepsis had microbiological confirmation of infection. Those in the sepsis cohort who survived to be discharged from ICU were consented for a further blood sample (sepsis recovery subgroup, n=10). Monocyte TF surface expression was measured with whole blood flow cytometry. Significance was calculated using paired and unpaired t-tests. Informed consent was taken when possible. Personal and professional consultees were used if an individual did not have capacity. Ethical approval was granted by a medical ethics committee.
Results: Both the sepsis and the critically ill groups were evenly matched, with no significant differences in age, sex, length of stay in ICU, organ support or monocyte count.
There was no difference between the total monocytic TF expressions between the critically ill and sepsis cohorts as well as between those with and without coagulopathy (platelet < 150 X 10^9/L, prothrombin time >15 sec, activated partial thromboplastin time >35 sec or fibrinogen < 1.5 g/L). Total monocyte TF surface expression did increase following recovery from sepsis (Fig 1). The sepsis cohort had higher TF surface expression on the intermediate and non-classical subsets compared to the critically ill group. Following recovery from sepsis, there was an increase in the classical monocyte surface expression of TF (Fig 2).
Conclusions: Monocytic subsets vary TF surface expression during and on recovery from sepsis. This was no correlation between monocyte TF surface expression and coagulopathy.

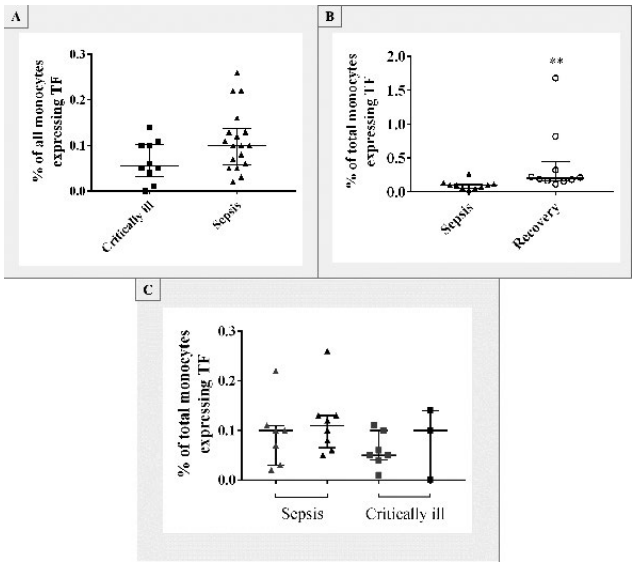

FIGURE 1 Total monocytic TF. A- critically ill & sepsis. B- sepsis & sepsis recovery **p<0.01. C- coagulopathic (red) & non-coagulopathic (black)

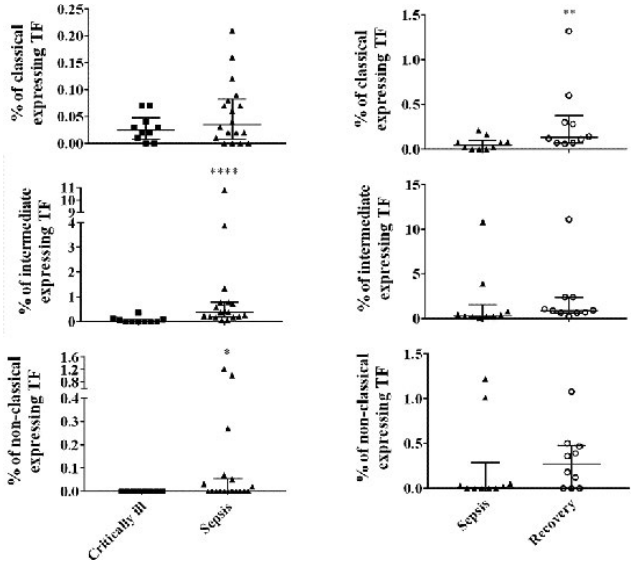

FIGURE 2 Monocyte subset TF surface expression during critical illness, sepsis and recovery from sepsis. *p<0.05, **p<0.01. ****p<0.0001

PB1987 | The Tissue Factor Expression of Monocyte Subsets and the Activation of Coagulation during Human Endotoxaemia

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Background: Monocytic surface expression of tissue factor (TF) is implicated in the development of sepsis-associated coagulopathy. Monocytes are divided into three subsets: classical, intermediate and non-classical.

Aims: This work investigates the surface TF expression between monocyte subsets during endotoxaemia.

Methods: Lipopolysaccharide (LPS, 1ng/kg) was administrated intravenously to healthy volunteers (n= 13). Informed consent was given. Ethical approval was granted by a medical ethics committee. Blood samples were taken at baseline, 1.5, 4, 6, 10, and 24 hours, and 7 days following LPS injection. Whole blood flow cytometry was used to measure monocytic TF surface expression. Markers of activated coagulation were measured at CARIM with in-house constructed ELISA's to quantify complexes between factor (F) XIa, FIXa, FXa or thrombin with antithrombin (AT), or FXIIa in complex with C1 esterase inhibitor (C1inh).

Results: Two types of TF response were identified: 8 participants had an increase in monocytic TF surface expression (responders), the remaining 5 had no change in TF (non-responders). Responses were consistent across each monocyte subset. Intermediate and non-classical monocytes expressed higher levels of surface TF compared to classical, although endotoxaemia induced a profound transient monocytopenia. Classical monocytes returned at 4 hours and intermediate and non-classical returned at 24 hours (Figure 1).

All participants post LPS injection, had activation of coagulation with elevated levels of thrombin-anti-thrombin and complexes between anti-thrombin and activated factors: IX, X and XI. There was no activation of FXII, assessed by means of the FXIIa:C1inh complex (Figure 2). Coagulation was similarly activated in both responders and non-responders.

Conclusions: Two groups were identified following endotoxaemia, responders who had an increase in monocytic TF surface expression and non-responders who did not. The TF surface response does not affect the activation of coagulation. Further work should investigate whether the marked variation of TF surface expression between individuals can alter the thromboinflammatory response.

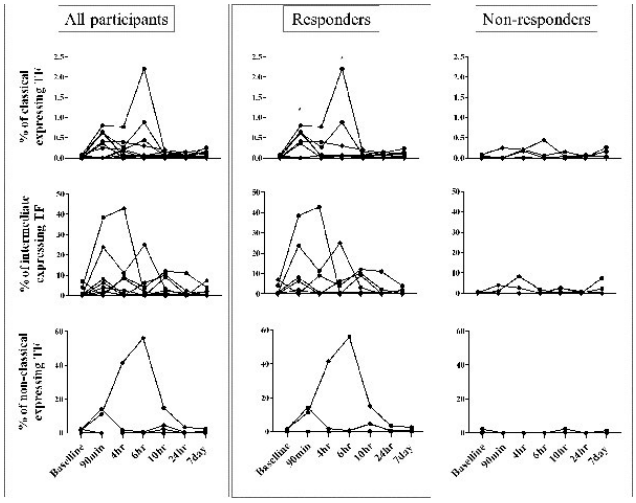

FIGURE 1 Monocyte subset TF surface expression. Significance was calculated used Friedman's and Dunn's post hoc test (*=p<0.05)

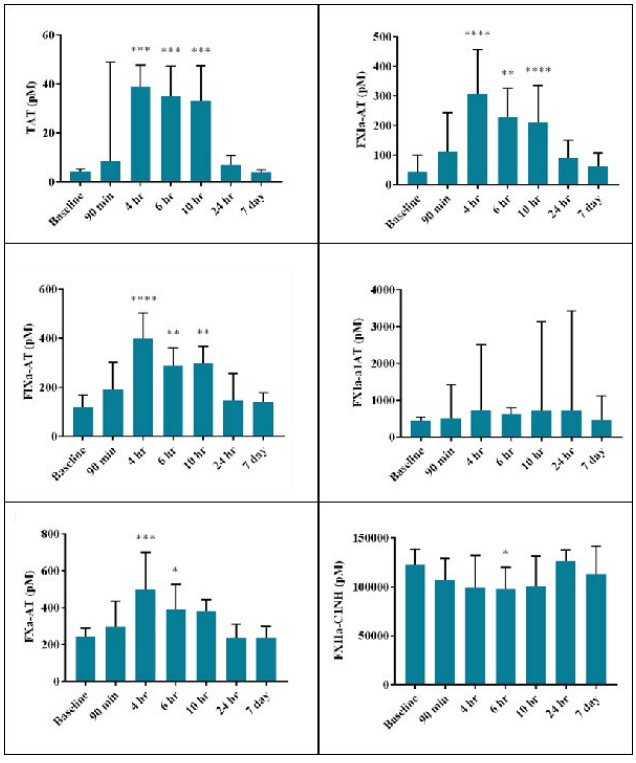

FIGURE 2 Markers of coagulation. Significance calculated used Friedman's and Dunn's post hoc test (*=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001)

PB1989 | Prevalence of Disseminated Microvascular Thrombosis, Unresolved Infection, and Lymphoid Depletion in Autopsies of Pediatric Sepsis-Induced Disseminated Intravascular Coagulation (DIC)

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Background: DIC contributes to multiple organ dysfunction syndrome (MODS) and mortality in sepsis. Sepsis induces DIC due to dysregulated host response to infection and unremitting systemic inflammation. A suggested cause of unremitting systemic inflammation is unresolved infection due to immune paralysis/lymphoid depletion syndrome. The frequency of this syndrome in sepsis-induced DIC is unknown.

Aims: We hypothesize that patients who died with sepsis-induced DIC have autopsy evidence of disseminated microvascular thrombosis, unresolved infection and lymphoid depletion.

Methods: We reviewed consecutive autopsy reports of children who died with a diagnosis of sepsis from 1/2017 to 4/2018 in a tertiary care university-based pediatric hospital. Septic patients were identified by Electronic Medical Record query using diagnostic codes for "sepsis" and "septic shock." Clinical DIC was diagnosed based on the International Society of Thrombosis and Haemostasis criteria.

Results: Out of 330 septic children identified, there were 79/330(24%) deaths. Clinical DIC was present in 110/330(33%) septic children, of whom 44/110(40%) died. Autopsies were performed on 16/79(20%) deaths. Clinical DIC was present in 11/16(69%) patients with autopsies. All 11/11(100%) DIC patients died with MODS. Patients with DIC had evidence of microvascular thrombosis in the kidneys 6/11(55%), lungs 6/11(55%), heart 6/11(55%), liver 3/11(27%), and brain 0/11(0%). 8/11(73%) patients with DIC had active infections found on autopsy categorized as: gram negative 4/8(50%) and gram positive 5/8(63%) bacteria, fungus 1/8(13%), and more than one pathogen 3/8(38%). Hypocellular bone marrow and/or lymphocyte depletion in the lymph nodes or thymus was found in 5/11(45%) patients with DIC.

Conclusions: All sepsis-induced DIC patients with autopsies died with MODS. Over half of clinical DIC autopsy reports showed evidence of microvascular thrombosis in the kidneys, lungs and heart. The majority of DIC deaths had unresolved infection suggesting a cause for unremitting systemic inflammation. Less than half of DIC patients had lymphocyte depletion suggesting immune dysregulation. Further studies are warranted.

PB1990 | Ca2+ Release from Stores Controlled by Sarco/Endoplamic Reticulum Ca2+-ATPase 3 (SERCA3) Is Involved in Endothelial Permeability and Inflammatory Response

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Background: while Ca^{2+} signaling plays a crucial role in regulating inflammatory response, the role plays by Ca^{2+} mobilization from intracellular stores remains unclear. SERCAs are pumps that control intracellular calcium (Ca^{2+}) storage and we have, recently, established that full platelet activation requires SERCA3, indicating a specific role for this pump. And SERCA3 is expressed in leukocytes, platelets and endothelial cells, which actively participate to inflammation.
Aims: Is SERCA3 important in two major inflammatory processes, endothelial hyperpermeability and leukocytes activation?
Methods: Wild-type (WT) and SERCA3 knockout (SERCA3-/-) mice were compared, lung vascular endothelial cells (mLECs) isolated from these animals and Human pulmonary artery endothelial cells (hPAEC). Different inflammation models were explored in mice and thrombin challenging was analysed in primary endothelial cells. Leukocytes behavior was explored *in vivo* using intravital microscopy for rolling/adhesion and myeloperoxidase assay for leukocytes recruitment. *In vitro* hyperpermeability and cytoskeletal reorganization of human and murin lung endothelial cells were investigated using transendothelial electrical resistance and actin/VE-cadherin immunostaining, respectively.
Results: In SERCA3-/-, early leukocyte mobilization in response to $FeCl_3$ injury was abolished independently of platelet activation, as similar results were observed in thrombocytopenia induced animals. Leukocyte rolling velocity and adhesion were also significantly reduced after LPS challenging. In Arthus inflammation model, an altered leukocyte recruitment was observed in platelet dependent way. Moreover, absence of platelets uncovered that lack of SERCA3 prevented inflammation-induced haemorrhage suggesting a role for endothelium.
SERCA3-/- mLECs studies showed that Ca^{2+} release and stress fiber formation in response to low concentration of thrombin were abolished. In thrombin-stimulated hPAEC, pharmacological inhibition of SERCA3, was also associated to reduced Ca^{2+} release, stress fiber formation, VE-cadherin disassembly and barrier disruption.
Conclusions: Our results suggest that lack of SERCA3-dependent Ca^{2+} release is associated to lower endothelial barrier disruption and weaker inflammatory responses.

PB1991 | Pro-Inflammatory Cytokines, Tissue Factor Gene Polymorphism (A603G, C1322T, C1812T, G1442C) and Tissue Factor Expression in Patient with Erysipelas

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Background: Erysipelas is an actual problem of health care. It is characterized by consistently high morbidity, increased tendency to recur, reduced the life quality and took important place in the structure of temporary disability. Erysipelas causes disorders of haemostasis and development of immune response. Activation of the macrophage system in the skin is accompanied by the expression of tissue factor, which leads to hypercoagulation in erysipelas.
Aims: The aim was to study the genetic polymorphism of TF (A603G, C1322T, C1812T, G1442C), concentration of pro-inflammatory cytokines IL-1β and TNFα and expression of tissue factor by peripheral blood monocytes in healthy individuals and patients with erysipelas in Transbaikalian region.
Methods: The study enrolled 104 patients with erysipelas and 94 healthy residents. Tissue factor gene polymorphism was detected by PCR method. Expression of tissue factor by monocytes was studied by the method of R.A. Santucci et al. (2000) in our modification.
Results: At was established that of homo- and heterozygous SNP of TF gene were spreading to Hardy-Weinberg equilibrium ($p>0.05$). It was founded that alleles and genotypes of SNP TF A603G, C1322T, C1812T, G1442C did not differ in healthy individuals and patients with erysipelas. Alleles and genotypes of SNP of TF gene didn't effect on tissue factor expression. In the same time there was a significant difference between stimulated bacterial LPS and unstimulated blood samples in healthy individuals. But the difference was decreased significantly in patients with erysipelas ($p < 0.001$), which indicates a high expression of tissue factor. At was established that a decrease in blood coagulation time occurred with an increase in the concentration of IL-1β and TNFα in patients with erysipelas, which also indicates a high expression of tissue factor.
Conclusions: Thus, the concentration of pro-inflammatory cytokines IL-1β and TNFα influence on TF activity in healthy individuals and patients with erysipelas.

PB1992 | Design and Characterization of Novel Activated Protein C (APC) Variants for the Proteolysis of Cytotoxic Extracellular Histone H3

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Background: Extracellular histone H3 can induce pathological effects *in vivo* including inflammation and cell death. The neutralization of histone H3 is therefore beneficial in various diseases, such as sepsis. Activated protein C (APC) can cleave histone H3, which reduces its cytotoxicity. However, due to the anticoagulant properties of APC, use of wildtype APC is not optimized for the treatment of histone-mediated cytotoxicity, because it may cause bleeding.

Aims: We aimed to investigate the molecular interaction between APC and histone H3 and subsequently generated rationally designed APC variants. These variants are predicted to have reduced anticoagulant activity, whilst also possessing an enhanced ability to bind and cleave histones, as compared to wildtype APC.

Methods: We used computational design to identify key residues that mediate the interaction between APC and histone H3. By rational design we introduced *in silico* mutations in APC, to obtain variants with optimized functional characteristics. Next, ability of the variants to function as an anticoagulant, to bind to histones and to cleave histones was tested, and correlated to the cytoprotective properties.

Results: Analysis of APC-mediated histone H3 proteolysis and structural modelling identified the cleavage sites in histone H3 and APC residues that are important for the interaction with histone H3. APC variants were engineered and generated by mutation of these identified key cationic residues to neutral or anionic residues, resulting in the novel 5D-APC and 5D2A-APC variants.

Compared to wildtype APC, both the 5D-APC and 5D2A-APC variant showed a significant decreased anticoagulant activity and increased binding to histone H3. In addition, the APC variants showed a similar ability to proteolyze histone H3 as wildtype APC.

Conclusions: It is possible to rationally design novel optimized APC variants that can be further developed and utilized as a therapy for histone-mediated disease, to reduce their associated cytotoxic properties, whilst not inducing an increased bleeding risk.

PB1993 | Inhibitory Effect of Heparin and Modified Heparin on Neutrophil Migration

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Background: The anticoagulant heparin has demonstrated anti-inflammatory properties *in vitro* by dampening elastase release and aggregation of neutrophils stimulated by *N*-formyl-methionyl-leucyl-phenylalanine (fMLP). However, the use of heparin as a potential anti-inflammatory agent in patients is precluded by its anticoagulant properties and so it would be of interest to investigate anti-inflammatory properties of modified heparins with different anticoagulant activities.

Aims: Modified non-anticoagulant heparins were studied for their ability to restrict the migration of neutrophils towards the chemotactic peptide, fMLP.

Methods: Heparin and modified heparin with anticoagulant activities ranging from 0 to 209 IU/mg were employed in this study (Table 1). Isolated neutrophils (> 90% purity) were incubated with 0.02, 0.2, 2, 20 and 200 µg/ml of heparin, before being placed in a fMLP chemotaxis system. The chemotaxis index was calculated for ≥10 donors and results were analysed by Dunnett's multiple comparison (* = $p < 0.05$) relative to fMLP treatment only.

Results: Broadly, the inhibition of migration by the heparins appeared to show a bell-shaped response. Unmodified heparin at the concentrations used significantly reduced the chemotaxis index ranging from 33 to 64% of fMLP alone (figure 1). The 2-O-desulfated and N-acetyl-O-desulfated were less effective at reducing chemotaxis, ranging from 32 - 51% and 17- 36% respectively, whilst N-acetyl heparin was of comparable effectiveness, 45 - 67% to unmodified

TABLE 1 Anticoagulant activities of heparin samples by antithrombin-dependent anti-IIa assay against the 6th IS for Unfractionated Heparin

Heparin Sample	Anti-IIa IU/mg (95% CL)
Unmodified	209 (196 - 223)
2-O-desulfated	43.4 (40.5 - 46.5)
N-acetyl	<1
N-desulfated	<1
N-acetyl-O-desulfated	<1

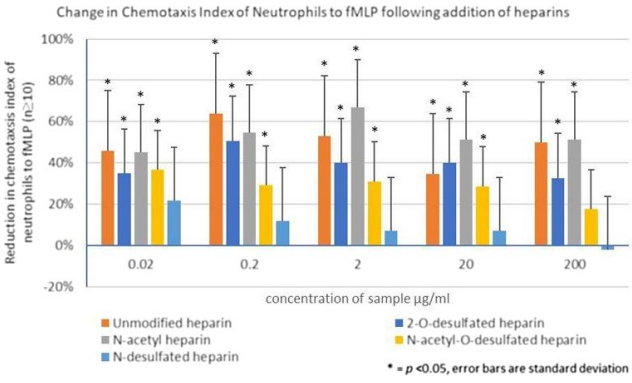

FIGURE 1 Change in chemotaxis index of neutrophils to fMLP following addition of heparin

heparin. No significant inhibition was observed for N-desulfated heparin.

Conclusions: Loss of the N-sulfate rendered heparin ineffective at preventing migration, whilst acetylation of this location restored effectiveness. A completely desulfated heparin, with N-acetylation was interestingly able to reduce neutrophil migration, indicating the importance of the "shielding" of the amine groups for heparin to elicit a reduction of fMLP induced neutrophil migration.

PB1994 | Significance of Macrophage Migration Inhibitory Factor and Toll-Like Receptor 4as Predictive Factors of Sepsis in Shock Patients

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Background: Many patients die from sepsis and multiple organ failure, even though proper management in shock patients. Early prediction of sepsis in shock patients is important and used in various ways, such as c-reactive protein (CRP), white blood cell (WBC) and procalcitonin (PCT), but they have some problems. Recently, toll-like receptor (TLR), macrophage migration inhibitory factor (MIF) have emerged as predictive factors.

Aims: Our study aims to explore the significance of TLR, MIF as predictors of sepsis in shock patients.

Methods: This study were conducted on prospective observational study patients who visited an emergency medical center in a university hospital from March 1, 2019 to December 31, 2019 and were intended for shock patients aged 18 or older. We measured WBC, CRP, PCT, MIF, TLR_4, TNF-α, Interleukin-6 (IL-6), and lactic acid with serum taken from the patient's blood. The definition of sepsis was defined as being part of SIRS criteria with infections within a week.

Results: 153 hemorrhagic shock patients were registered in emergency department, 44 of whom had sepsis within a week. The PCT, WBC, TNF-α, IL-6 did not differ in the comparison between sepsis and non-sepsis patients, while the CRP was somewhat high in sepsis patients (133.95±16.24mg/L > 60.46±3.57mg/L). However, MIF was significantly elevated in sepsis (2633±710pg/mL) to non-sepsis group (1460±680pg/mL). and also TLR_4 was elevated in sepsis (16.27±2.05) to non-sepsis group (7.78±0.59). There was no correlation between MIF, TLR_4 and lactic acid, which is the diagnostic criteria of shock.

Conclusions: It is believed that MIF amd TLR_4 may be used as predictors of sepsis in shock patients. However, more research on the occurrence of MIF and TLR_4 are thought to be necessary.

PB1995 | Significance of Macrophage Migration Inhibitory Factor for Sepsis in Hemorrhagic Shock Patients

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[1]*Korea University Guro Hospital, Seoul, Korea, Republic of;* [2]*Injae University Ilsan Baek Hospital, Goyang, Korea, Republic of*

Background: Many patients die from sepsis and multiple organ failure, even though proper management in hemorrhagic shock patients. Early diagnosis of sepsis in hemorrhagic shock patients is important and used in various ways, such as CRP and WBC, procalcitonin (PCT), but they have some problems. Recently, macrophage migration inhibitory factor (MIF) have emerged as predictive factors.

Aims: Our study aims to explore the significance of MIF as a predictor of sepsis in hemorrhagic shock patients.

Methods: This study was conducted on prospective observational study patients who visited an emergency medical center in a university hospital from March 1, 2019 to December 31, 2019 and were intended for hemorrhagic shock patients aged 15 or older. We measured WBC, CRP, PCT, MIF, TNF-α, Interleukin-6 (IL-6), and lactic acid with serum taken from the patient's blood. The definition of sepsis was defined as being part of SIRS criteria with infections within a week.

Results: 180 hemorrhagic shock patients were registered in emergency department, 28 of whom had sepsis within a week. The CRP, WBC, TNF-α, IL-6 did not differ in the comparison between sepsis and non-sepsis patients, while the PCT was somewhat high in sepsis patients (0.24±0.1ng/mL > 0.18±0.07ng/mL), but with had no statistical significance. However, MIF was significantly elevated in sepsis (2633±710pg/mL) to non-sepsis group (1460±680pg/mL). There was no correlation between MIF and lactic acid, which is the diagnostic criteria of shock.

Conclusions: It is believed that MIF may be used as a measure of sepsis in hemorrhagic shock patients. However, more research on the occurrence of MIF is thought to be necessary.

PB1996 | Defibrotide Prevents Sepsis Induced Endothelial Cell Activation

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Background: Endothelial dysfunction plays a key role in sepsis physiopathology, leading to multiorgan failure and death. Defibrotide (DF) is a product effective in the protection of endothelial cells (ECs). Although the mechanism of action of this drug is uncertain, it may have a role as potential treatment for sepsis complications.

Aims: Characterize *in-vitro* the activation and damage of ECs in different sepsis syndrome and determine the influence of DF in these alterations.

Methods: Humanumbilical vein endothelial cells (HUVEC) were grown in the presence of 20% pooled sera collected from patients with septic syndrome (sepsis, severe sepsis and septic shock) or systemic inflammatory response syndrome (SIRS) within the first 24 hours of symptoms onset. Sera from healthy volunteers was used as control. We assessed changes in the expression of ECs receptors (ICAM-1 and VCAM-1), the presence of extracellular adhesive proteins such a Von Willebrand factor (VWF) and the thrombogenicity of the extracellular matrix (ECM) generated by these cells. To explore the effect of DF, ECs were incubated with DF (100 µg/mL) for 24 hours before being exposed to the sera and every 24 hours after the exposition.

Results: EC activation, determined by an increase in expression of ICAM-1 and VCAM-1 and an increase in expression of VWF and platelet adhesion on the ECM, was higher in septic syndrome compared with SIRS and control group ($p< 0.001$) with a progressive increase correlated with sepsis severity (sepsis < severe sepsis ≤ septic shock) ($p< 0.05$ for VCAM-1, ICAM-1 and VWF; $p< 0.001$ for platelet adhesion). Previous exposure and continuous incubation of ECs with DF modified the endothelial response by significantly reducing VWF, ICAM-1, VCAM-1 and platelet adhesion induced by sera from patients with septic syndrome in all gropus ($p< 0.001$).

Conclusions: DF prevents activation and endothelial damage induced in septic syndrome in an *in-vitro*model of sepsis.

PB1997 | The Role of α1AMP-Activated Protein Kinase (α1AMPK) in Haemostatic Dysfunctions Induced by Sepsis

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Background: Sepsis is a pathological circumstance whose outcome is associated with organ dysfunction and death. Vascular hyperpermeability and haemostasis impairment, both play a key role in the onset of organ failure. Our previous work highlighted the protective role of α1AMPK against sepsis-induced hyperpermeability. However, the impact of α1AMPK on haemostasis has never been investigated in this condition.

Aims: This study aims to characterize the interaction of α1AMPK with haemostasis in sepsis.

Methods: C57BL6J mice and α1AMPK wild-type (WT) and knock-out (KO) mice were challenged with intraperitoneal injection of lipopolysaccharide (LPS) from Escherichia coli O55B5 ($10mg.kg^{-1}$) during 6 hours. Plasmatic markers of coagulation, platelet and NETosis activation were measured by ELISA and membrane markers of platelet activation were analysed by flow cytometry. The dsDNA analysis of NETosis was performed by fluorometry. *Ex vivo* platelets activation was assessed by flow cytometry on platelets α1AMPK WT and KO, incubated in a medium with 5µg/mL of LPS.

Results: In C57BL6J mice, haemostasis was activated 6 hours after LPS administration, as reflected by the increase in fibrinogen, plasminogen activator inhibitor and tissue plasminogen activator. In addition, platelets isolated from LPS-treated mice were activated as demonstrated by the increased exposure of CD62P and αIIbβIII on platelet surface and soluble CD62P detection in plasma. Finally, we measured a significant increase in myeloperoxidase, neutrophil elastase and plasmatic dsDNA, all markers of NETosis. Same parameters were measured in α1AMPK WT and KO mice. We brought out an increase of fibrinolysis impairment and heightened a trend of NETosis in α1AMPK undergoing sepsis. Furthermore, platelets of α1AMPK KO mice exhibited a more important activation *ex vivo*, in response to LPS.

Conclusions: These results suggest that α1AMPK is involved in the regulation of haemostasis disturbances following sepsis.

PB1999 | Systemic Inflammation in Sepsis Patients Is Demonstrated by Levels of IL-8, IL-13, and TAFI

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Background: Despite extensive research, the pathophysiology of inflammation in sepsis patients is partially understood.

Aims: This study measured levels of IL-8, a major mediator of the inflammatory response, IL-13, an immunosuppressive cytokine that reduces the production of inflammatory cytokines, and thrombin activatable fibrinolysis inhibitor (TAFI), which stabilizes the clot in a population of sepsis patients with organ dysfunction.

Methods: Citrated blood samples were collected from 78 sepsis patients with suspected or confirmed infection, and organ dysfunction as defined by a SOFA above baseline. Under an IRB approved protocol, samples were collected within 72 hours of ICU admission in conjunction with an ongoing trial at the University of Utah and George E. Wahlen Department of Veterans Affairs Medical Center. Normal controls were comprised of commercially available citrated plasma samples (25 male and 25 female; George King Biomedical, Overland Park, Kansas City). Levels of IL-8, IL-13 and TAFI were measured using commercially available sandwich ELISA methods. All results were compiled as mean± SEM.

Results: Levels of IL-8 and IL-13 were significantly increased in sepsis patients (44.1±14.1 pg/ml, and 181±7.1 pg/ml, respectively) compared to normal human plasma (0.0pg/ml and 113.3pg/ml, respectively). In contrast, the mean TAFI level was lower in sepsis patients (62.4±5.2%) when compared to normal plasma (109.1±14.7%).

There was no correlation between IL-8 and IL-13 (r=0.007), IL-13 and TAFI (r=0.006) or IL-8 and TAFI (r=0.027) levels.

Conclusions: Sepsis patients demonstrated elevated levels of IL-8 and IL-13 in comparison to the control group, indicating the complex role of cytokines in the mediation of inflammatory processes in this syndrome. A decrease in TAFI level suggests an enhanced level of fibrinolysis in septic patients. Understanding the inflammatory response is essential for determining septic patients' outcomes.

PB2000 | Immunization with Recombinant Atherogenic Antigen (AHC) Does Not Enhance Susceptibility to Sepsis

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Background: Sepsis is a state of disrupted inflammatory homeostasis that is often initiated by infection with a mortality ranging from 28-47%. The development and progression of sepsis are multi-factorial and affects the cardiovascular, immunological and endocrine systems of the body. Although recombinant atherogenic antigen (AHC) containing epitopes derived from ApoB, heat shock protein (HSP) 60, and *Chlamydia pneumoniae* outer membrane protein would represent the most successful vaccine candidate to reduce autoantigen (such as ApoB and HSP)-associated inflammation towards atherosclerosis, little is known about the potential effect of the immune response induced by AHC construct containing autoantigen epitopes on sepsis.

Aims: To assess whether immunization of C57BL/6 mice with the recombinant atherosclerotic antigen AHC has an effect on the outcome of induced sepsis.

Methods: 6-week old C57BL/6 mice (N=6 per group) were immunized with AHC and a non-immunized group served as control. Two weeks after the first immunization, the mice were subjected to cecal ligation and puncture (CLP) surgery to induce sepsis. Blood samples were collected at 6, 24, 48 and 72 h post CLP. Blood was taken at each time point and the level of plasma cytokines was measured by ELISA.

Results: Similar plasma levels of proinflammatory cytokines (IL-6 and TNF-alpha) and anti-inflammatory cytokine (IL-10) were observed in both AHC-immunized mice and non-immunized controls. Interestingly, the AHC-immunized mice showed better survival rate than non-immunized mice (5% versus 30%).

Conclusions: Immune response to AHC did not contribute to promotion of sepsis-induced inflammation in tested mice, indicating that CLP-induced inflammation may involve a different signal pathway from that of autoantigen (such as ApoB and HSP)-induced inflammation.

PB2001 | Cell-free DNA and Citrullinated Histone H3 Plasma Levels Improve the Prediction of Mortality in Septic Patients

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Background: Despite advances in critical care management and in the understanding of its physiopathology, sepsis remains a serious problem. In sepsis, thrombus formation is a complication that increases the risk of death and the severity of organ dysfunction. Due to SOFA score limitations, it is imperative to find reliable biomarkers to improve it or generate a better predictive score.

Aims: Our aims were to study two plasmatic biomarkers derived from different cell death mechanisms: *cell-freeDNA* (cfDNA) and citrullinated histone H3 (*H3cit*) within the first hours of admission in the ICU to evaluate their predictive value in the prognosis of sepsis.

Methods: 203 septic patients (median age:45 years[33.5-54.5];women: 37.1%) have been recruited, of whom 94 survived (median age:42.5 years[28.75-53.50];women: 35.72%) and 109 died (median age:46 years [35-55];women: 42.18%). As a reference, cfDNA and H3cit levels were measured in 85 selected healthy controls (median age:30 years[25-39];women: 56.5%).

Results: Both *cf*DNA and H3cit median plasmatic levels were significantly higher in septic patients that survived than in those patients who died in ICU (Figures 1/2). Moreover, *cf*DNA and H3cit levels showed a positive correlation between them (rho Spearman's correlation coefficient: ρ=0.445; p< 0.01).

We found that having cfDNA levels higher than 2.27 ng/ul (the 75th percentile among patients) would significantly increase the odds of sepsis associated mortality (OR=3.08 [1.53-6.10]; p=0.002; binary logistic regression).

Finally, we made a preliminary analysis to compare the traditional SOFA score, *cf*DNA and H3cit categorized levels as predictors of

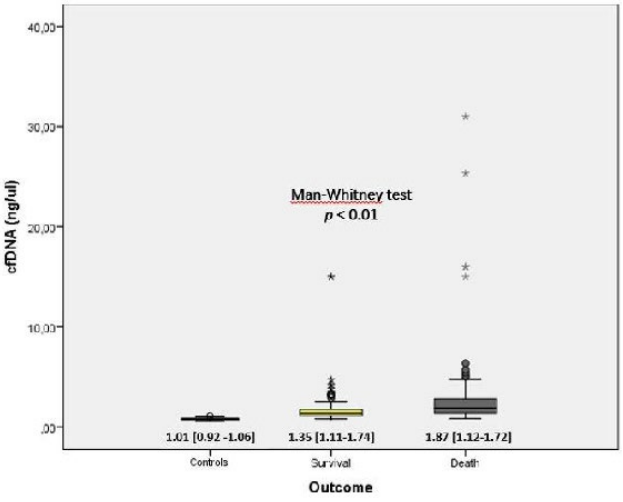

FIGURE 1 Plasma levels of cfDNA (ng/ul) in septic patients according their outcome. Control group is shown as a reference

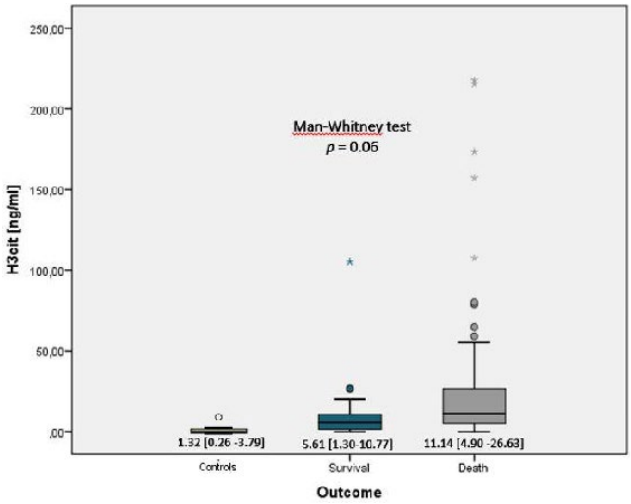

FIGURE 2 Plasma levels of H3cit (ng/ml) in septic patients according their outcome. Control group is shown as a reference

mortality in sepsis. So far, SOFA score seemed to be a better predictor (AUC=0.780 vs AUC=0.732 vs AUC=0.683, respectively). However, adding these plasmatic biomarkers to the SOFA score slightly increase its predictive ability (AUC=0.796).

Conclusions: Our results could be promising to consider the measurement of cfDNA and H3cit levels as useful biomarkers to be incorporated to the SOFA score for sepsis prognosis.

PB2002 | Fibrinolytic Activity in Patients with Granulomatosis with Polyngiitis (GPA)

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Background: Granulomatosis with polyangiitis (GPA) is a multisystem inflammatory autoimmune disease characterized by the presence of necrotizing vasculitis of small and medium vessels with granuloma formation in various organs associated with antineutrophil cytoplasmic antibody (ANCA) presence.

Aims: Assessment of fibrinolytic activity in GPA patients to explain mechanisms that might be responsible for thrombotic manifestations of the disease.

Methods: Assessment of fibrinolytic activity in GPA patients to explain mechanisms that might be responsible for thrombotic manifestations of the disease.

Results: In GPA patients CLT, plasminogen activity and tPA antigen concentration were significantly higher than those of the healthy controls (119.00 vs 95.8; 109.95 vs 98.90 and 9.73 vs 7.02 respectevily; p< 0.05). There were no significant differences between PAI-1 antigen concentrations and alfa2-antiplasmin activity. Similarly in patients in remission, the same parameters were significantly higher (p< 0.05) than in controls. Clot lysis time and plasminogen activity were significantly higher only between patients in acute phase of the disease and controls (p< 0.05). There were no significant differences in parameters measured between patients in remission those in acute phase of their disease.

Conclusions: We observed an enhanced activity of the fibrinolytic parameters in patient with GPA, irrespective of the disease activity which may be secondary to an increased thrombotic activity in GPA.

PB2003 | Megakaryocytes Are Reprogrammed by Lipopolysaccharide Exposure during Bacterial Sepsis

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Background: Sepsis is a complex life-threatening inflammatory reaction. In bacterial sepsis, lipopolysaccharide (LPS) enters the patients' blood circulation. However, it is unknown whether LPS can reach the bone marrow and stimulate resident megakaryocytes. In our proof-of-principle study, we particularly focus on interleukin 6 (IL6), a severity marker for sepsis, as a representative gene.

Aims: To assess if megakaryocytes are exposed and respond to LPS during bacterial sepsis.

Methods: We used human cord blood-derived megakaryocytes to study changes in mRNA expression with RNA sequencing and to assess IL6 secretion with ELISA. Megakaryocyte-specific IL6 protein expression was tested with immunocytochemistry. Plasma of septic patients and healthy donors were subjected to multiplex cytokine analysis. A murine polymicrobial sepsis model (cecal ligation and puncture; CLP) was used to examine IL6 mRNA expression in sorted bone marrow megakaryocytes by qPCR. LPS levels were measured by chromogenic endotoxin quantitation.

Results: In human megakaryocytes, 192 transcripts were regulated (LPS vs. PBS, n=3; $P_{adj} < 0.05$). IL6 was among the most upregulated cytokines (P_{adj}=4.1E-12) and was secreted into the supernatant. Human megakaryocytes co-stained for CD41 and IL6 suggesting megakaryocyte-specific expression. IL6 was elevated in plasma of septic patients compared to healthy controls (n≥8; $P_{adj} < 0.01$). LPS levels were increased in plasma and bone marrow suspensions of CLP mice compared to non-treated controls (n≥10; P < 0.001). IL6 mRNA was upregulated in megakaryocytes from CLP mice compared to non-treated mice (2.2-fold; n≥3; P < 0.05).

Conclusions: We demonstrate both *in vitro* and *in vivo* that LPS reaches the bone marrow and changes the gene expression profile of megakaryocytes in bacterial sepsis. We hypothesize direct consequences in the bone marrow milieu which could become impactful in disease.

PB2005 | Neutrophilic Extracellular Traps (NETs) Are a Subset of Smudge Cells Identifiable by Peripheral Smear Autoanalyzers

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Background: Neutrophil Extracellular Traps (NETs) are composed of extracellular decondensed chromatin networks that play an important role in immunity. Simple and reliable identification of NETs has been challenging. Automated hematology analyzers such as the CellaVision® classify cell-derived remnants as smudge cells. We hypothesize that, in addition to the typical degenerated lymphocytes (DLs) forming smudge cells, a proportion of smudge cells as identified by the CellaVision® are actually NETs.

Aims: To study the correlation of high NET percent on blood smears with infection.

Methods: To test our hypothesis, we used morphologic characteristics, immunohistochemistry, immunofluorescence and flow cytometry to differentiate NETs from DLs. Smears with ≥20% smudge cells were classified morphologically as NETs vs. DLs; compared to control group, ≤ 5% smudge cells. Medical chart review performed by blinded investigators, included patient demographics, presence of bacterial or viral infection occurring ≤ 1 week of sample collection, and other comorbidities. Statistical analyses included two-sided *t*-test and chi-square.

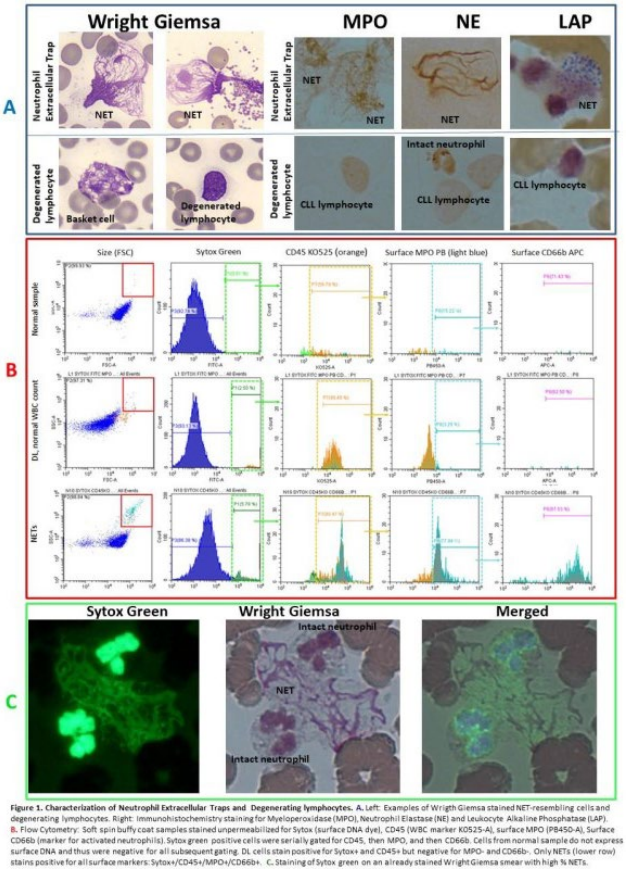

Figure 1. Characterization of Neutrophil Extracellular Traps and Degenerating lymphocytes. A. Left: Examples of Wrigth Giemsa stained NET-resembling cells and degenerating lymphocytes. Right: Immunohistochemistry staining for Myeloperoxidase (MPO), Neutrophil Elastase (NE) and Leukocyte Alkaline Phosphatase (LAP). B. Flow Cytometry: Soft spin buffy coat samples stained unpermeabilized for Sytox (surface DNA dye), CD45 (WBC marker KO525-A), surface MPO (PB450-A), Surface CD66b (marker for activated neutrophils). Sytox green positive cells were serially gated for CD45, then MPO, and then CD66b. Cells from normal sample do not express surface DNA and thus were negative for all subsequent gating. DL cells stain positive for Sytox+ and CD45+ but negative for MPO- and CD66b-. Only NETs (lower row) stains positive for all surface markers: Sytox+/CD45+/MPO+/CD66b+. C. Staining of Sytox green on an already stained Wright Giemsa smear with high % NETs.

FIGURE 1 Characterization of Neutrophil Extracellular Traps and Degenerating lymphocytes

TABLE 1 Demographics and comorbidity analysis for <5% smudge cells and >20% smudge cells, sub-classified as NET vs. DL(>80% cells in that category)

	< 5% Smudge Cells n=59	> 20% Smudge Cells n=96 NETs n=88	> 20% Smudge Cells n=96 DLs n=8	T-test < 5% vs. > 20% Smudge Cells	T-test < 5% vs. > NETs	T-test < 5% vs. DLs	T-test NETs vs. DLs
Bacterial Infection, n (%)	11 (18.6%)	35 (39.8%)	1 (12.5%)	**0.0091**	**0.0045**	0.66	0.073
Viral Infection (includes HIV, HBV, HCV), n (%)	8 (13.6%)	29 (33.0%)	2 (25.0%)	**0.0085**	**0.0081**	0.53	0.663
Lymphoproliferative Disorder, n (%)	12 (20.3%)	6 (6.81%)	5 (62.5%)	0.16	0.17	0.17	0.019
Myeloproliferative/ MDS, n (%)	9 (15.3%)	5 (5.7%)	2 (25.0%)	0.15	0.076	0.58	0.28
VTE/ATE, n (%)	5 (8.5%)	10(11.4%)	1 (12.5%)	0.544	0.56	0.76	0.93
WBC (x109/L), mean (SD)	7.4 (4.8)	8.5 (5.75)	84.05 (69.6)	0.014	0.18	0.017	0.018
Neutrophil (x109/L), mean (SD)	5.24 (4.09)	4.50 (4.0)	5.35 (5.5)	0.33	0.28	0.959	0.68
Age, median yrs (IQR)	61 (68.1-43.5)	48.0 (63.1-7.3)	69.6 (80-65.2)				
%Female	42.4%	44.3%	50%				

Results: Within the study group of ≥20% smudge cells, 88 were morphologically designated as NETs, 8 as DL vs. 59 as control group. Smears from ≥20% smudge cells (NET-subclassification) stained strongly with myeloperoxidase (MPO), neutrophil elastase, leukocyte alkaline phosphatase, whereas DLs were negative. On Wright-Giemsa stain, cell remnants that were morphologically classified as NETs also stained for Sytox-Green. On flow cytometry, NETs are large and display extracellular DNA and MPO. Comparing patients with ≥20% smudge and NET sub-classification to ≤ 5% smudge cells, the formers had higher incidence of bacterial and viral infections (p=0.009/0.005 and p=0.009/0.008).

Conclusions: This study is the first to identify NETs on peripheral smear evaluations as performed by a routine Hematology Autoanalyzer and to differentiate them from DLs using a reliable set of morphologic characteristics, immunohistochemical stains, and flow cytometry probes. This study also supports data that associate NETs with infections even in the absence of leukocytosis.

PB2006 | Promising Therapeutic Effects of a Novel RNA Aptamer, Apta-1, in a Severe Systemic Inflammation Model on Non-human Primates

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Aptahem AB, Malmo, Sweden

Background: The Apta-1 aptamer is an RNA oligonucleotide sequence selected to bind with high affinity and selectivity to a specific functional molecular motif. Since Apta-1 binding sequence is present on different proteins, Apta-1 displays multiple effects in various animal models. This unique feature makes Apta-1 a suitable candidate for treatment of complex pathophysiological conditions, such as Systemic Inflammatory Response Syndrome (SIRS) or sepsis.

Aims: The effects of Apta-1 on severe systemic inflammation were investigated *in vivo* in non-human primate animal model.

Methods: The 15 mg/kg of LPS endotoxin (strain K-235) was administered intravenously to all Cynomolgus monkeys. Apta-1 treated animals received Apta-1 intravenously in two doses, 20 mg/kg at 15 min and 10 mg/kg at 120 minutes post LPS infusion. Untreated animals received saline solution at the same time points. Plasma and serum samples were collected from all animals at 3, 6, 12, 24 and 48 hours after LPS infusion. Selected markers of vascular damage, inflammation and coagulation were analyzed.

Results: Markers of vascular damage, i.e. myoglobin, metalloprotease 9 and damage associated endogenous patterns (S1008/MRP8 and HMGB1) were induced in blood samples after LPS infusion. Animals treated with Apta-1 showed decreased levels of pro-inflammatory markers, i.e. IL-6, IL-1beta, TNF-alpha and serum amyloid A (SAA); as well as complement fragments C3a and C4a. Furthermore, anti-inflammatory cytokine IL-10 levels were 2-fold increased. Administration of Apta-1 also resulted in prolonged blood coagulation (aPTT). Most importantly, Apta-1 induced plasma levels of Pentraxin 3 (PTX3) up to 5- fold. Pentraxin 3 is a known complement modulator.

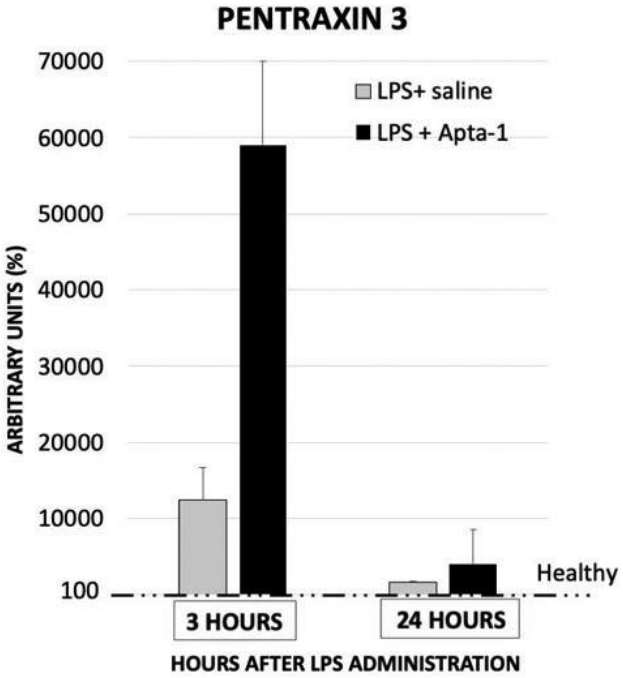

FIGURE 1 Plasma levels of Pentraxin 3 in untreated and Apta-1 treated animals

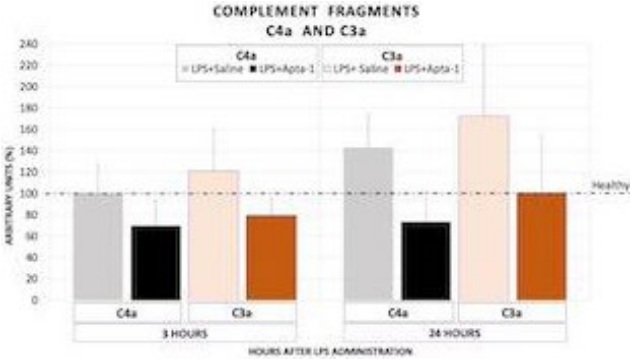

FIGURE 2 Decrease of complement fragments C4a and C3a in plasma of Apta-1 treated animals

Conclusions: In this study, treatment with the novel RNA aptamer Apta-1resulted in suppression of inflammation, prolongation of coagulation and induction of tissue repair mediated via upregulation of Pentraxin 3. The multifactorial activity of Apta-1 affects different components of dysregulated immune response, thus restoring its protective function.

PB2007 | Cytokine Network Architecture at the Endothelial Interface

A.J. Hoogendijk[1]; E.F.J. Janssen[1]; B.L. van denEshof[1]; F.P.J. vanAlphen[1]; C. van derZwaan[1]; A.B. Meijer[1,2]; M. van denBiggelaar[1]
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Background: In acute or chronic inflammation, the vascular endothelium actively regulates hemostasis as well as immune cell trafficking to enable clearance of infections and cellular damage. Cytokines form the foundation of the communication network that controls the immune system and orchestrates endothelial responses. Although it is generally acknowledged that immune system activation results in the release of a cocktail of cytokines, the current paradigm of vascular inflammation is based on studies using a limited set of individual cytokines, most notably TNFα and IL1β.

Aims: We aim to reveal the distinct cytokine signals that initiate unique adaptive endothelial responses and thereby drive selective immune cell trafficking between blood and tissues by integrating phosphoproteomics, transcriptomics and proteomics.

Methods: Based on ImmuneXpresso datamining a library of recombinant human cytokines was obtained. Blood outgrowth endothelial cells were stimulated with these cytokines for 24 hours and proteomes were determined using quantitative mass spectrometry. Cytokines that displayed distinct endothelial responses were applied as concatenated mixes and sampled for RNAseq and (phospho)proteomic analyses.

Results: To dissect the diversity and selectivity of endothelial inflammation, we studied endothelial responses to a library of 70 cytokines using mass spectrometry based quantitative proteomics. This resulted in the quantification of 4331 proteins of which 261 were statistically significant. These proteomic profiles could be categorized in four main response types. Based on these, we prepared cytokine mixtures to determine their synergistic potential. This revealed that synergistic responses were indeed induced, most notably by the combination of TNFα and IFNγ. Using an expression correlation approach, we highlight a distinct set proteins (*e.g.* CCL5) that are affected by synergism.

Conclusions: Taken together, we provide a comprehensive map of the endothelial-cytokine interactions that may pave the way for rational design of therapeutic strategies aimed to selectively modulate immune cell trafficking across the vessel wall.

PB2008 | Biomarkers of Platelet Activation and their Prognostic Value in Patients in Sepsis Associated Coagulopathy

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Background: Sepsis-associated disseminated intravascular coagulation (SAC) is associated with marked hemostatic changes including transient thrombocytopenia due to their enodogenous activation / consumption. The widespread activation of platelets contribute to vascular occlusions, fibrin deposition, multi-organ dysfunction, contributing to a two-fold increase in mortality.

Aims: The purpose of this study was to measure markers of platelet function in the plasma of patients with clinically established SAC and to determine their association to disease severity and outcome.

Methods: Plasma samples from 103 adult intensive care unit (ICU) patients with sepsis and suspected disseminated intravascular coagulation (DIC) were acquired from the University of Utah Hospital at the time of admission. Samples were shipped to Loyola University Chicago and stored at -80°C prior to analysis. Plasma levels of CD40L, von Willebrand Factor (vWF), platelet factor-4 (PF-4), and microparticles (MP) were quantified using commercially available ELISAs.

Results: Markers of platelet activation were significantly elevated in patients with sepsis alone and with DIC compared to normal controls on ICU ($p < 0.001$). PF-4 was significantly decreased in non-survivors compared to survivors ($p = 0.0156$). Since platelet count is often reduced in patients with DIC, the platelet count and levels of platelet biomarkers were also evaluated. Markers of platelet activation were significantly elevated in patients with SAC compared to healthy volunteers, independent of platelet count, underscoring the importance of activation processes to this disease process. Of the markers studied, PF4 showed a significant difference based on DIC score and mortality, and differentiated the non-survivors compared to survivors. Levels of CD40L, vWF, PF4, and MP showed significant association with platelet count.

Conclusions: These studies suggest that the markers of platelet activation as primarily regulated by the number of circulating platelets and may be independent of the factors leading to their endogenous consumption.

TABLE 1 Markers of platelet activation on day 0 vs. platelet count

	<50 (k/ul)	50-99 (k/ul)	100-149 (k/ul)	>150 (k/ul)	P (Kruskal-Wallis ANOVA)
CD40L (pg/ml)	127.0 ± 46.0	158.0 ± 42.0	274.0 ± 37.0	496.0 ± 94.0	0.0063
vWF (%)	218.0 ± 35.0	268.0 ± 18.0	274.0 ± 16.0	232.0 ± 8.6	0.0452
PF-4 (ng/ml)	46.0 ± 3.4	52.0 ± 5.6	81.0 ± 8.2	84.0 ± 4.6	<0.0001
MP (nM)	20.0 ± 4.8	20.0 ± 5.5	29.0 ± 4.3	47.0 ± 4.0	<0.0001
n	5	16	20	61	N/A

PB2009 | Human Vascular Endothelial Cell Derived Exosomes Contribute to the Excessive Inflammatory Response Observed in Sepsis through a Dysregulated MicroRNA Expression Profile

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Background: The pathophysiology of sepsis and its accompanying hyper-inflammatory response are key events that lead to multi-organ failure and death. A growing body of literature now suggests that the vascular endothelium plays a critical role in driving sepsis progression. Upon bacterial infection, the endothelium releases exosomes, which harbour microRNA. A dysregulated exosomal miRNome may be a key driver of the sustained inflammatory response observed in sepsis.

Aims: We aim to identify the role of endothelial exosomes in driving a pro-inflammatory phenotype in leukocytes.

Methods: Exosomes were identified by western blot, flow cytometry and fluorescent imaging. Exosomal miRNA profiling was performed using TaqMan® Microarray Cards. The inflammatory state of monocytes was determined by cytokine analysis, inflammatory marker cytometry, and western blot.

Results: Activation of monocytes was determined by flow cytometry of CD11b. Exosomes, from healthy endothelial cells, were delivered to monocytes and failed to affect CD11b expression. Exosomes isolated from *Staphylococcus aureus* infected endothelial cells induced a ~2; ~4 fold increase in CD11b expression after t=24; 120 hours respectively (N=5; ±SEM; $p< 0.05$). These exosomes resulted in heightened production of IL-6 and reduction in IL-10 over 24 hours (N=3; ±SEM; $p< 0.05$). Our data suggests that exosomes contain RNA capable of influencing monocyte function. Microarray analysis of exosomal RNA identified 17 up-regulated miRNA, including the targets of interest, miR-99a/b. Bioinformatics analysis identified >200 potential miR-99a/b targets that were critically affected following infection including mTOR. Absolute quantification of miR-99a/b in monocytes revealed that these miRNA are poorly expressed (N=3; ±SEM; p=NS). Knockdown of mTOR (N=3; ±SEM; $p< 0.05$) using miR-99a/b mimetics resulted in an increase in IL-6 production and a decrease in IL-10 production comparable to that of exosome delivery data (N=3; ±SEM; $p< 0.05$).

Conclusions: Our results suggest that upon infection, endothelial cells release exosomes harbouring miRNAs capable of driving a pro-inflammatory phenotype in monocytes.

PB2010 | *Candida albicans* Expresses an Integrin-like Protein that Is Involved in Yeast Adhesion to Endothelial Cells

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Background: Acute vascular endothelial dysfunction is a central event in the pathogenesis of sepsis and leads to increasing vascular leak, tissue oedema and compromising regional perfusion in critical organs. While bacteria remain the primary cause of sepsis, current etiologic evidence indicates that the incidence of fungal infection in septic patients is increasing at a rapid rate. There is a paucity of information on the molecular mechanism through which fungus interact with endothelial cells, specifically by the commensal yet opportunistic pathogen, *Candida albicans*.

Aims: Our aim is to elucidate the factors by which *C. albicans* interacts with vascular endothelial cells to result in disseminated candidiasis.

Methods: Human endothelial cells were sheared at physiological rates and fungal attachment was examined through phase-contrast and immunofluorescence microscopy. Changes in vascular permeability were measured by transwell paracellular permeation.

Results: *C. albicans* binds to both static (9.61 yeast cells/mm^2) and sheared (84.41 yeast cells/mm^2) human endothelial cells, although binding was significantly stronger to sheared endothelial cells ($p< 0.0001$) and with human plasma ($p< 0.05$). Binding was dose-dependently inhibited using the $\alpha V\beta 3$ antagonist, cilengitide (23.98 yeast cells/mm^2 with drug, 84.41 yeast cells/mm^2 without drug, $p< 0.005$). Attachment resulted in disturbances in endothelial barrier integrity, as determined by loss of tight junction protein staining and an increase in permeability. Interestingly and consistent with previous observations, using immunofluorescence we demonstrated that *C. albicans* expresses an integrin $\alpha V\beta 3$-like protein on its surface which we believe binds directly to plasma fibrinogen and cross-links the fungus to human endothelial cell $\alpha V\beta 3$.

Conclusions: Our data for the first time demonstrates a mechanism through which *C. albicans* attaches to the vascular endothelium, to promote further dissemination upon barrier penetration. Additionally, inhibition of *C. albicans* binding to endothelial $\alpha V\beta 3$ using cilengitide prevents endothelial dysfunction and therefore may present as a novel therapeutic approach for the treatment of fungal sepsis.

PB2011 | Diabetes Mellitus Increases Thrombin and Activated Protein C Effects on Mast Cell and Macrophages

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Background: Proteases of the blood coagulation, thrombin and activated protein C (APC) are involved in the development of inflammatory processes. Diabetes mellitus (DM) is characterized by a high glucose in blood, a hyperglycemia. It contributes to vascular damage and leads to the development of DM complications such as neuropathy, angiopathy, diabetic foot and others. The understanding of the mechanism of haemostasis proteases involvement in the inflammation accompanied many diseases is one of the fundamental problems of physiology.
Aims: The aim of this study to investigate the features of thrombin and APC-induced regulation of mast cell and macrophage(MP) activity in diabetes, inflammation and high glucose.
Methods: Peritoneal mast cells and MP isolated from rat with streptozotocin-induced DM, thioglycolate-induced inflammation and RBL-2H3 were analyzed. The evaluation of proliferation and accumulation of nitrites, intracellular calcium, secretion and actin cytoskeleton were performed for comparing effects of thrombin and APC on cells.
Results: At the first time has been shown that the effect of thrombin on mast cell secretion is dose-dependent in both experimental diabetes and inflammation. It has been shown that high glucose and DM reduce baseline proliferation and the production of NO by MP. Proinflammatory activity of MP is potentiated by hyperglycemia, one of the main pathological factors of diabetes. A high glucose impacts proliferation and NO production and increases the thrombin and APC-induced effects on cells. The PKC activator - phorbol ester abolished the pro-inflammatory effect of thrombin, that indicates the involvement of PKC in protease effects.
Conclusions: Thus, this study points to the leading role of glucose in the regulation of MP and mast cells activation. In contrast to inflammation, diabetes is a more significant factor that changes the effect of protease on mast cells, probably due to a change in the affinity of the PAR-1 receptor.
This work was supported by RFBR 19-015-00529.

PB2012 | Altered Coagulation Parameters and D-Dimer Measurements in Sepsis Are Useful in Scoring the Risk Stratification

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[1]*Loyola University Medical Center, Maywood, United States;* [2]*University of Utah School of Medicine, Salt Lake City, United States*

Background: Sepsis is characterized by a simultaneous activation of inflammation and hemostasis in response to microbial infection. This systemic inflammatory response is due to the release of pro-inflammatory cytokines, pro-coagulants and adhesion molecules from immune cells and/or damaged endothelial tissue.
Aims: This study was designed to profile coagulation parameters in a defined clinically confirmed sepsis population in conjunction with an IRB approved clinical trial.
Methods: Plasma samples from septic shock patients (n=78) were collected in citrated tubes within 72 hours of ICU at the University of Utah and Veteran's Affair FFC Health Care System VAMC. Normal controls were comprised of 25 male and 25 female samples of citrated plasma (George King Biomedical, Overland Park, Kansas City). D-Dimer was measured using a commercially available sandwich ELISA methods. PT/INR, aPTT and fibrinogen measurements were based on clot-based assays. All results were compiled as mean ± SD and SEM.
Results: D-Dimer levels were markedly increase in sepsis patients (23-fold) in comparison to control. Significant elevation of clotting parameters such as PT/INR (2.0-fold), aPTT (2.5-fold) and fibrinogen (2.0-fold) was noted in the sepsis patients in comparison to controls. These differences were significant (p value ≤0.0009) for all of the parameters except fibrinogen (p value 0.4694). There was no correlation between D-dimer and fibrinogen in the sepsis patients.
Conclusions: The marked prolongation of PT and aPTT are suggestive of both the extrinsic and intrinsic pathway defects and consumption of clotting factors. The fibrinogen levels were also elevated and nearly 1/3 of the patients showed >1000 mg/dL levels. Marked elevation of D-Dimer is indicative of endogenous fibrin formation and its consumption consistent with activation of fibrinolysis. These results further underscore the importance of including the clotting parameters and D-dimer in scoring the coagulopathies associated with sepsis such as disseminated intravascular coagulation.

INNATE AND ADAPTIVE IMMUNITY

PB2013 | Activation of ERK Signaling Pathway by Plasma IgG in Venous Thromboembolism Patients without Antiphospholipid Antibodies

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Background: Venous thromboembolism (VTE) is still a huge challenge for Public Health. Autoimmune diseases play an increasing role in human pathologies, antiphospholipid antibody (aPL) syndrome being one of the models for VTE pathophysiology. aPL have been found to

activate several signalling pathways, but we lack data on circulating antibodies-related cell activities in aPL-negative patients with VTE.

Aims: We hypothesized that certain circulating immunoglobulins G (IgG) in VTE patients might stimulate the signalling pathways involved in monocyte activation, even in aPL-negative patients (clinicaltrials.gov identifier: NCT02713581).

Methods: Plasma IgG from 13 VTE patients and 20 controls were purified by column chromatography. These purified IgG were added to a THP-1 monocyte culture medium, and proteins were extracted for subsequent western-blot analysis. Quantification of actin-normalized phospho-ERK, phospho-P38 and phospho-AKT allowed us to characterize the monocyte activation profile for each patient. Modulation of signalling pathways between groups was assessed per pathway by two-way ANOVA which also takes into account the experiment effect.

Results: Our results show a significant increase in the phospho-ERK/Actin ratio ($p< 0.0001$). There was no group effect concerning total protein ERK, demonstrating that activation was only due to an increase in ERK-phosphorylation ($p=0.419$). No significant differences were found for the other pathways ($p=0.964$ and $p=0.174$ respectively for phopsho-p38 and phospho-AKT).

Conclusions: Our pilot study shows that total IgG from patients with a history of VTE may activate the ERK pathway in the absence of antiphospholipid antibodies. The impact of this IgG-related activity on individual risks of VTE deserves to be investigated.

PB2014 | Determinants of Neutrophil Extracellular Trap Formation in the General Population: Based on the Rotterdam Study Cohort

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Background: Neutrophil extracellular traps (NETs) are formed when activated neutrophils secrete decondensed intracellular DNA together with antimicrobial enzymes such as myeloperoxidase (MPO). Since NETs are involved in the development of thrombosis, it is relevant to know which demographic and clinical characteristics are associated with the formation of NETs.

Aims: To determine the demographic and clinical characteristics that are associated with NET formation in the general population.

Methods: Citrated plasma was collected from 6449 participants (aged ≥55 years) included in the prospective population-based Rotterdam Study. Levels of NET formation were quantified by measuring levels of MPO-DNA complexes using an enzyme-linked immunosorbent assay. We used linear regression to determine the associations of MPO-DNA complex with age, sex, cardio-metabolic risk factors (e.g. hypertension, cholesterol, diabetes), a history of cardiovascular disease, and blood markers of inflammation and coagulation.

Results: The median age of the participants was 68.6 years (Interquartile Range (IQR) 62.8-75.3 years) and 56.3% of the participants were female. Median MPO-DNA complex levels were 0.05 AU/mL (IQR 0.04-0.09). We found an inverse association between MPO-DNA complex levels and the following characteristics: age (β -0.04 AU/mL/10 years, 95% confidence interval (CI) -0.06;-0.02), a history of coronary heart disease (CHD) (β -0.10 AU/mL, 95% CI -0.17;-0.03), the use of lipid-lowering drugs (β -0.06 AU/mL, 95% CI -0.12;-0.01), and levels of HDL-cholesterol (β -0.07 AU/mL/ mmol/L, 95% CI -0.12;-0.03). MPO-DNA complex levels were not related to sex, other cardiometabolic risk factors, and markers of inflammation and coagulation.

Conclusions: Our results suggest that increasing age, a history of CHD, the use of lipid-lowering drugs and higher HDL-cholesterol levels are associated with lower levels of MPO-DNA complexes, a marker of NET formation. These results can lead to a better understanding of individuals who are at risk of developing NET-associated thrombotic diseases.

PB2015 | Ageing Affects Neutrophils - Changes in Activation, DNA Release and Neutrophil Extracellular Formation of Neutrophil Subpopulations with Human Age

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Background: The heterogeneity of neutrophils and their functions, especially production of neutrophil extracellular traps (NETs), are important foci of thrombosis research. Neutrophils can be subdivided into high and low density neutrophils (HDNs/LDNs) and they are thought to react differently to stimuli. Studies have shown differences in neutrophil subpopulations and functionality in patients but data on healthy subjects are scarce.

Aims: We aimed to investigate age-related changes in neutrophil activation, DNA release and NET formation.

Methods: HDNs and LDNs from 25 healthy individuals, subdivided in 3 age groups (< 45 years, n=8; 45-54 years, n=9; >54 years, n=8) were isolated. Potential of activation (increase of CD11b surface expression), DNA release (DNA release assay) and NET production (measured by citrullinated histone H3 (H3Cit)) of HDNs/LDNs were investigated after stimulation with ionomycin (IO) and phorbol 12-myristate 13-acetate (PMA).

Results: No differences in isolated HDN or LDN counts were observed among the age groups. HDNs from older individuals had less increase of activation markers (MFI CD11b) than HDNs from younger subjects (Fig. 1A) but they showed significantly increased DNA release compared to those from younger participants (Fig. 1B). NET release was higher in LDNs (Fig. 1C) compared to HDNs (Fig. 1D) and with regard to age groups the highest NET release at baseline but also after 3h of stimulation with IO was seen in LDNs of the age group between 45-54 years (Fig. 1C).

Conclusions: We conclude that neutrophils and their ability of activation, DNA release and NET formation change during the ageing process and that this might be associated with a higher thrombotic risk and increased vulnerability to infections at advanced age. Furthermore,

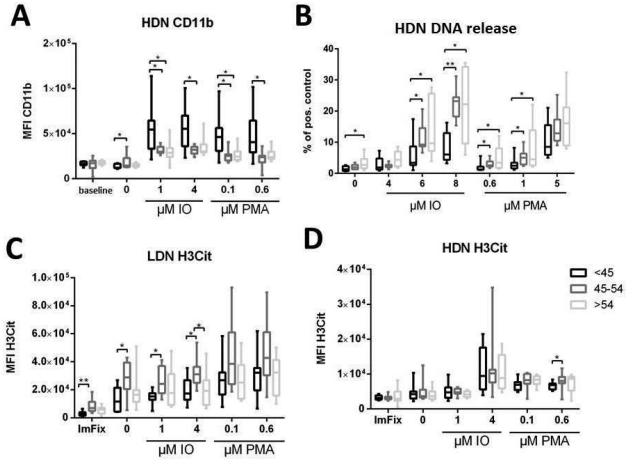

FIGURE 1 Potential of activation (A), DNA release (B) and NET formation (C/D), measured by H3Cit, in neutrophils (HDNs/LDNs) of healthy volunteers

these results highlight the importance and necessity of correct age-matching in studies, especially in studies focusing on neutrophils.

PB2016 | The Gut Microbiota Restricts NETosis in Acute Mesenteric Ischemia-Reperfusion Injury

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Background: Acute mesenteric ischemia (AMI) is a life-threatening clinical situation, potentially leading to bowel wall necrosis and multiple organ failure. Intestinal ischemia, caused by the occlusion of the superior mesenteric artery, breaks the intestinal barrier and results in the translocation of bacterial products. The impact of the gut microbiota as a trigger of Toll-like receptor (TLR) signaling for the recruitment of leukocytes to ischemia/reperfusion (I/R) damaged mesenteric venules and its influence on the formation of neutrophil extracellular traps (NETs) is unexplored.

Aims: We studied gnotobiotic mouse models to resolve the role of gut commensals on NETosis in AMI.

Methods: We applied a mesenteric I/R injury model to germ-free (GF) and C57BL/6J mice colonized with a gut microbiota, *Escherichia coli*, or *Bacillus subtilis*. By intravital imaging, we quantified leukocyte adherence and NET formation in I/R-injured mesenteric venules. Comparing GF with conventionally raised (CONV-R) mice, lipopolysaccharide (LPS)-triggered NETosis was analyzed in vivo and with bone marrow-derived neutrophils ex vivo.

Results: Colonization with a complex gut microbiota as well as monocolonization with *Escherichia coli* JP313 and *Bacillus subtilis* augmented the adhesion of leukocytes to I/R-injured mesenteric venules, which was dependent on the Toll-like receptor-4 (TLR4) / TIR-domain-containing adapter-inducing interferon-β (TRIF) pathway. Conversely, antibiotic depletion of gut commensals reduced leukocyte adhesion. In contrast, NETosis was significantly increased in I/R-injured mesenteric venules of GF mice and in an ex vivo assay. Likewise, neutrophils from CONV-R mice treated with broad-spectrum antibiotics showed increased NETosis. LPS administration to GF mice suppressed I/R-induced NETosis. In line with augmented TLR4 / TRIF signaling, isolated bone marrow-derived neutrophils from GF mice showed enhanced LPS-induced NETosis. Furthermore, NETosis in I/R injury was reduced in *Trif*$^{-/-}$ and *Myd88*$^{-/-}$*xTrif*$^{-/-}$ mice, but not in *Myd88*$^{-/-}$ mice.

Conclusions: Collectively, our results identified that the gut microbiota restricts NETing neutrophils in mesenteric I/R injury, while ensuring immunovigilance by enhancing neutrophil recruitment.

PB2017 | Specialized Regulatory T Cells Control Venous Blood Clot Resolution

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Background: Deep vein thrombosis is associated with long-term complications, including venous thromboembolism, post-thrombotic syndrome and chronic thromboembolic pulmonary hypertension. $CD4^{+}Foxp3^{+}$ regulatory T (Treg) have a crucial role in tissue healing and maintenance -distinct from their role in suppression of immune responses. Less is known about the composition and function of infiltrating T cell populations, especially the contribution of Treg cells in post thrombotic inflammation.

Aims: Based on the observation that Treg accumulate in venous thrombi, we aimed to systematically clarify their functional role in venous clot resolution.

Methods: Experimental approaches included the selective manipulation of Treg numbers during thrombus resolution, comprehensive characterization of the histological and cellular changes induced by them, generation of Treg expression profiles and the identification of their clot-dissolving function.

Results: We describe a specialized population of resident 'clot-busting' Treg that develops in venous blood clots, is activated by monocyte-derived cytokines, and regulates thrombus resolution by imposing phenotypic and functional changes in the myeloid compartment.

Conclusions: Our study reveals a critical role of specialized Treg in order to blood clot resolution. These findings suggest that Treg cells and their function may offer new therapeutic opportunities for thrombus repair. Increasing Treg activity appears to be an attractive therapeutic approach for improving thrombus resolution and restoring organ function in chronic thromboinflammatory diseases.

PB2018 | Neutrophil Extracellular Traps (Nets) Increase the Pro-Tumoral Properties of the Human Breast Cancer Cell Line, MCF7

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Background: Several different host cells found in the tumor microenvironment, including immune cells such as neutrophils, seem to facilitate tumor progression. Neutrophils may undergo a cellular program that leads to the extrusion of a web-like material named Neutrophil Extracellular Traps (NETs) that are composed of DNA and proteins such as elastase, histones, and myeloperoxidase. NETs have been proposed as possible effectors in the tumor progression.

Aims: Herein, we evaluated the effects of isolated NETs on the human breast cancer cell line, MCF7.

Methods: Neutrophils were isolated from the blood of health donors and further stimulated with PMA to generate NETs. MCF7 cells were starved in serum-free medium, incubated with NETs for 16h, and samples were processed for qPCR, flow cytometry, and fluorescence microscopy analyses.

Results: Treatment of MCF7 with purified NETs promoted remarkable morphological changes in the tumor cells. In addition, the incubation of MCF7 with NETs dramatically increased the migratory properties of the tumor cells. NETs upregulated the mRNA expression of interleukin-6, interleukin-8 and matrix metalloprotease 9 (MMP9) genes, as assessed by real-time qPCR analyses, which was approximately 10, 12 and 100-fold higher than the control condition, respectively. In order to evaluate if NETs could promote β-catenin translocation from the membrane to the nucleus, we employed immunocytochemistry assays. NETs promoted the nuclear localization of β-catenin in MCF7 cells. Morevover, the expression of the cancer stem cell marker, CD44, was upregulated upon incubation of MCF7 cells with NETs, as demonstrated by qPCR and by flow cytometry analyses.

Conclusions: Collectively our data demonstrate that NETs increase the pro-tumoral properties of the human carcinoma cell line, MCF7. We propose that NETs may participate in the primary breast cancer progression.

PB2019 | In Vitro Disruption of Endothelial Barrier Integrity by Heme and by Serum from Patients with Sickle Cell Disease

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Background: Free extracellular heme has been shown to activate several compartments of innate immunity, acting as a danger-associated molecular pattern (DAMP) in diseases associated with hemolysis. Although localized endothelial barrier (EB) disruption is an important part of innate immunity activation that allows circulating leukocytes to reach inflamed tissues, non-localized/deregulated disruption of the EB can lead to widespread microvascular hyperpermeability and secondary tissue damage, such as observed in sepsis. In mice models of sickle cell disease (SCD), EB disruption has been associated with the development of a form of acute lung injury that closely resembles acute chest syndrome (ACS), and that can be elicited by the acute infusion of heme.

Aims: Here we explored the effect of heme on EB integrity using human endothelial cell monolayers, in experimental conditions that include elements that normally trigger acute SCD complications in humans.

Methods: EB integrity was assessed by electric cell-substrate impedance sensing in the presence of varying concentrations of heme, LPS and sera from SCD patients or healthy volunteers.

Results: Heme caused a dose-dependent decrease of the electrical resistance of cell monolayers, consistent with EB disruption, which was confirmed by staining of *adherens* junction protein VE-cadherin. LPS further increased this effect. Sera from SCD patients, but not from healthy volunteers, were also capable to induce EB disruption. Finally, the EB-disrupting effects of heme were still observed in the presence of serum from SCD patients but not from healthy volunteers.

Conclusions: Together our results provide new information about the pathogenesis of diseases in which EB disruption occurs in association with hemolysis, such as SCD and sepsis, and reinforce the concept that heme can act as a DAMP in hemolytic conditions.

PB2020 | mir-146a Deficiency Promotes Neutrophil Aging and Increases their Sensitivity to Form Neutrophil Extracellular Traps (NETs) in a Murine Model

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Background: Neutrophil extracellular traps (NETs) are released by neutrophils under diverse pro-inflammatory *stimuli*. Our group showed that miR-146a deficiency in a murine model increased the intrinsic ability of neutrophils to form NETs *in vitro*.

Aims: Since it has been described that neutrophils display a wide phenotypic diversity accompanied by important functional changes, we investigate whether the greater capacity of miR-146a-deficient neutrophils to form NETs is associated with neutrophil phenotype.

Methods: Peripheral blood (PB) was extracted from wild type (WT) and miR-146a$^{-/-}$ mice at baseline and 4 hours after intraperitoneal injection of LPS (1mg/Kg). NET components were measured in plasma from these mice: neutrophil elastase (NE) by ELISA, circulating free DNA (cfDNA) by SYTOX Green, and citrullinated Histone H3 (citH3) by western blotting. Ly6G, CD11b, CD62L, Cxcr4, and Tlr4 were measured by flow cytometry in PB neutrophils.

Results: miR-146a$^{-/-}$ mice showed higher plasma levels of all the NET markers analyzed (NE, cfDNA, and citH3) than WT mice after LPS injection. When we compared baseline levels of neutrophil activation markers, we found that miR-146a$^{-/-}$ neutrophils had higher levels of CD11b (MFI 44809 vs. 26011, $p< 0.05$), and lower levels of CD62L (MFI 2641 vs. 6022, $p< 0.01$) than WT neutrophils. However, after LPS stimulation both markers reached similar levels, suggesting that miR-146a deficiency confers a pre-activated state. In addition, miR-146a$^{-/-}$ neutrophils had higher levels of Cxcr4 (MFI 1285 vs. 965, $p< 0.01$) and Tlr4 (MFI 1015 vs. 852, $p< 0.05$) at baseline, an event that has been associated with an aged phenotype and an increased sensitivity to form NETs.

Conclusions: miR-146a$^{-/-}$ neutrophils produce higher levels of NETs than WT neutrophils upon *in vivo* stimulation with LPS. We describe that miR-146a deficiency promotes the acquisition of an aged neutrophil phenotype defined by CD11b^{high} CD62L^{low} Cxcr4^{high} Tlr4^{high} markers and an increased ability to form NETs via Tlr4 stimulation.

Funding: PI17/0051,PFIS18/0045,CD18/00044(ISCIII&FEDER),19873/GERM/15(Fundación-Séneca),Premio-López-Borrasca-SETH.

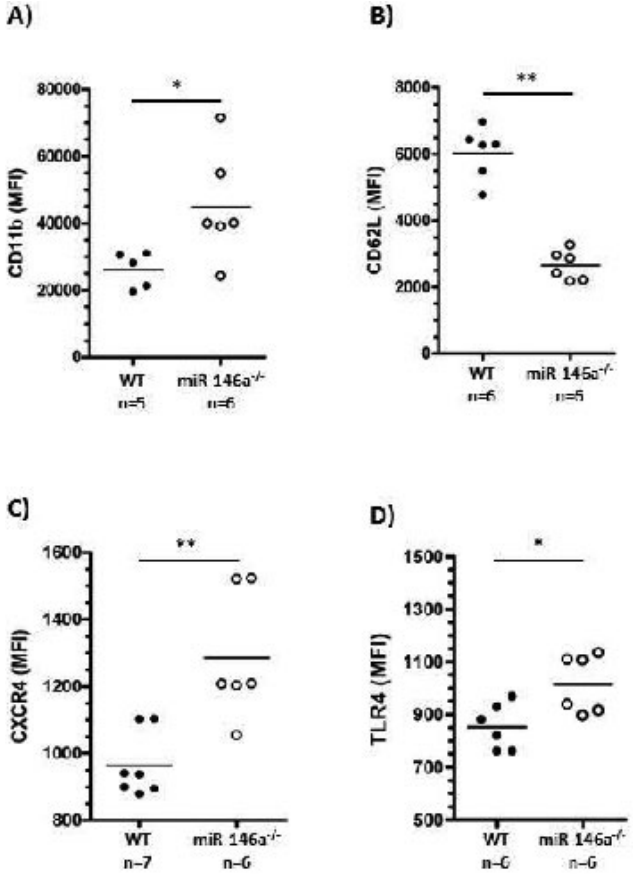

FIGURE 1 Flow cytometry analysis of CD11b, CD62L, Cxcr4, and Tlr4 in WT and miR-146a-/- neutrophils

PB2021 | Stabilin-2 Promoter Modulates Immune Response in the Liver of Lentiviral Vector Injected Mice

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Background: Liver sinusoidal endothelial cells (LSECs) are specialized cells with unique morphology and scavenger activity related to their endocytic capacity taking part to liver tolerance. This activity relies also on the ability of stabilin-2 (Stab2), expressed on LSECs in liver, to mediate the binding of several ligands, among which are coagulation factors (e.g. vWF-FVIII complex). Since LSECs are the main source of FVIII, they could be an optimal target for gene therapy of hemophilia A, and the Stab2 promoter could be used for directing specific expression of FVIII in LSEC upon lentiviral vector (LV) delivery.

Aims: To test the hypothesis that targeting transgene expression to LSECs via Stab-2 promoter will promote robust tolerance to the transgene, thus resulting in transgene expression.

Methods: To test our hypothesis, we adoptively transferred GFP-specfic CD8^{+} Jedi T cells (Jedi-CD8), which are T cells that recognize the immunodominant epitope of GFP ($GFP_{200-208}$), in mice previously injected with LVs carrying GFP under different promoters. Four days after Jedi-CD8 injection (14 days from LV delivery), we analyzed the expression of GFP in liver and spleen by immunofluorescence

through co-staining with specific cell surface markers, while immune cell recruitment and activation was assessed by flow cytometry.

Results: The Stab2 promoter was able to restrict GFP expression mainly to LSECs in different strain of mice, where the expression was sustained for over three months. Strikingly, when we performed the adaptive transfer of Jedi-CD8, only the mice receiving LV.Stab2-GFP maintained GFP^+ cells in the liver while LV.PGK-GFP injected mice showed total absence of GFP^+ cells.

Conclusions: These studies demonstrate that the Stab-2 promoter mediates specific transgenes expression to LSECs. These data support the hypothesis that antigen presentation by LSECs can promote immunological tolerance to a vector-derived antigen, providing relevant new data into the biology of LSECs and a promising strategy for LSEC-directed gene therapy.

PB2022 | Antisense Oligonucleotide Targeting of Thrombopoietin Represents A Novel Platelet Depletion Method to Assess the Role of Platelets During Inflammation Resolution

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Background: Platelets are effector cells of the innate and adaptive immune system; however, understanding their role during inflammation can be challenging due to drawbacks associated with current platelet depletion methods. The generation of antisense oligonucleotides (ASO) directed to thrombopoietin (*Thpo*) mRNA represents a novel method to reduce platelet count to aid in elucidating the role of platelets during inflammation.

Aims: To understand if Thpo-targeted ASOs represent a viable strategy to reduce platelet count in mice, and to delineate the role of platelets to inflammation resolution during lower airway dysbiosis.

Methods: Mice were treated with a Thpo-targeted ASO and the abundance of platelets, blood cells and bone marrow hematopoietic progenitor cells assessed. Additionally, platelet responsiveness to agonists and surface expression of P-selection and JON/A was measured. To assess the contribution of platelets to inflammation resolution Thpo-ASO and control-ASO treated mice were challenged with a bacteria cocktail to model lower airway dysbiosis. Thpo-ASO mediated platelet depletion was compared to anti-CD42b platelet depletion in the lung dysbiosis model.

Results: ASO-mediated silencing of hepatic *Thpo* reduces platelet, megakaryocyte, and megakaryocyte progenitor count by 50% relative to control ASO treated mice. Thpo-ASO treatment does not alter platelet reactivity to agonists, or platelet size. Following bacterial inoculation, we found a significant increase in lung platelet-leukocyte aggregates and consistent with a response to inflammation an increase in lung $Ly6C^{hi}$ monocytes and macrophages in inoculated mice. Platelet depletion, by either Thpo-ASO or anti-CD42b treatment, reduces the accumulation of lung inflammatory immune cells, including monocytes ($Ly6C^{hi}$) and macrophages ($CD45^+CD11b^+F4/80^+$). Furthermore, we found that in contrast to anti-CD42b platelet depletion, Thpo-ASO mediated depletion allows for introduction of new platelets.

Conclusions: Thpo ASO-mediated platelet depletion represents a viable approach to reduce platelet count. Platelet count directly impacts lung inflammation resolution during lower airway dysbiosis demonstrating the essential role of platelets in pulmonary immune defense.

NON-CODING RNAS

PB2023 | Low Levels of Circulating Extracellular Vesicle-Encapsulated HULC Are Associated with Left Ventricular Hypertrophy in Japanese

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Background: Altered expression of long (>200 nucleotides) non-coding RNAs (lncRNAs) is often associated with various human diseases as well as development. Some lncRNAs are transferred by extracellular vesicles (EV) and thus may serve as biomarkers. Highly upregulated in liver cancer (HULC) is a 500 bp lncRNA and is overexpressed in patients with liver cancer. We have previously reported that circulating EV-encapsulated HULC is a potential biomarker of pancreatic cancer. HULC is multifunctional and has pro-angiogenic function in various cancers.

Aims: To assess the roles of HULC as biomarker the present study aimed to determine the association of EV-encapsulated HULC with physiological and biochemical laboratory test results.

Methods: The study involved 13 apparently healthy subjects (7 females and 6 males; mean age 73 years). Serum samples were obtained from these individuals. Serum EV-encapsulated HULC was analyzed by digital PCR. Left ventricular hypertrophy (LVH) was diagnosed with ECG Cornell Product (1,700 mm x msec) or echocardiography. Vascular function was assessed by measuring ankle-brachial-index and brachial-ankle pulse wave velocity.

Results: Circulating levels of EV-encapsulated HULC were 0.629±0.460 copies/μl in subjects without LVH (ECG Cornell Product 1,303 mm x msec, n=7). EV-encapsulated HULC levels tended to be decreased in subjects with LVH (0.328±0.299 copies/μl) (ECG Cornell Product 2,320 mm x msec, n=6). Values of ankle-brachial-index (n=7) and brachial-ankle pulse wave velocity (n=6) were not associated with HULC levels. HULC levels were not associated with liver function tests, fasting blood sugar, HbA1c, CRP or creatinine.

Conclusions: Diminished HULC may impair cardiac angiogenesis and disrupt coordination between blood supply and adaptive hypertrophic response. Further studies are necessary to determine if

EV-encapsulated HULC and other lncRNAs are useful as biomarkers of cardiovascular diseases.

PB2024 | MiR-146a Regulates Platelet Function and Thrombosis

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Background: Platelets are crucial immunomodulatory elements linking inflammatory and thrombotic processes. Platelet-leukocyte aggregates increase the pro-inflammatory functions of leukocytes causing the release of neutrophil extracellular traps (NETs). NETs and platelets activate each other promoting a prothrombotic phenotype. MiR-146a acts as a brake on inflammatory and immunological reactions. Our previous results showed the involvement of miR-146a in thrombo-inflammatory diseases by regulating NET formation.

Aims: To evaluate the role of miR-146a in platelet functionality and thrombosis.

Methods: Wild type (WT) and *miR-146a*$^{-/-}$ (KO) mice were evaluated for:

1) Tail bleeding (n=12/group);
2) Fibrinogen (Fg) binding and P-selectin exposure (P-Sel) after platelet activation (PAR4, CRP, and PMA) (n=4-7/group) by flow cytometry;
3) Platelet metabolism by Seahorse analyzer;
4) Pulmonary thromboembolism (PTE) by monitoring time to death (30min) (n=27 WT, n=30 KO). Lung obstruction was assessed by injecting Evans-Blue;
5) $FeCl_3$ induced-arterial thrombosis (AT) by measuring blood flow occlusion time (OT) (n=17 WT, n=24 KO). NETs were quantified in AT by immunofluorescence [DNA/citrullinated-histone3 (citH3)] (n=10/group).

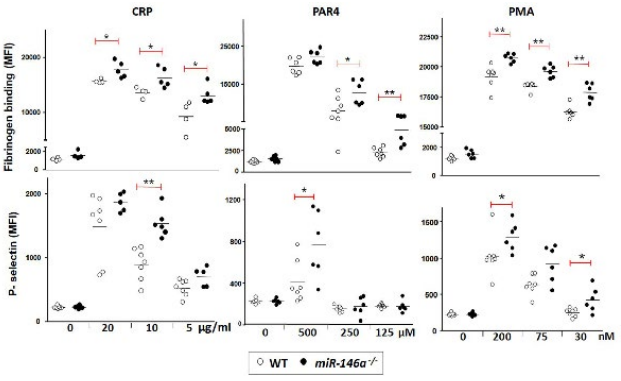

FIGURE 1

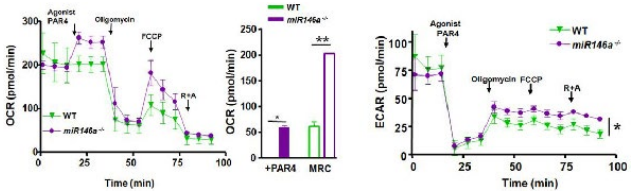

FIGURE 2

Results: Bleeding time was shorter in KO *vs.* WT mice (p=0.02). Fg binding and P-Sel exposure were higher in KO *vs.* WT platelets after activating with different concentrations of PAR4, CRP and PMA (Fig. 1). Oxidative phosphorylation and glycolysis were also higher in unstimulated KO platelets (p< 0.01) and following PAR4 stimulation (Fig. 2). KO mice succumbed earlier than WT after PTE induction (p=0.06). Obstruction of pulmonary circulation was higher in KO mice (p=0.01). OT was shorter in KO *vs.* WT mice (p=0.03) after AT induction. Moreover, thrombi extracted from KO mice showed more DNA/citH3 positive cells than WT (p< 0.01).

Conclusions: Platelets from KO mice are hyperreactive, which causes a prothrombotic phenotype that favors venous and arterial thrombosis. Increased platelet reactivity due in part to alterations in energy production profile could contribute to increase NET formation, supporting miR-146a involvement in immunothrombosis.

Funding: PI17/00051,PFIS18/0045,CD18/00044(ISC III-FEDER),9873/GERM/15,SETH.

PB2025 | Microrna-365a-3p, a Novel Modulator of Tissue Factor Protein Expression and Function

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Background: Molecular regulation of oestrogen (E_2) related venous thromboembolism is poorly understood. Our laboratory has identified several microRNAs (miRs) that respond to E_2 signaling to further modulate miR targeted haemostatic genes. One candidate miR, miR-365a-3p, was demonstrated as critical for tissue factor (TF) mRNA and protein expression.

Aims: To investigate miR-365a-3p's role in regulating TF protein function and TF-dependent thrombin generation.

Methods: HuH-7 hepatocarcinoma cells was transfected with 50 nM miR-365a-3p precursor, or negative control. After 48h treatment cells were evaluated for TF surface expression by flow cytometry (FACS) and TF activity by chromogenic assay. Cellular TF function was further investigated by measuring cell-surface TF -dependent thrombin generation (Ceveron® alpha) in the presence or absence of 3-5 pM

recombinant TF (rTF). Paired t-test was used to determine statistical significance.

Results: Expression of miR-365a-3p significantly reduced both cell surface TF expression and TF activity by 18% (p=0.02) and by 11% (p=0.04), respectively. Similarly in thrombin generation assays, miR-365a-3p transfected samples showed significantly lower thrombin generation. In the presence of rTF, there was prolonged lag time (6-fold, P=0.03) and time to peak (2-fold, P=0.001), and reductions in peak height (8-fold, P=0.001) and endogenous thrombin potential (10-fold, P=0.0001) (n=8). And in the absence of rTF, thrombin generation also had decreased peak thrombin (5-fold, P=0.02) and total thrombin (9-fold, P=0.02) in treated cells (n=3).

Conclusions: In HuH-7 cells, miR-365a-3p knocks down TF protein levels and activity, and attenuates TF-dependent thrombin generation. Consistent with our previous data, this suggests an E_2-responsive decrease in miR-365a-3p regulates both TF protein expression and function and thrombin generation. The miR-365a-3p and TF interaction provides additional evidence supporting the existence of an E_2-driven miR regulatory network in thrombosis and haemostasis.

PB2026 | Platelet miRNoma and Variability in the Responsiveness to Low-dose Aspirin in Patients with Type 2 Diabetes mellitus

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Background: Type 2 diabetes mellitus (T2DM) is a major cardiovascular risk factor. Platelet activation plays a key role in atherothrombosis and low-dose aspirin, that induces a permanent inactivation of platelet cyclooxygenase (COX)-1, may be less effective in T2DM patients. The variable turnover rate of the drug target (platelet COX-1) represents the most convincing determinant of the interindividual variability in ASA response. Diabetes-associated metabolic abnormalities may affect PLT and circulating miRNA profiles.

Aims: To characterize the signature of intra-PLT and circulating miRNAs expression associated with a suboptimal ASA response in T2DM patients.

Methods: One hundred patients with type 2 diabetes mellitus (T2DM) in chronic treatment with ASA (100 mg/day), for cardiovascular prevention, were enrolled. Blood sampling was performed at 10 hours (T10) and 24 hours (T24) after a witnessed ASA administration. Patients were stratified in tertiles according to serum TXB_2 (ex vivo index of COX-1-dependent TXA2 production) slope. First and third tertiles (good responders *vs.* bad responders) were compared. A panel of 48 PLT-miRNAs was measured by real-time PCR. For circulating miRNAs, custom array cards were applied to assay the expression levels of 14 miRNAs in plasma.

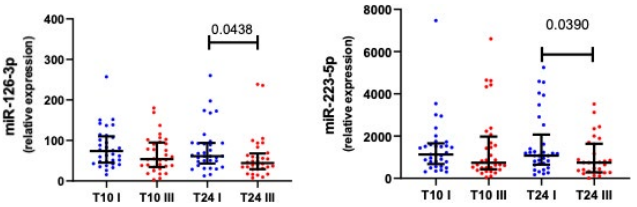

FIGURE 1

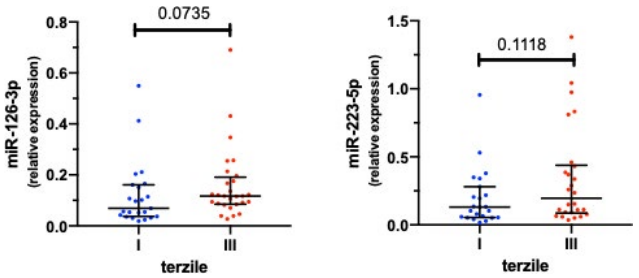

FIGURE 2

Results: Two miRNAs, miRNA-126-3p and -223-5p, were significantly lower in "bad responders" as compared to "good responders" at 24 hours after ASA administration (p=0.0438, p=0.0390 respectively; Fig. 1). *Vice versa*, circulating miRNA-126-3p and -223-5p at 24 hours were significantly higher in subjects in the third $sTXB_2$ slope tertile in comparison to the first tertile (p=0.0735, p=0.1118 respectively; FIG. 2).

Conclusions: Proteomic comparison of platelets proteins in bad *vs.* good responders can shed light on the possible proteins regulated by the two miRNAs, revealing the indirect molecular mechanisms behind COX-1 activity recovery. Circulating miRNA signature associated with accelerated COX-1 recovery may be tested as potential biomarker to predict the responsiveness to ASA treatment.

PB2027 | High Efficiency Unassisted Transfection of Platelets with Naked Double-stranded miRNAs Modulates Signal-activated Translation and Platelet Function

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Background: Anucleate platelets are highly enriched in microRNAs (miRNAs), 19-22 nucleotide non-coding RNAs which function as translational suppressors by multiple mechanisms collectively referred to as RNA inhibition (RNAi). The ability to over-express or antagonize miRNAs ectopically in platelets is of substantial value for both research and clinical applications. Transfection of miRNAs in *ex vivo* platelets has been achieved using coupling reagents such as liposome carriers which are standard reagents for transfection of nucleated cells; however, transfection efficiencies with these approaches have generally been low as reported by us and others, limiting their utility.

Aims: We sought novel approaches to improve transfection efficiencies of microRNAs (miRNAs) in platelets, and to apply these approaches to investigate roles of miRNAs in regulating signal-activated protein translation and functional effects.

Methods: We found that *ex vivo* human platelets support gymnosis - internalization of ectopic miRNAs following co-incubation in the absence of conventional transfection reagents or schemes - and subsequently incorporate transfected miRNA into ARGONAUTE2 (AGO2)-based RNA-induced silencing complexes (RISC).

Results: Thrombin/fibrinogen stimulation activated translation of miR-223-3p target SEPTIN2, which was suppressed by miR-223-3p transfection in an AGO2/RISC-dependent manner. Thrombin/fibrinogen-induced exosome and microvesicle generation was inhibited by miR-223-3p transfection, and this effect was reversed with a RISC inhibitor. Platelet gymnosis of naked miRNAs was mediated in part by endocytic pathways including clathrin-dependent and fluid-phase endocytosis and caveolae.

Conclusions: These results demonstrate the ability of *ex vivo* platelets to internalize ectopic miRNAs by unassisted transfection, and utilize them to modulate signal-activated translation and platelet function. Our results identify new roles for miR-223-3p in extracellular vesicle generation in stimulated platelets. High efficiency gymnotic transfection of miRNAs in *ex vivo* platelets may be a broadly useful tool for exploring molecular genetic regulation of platelet function.

PROTEASE ACTIVATED RECEPTORS

PB2029 | PAR1-Derived and PAR3-Derived Peptides and their Variants Synergistically Inhibit Human Macrophage NLRP3-Dependent Inflammasome Activity

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Background: Inflammasomes are intracellular multiprotein complexes of the innate immune system that can provide NLRP3-dependent activation of caspase-1 (casp-1) which enables release of proinflammatory cytokines, including interleukin (IL)-1β which is implicated in immunothrombosis. Activated protein C (APC) suppresses NLRP3-inflammasome activity in vivo, in part by signaling via protease activated receptor (PAR)1.

Aims: To determine the anti-inflammatory effects of APC and of PAR1-derived peptide (residues 47-66(P1-47)) and/or PAR3-derived peptide (residues 42-65(P3-42)) on phorbol myristate acetate-differentiated human monocytes that are stimulated to induce casp-1 activity and IL-1β, markers of inflammasome activity.

Methods: Monocytes (THP-1 cells) were differentiated into macrophages, then pretreated with APC or PAR1/PAR3 peptides prior to stimulation with LPS and ATP to induce casp-1 whose activity was measured using a luminescent substrate.

Results: APC, P1-47 or P3-42 each dose-dependently reduced NLRP3-dependent casp-1 activity and IL-1β release in differentiated, stimulated THP-1 cells. The P1-47 and P3-42 peptides at low concentrations where either peptide alone had little inhibitory activity synergistically inhibited generation of casp-1 and IL-1β. Studies showed that the following variations of PAR1/PAR3 peptides retained anti-inflammatory activity (e.g., reduction of casp-1 activity in stimulated THP-1 cells): shortened P1-47 variants with ≥8 amino acids; P1-47 variant with the N-terminal Asn47Gln replacement; or a shortened P3-42 of medium length. Moreover, combinations of low doses of a PAR1-derived variant peptide and a PAR3-derived variant peptide synergistically inhibited casp-1 activity generation.

Conclusions: APC or a non-canonical PAR1-agonist peptide or PAR3-agonist peptide are anti-inflammatory by reducing NLRP3-driven casp-1 activity in human THP-1 cells. Remarkably, combinations of low doses of P1-47 and P3-42 peptides, or of their variants, act synergistically to provide anti-inflammatory activity. The synergy observed for simultaneous agonism of PAR1 and PAR3 likely reflects the previously suggested existence of PAR1-PAR3 heterodimers. Our results suggest that combinations of PAR1- and PAR3-derived peptides have significant translational implications.

PB2030 | Thrombomodulin Makes the Difference - Novel Role of Protease-Activated Receptor 4 as Low-Dose Thrombin Receptor

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Background: Thrombin, the key clotting protease, acts on coagulation and inflammation via protease-activated receptors (PARs). PAR1 and PAR4 are major regulators of human platelet activation and vascular barrier integrity. Thrombin directly binds and cleaves PAR1 at very low nanomolar concentrations, while PAR4 lacks a thrombin binding site and therefore very high nanomolar concentrations of thrombin are required to cleave PAR4. We recently published that thrombomodulin, a co-factor expressed on both endothelial cells and leukocytes, improves PAR2 activation by thrombin, resulting in increased activation of inflammatory pathways. Thrombomodulin-dependent PAR4 activation was not addressed so far.

Aims: To test whether thrombomodulin recruitment of thrombin to the cell surface leads to a more efficient cleavage and activation of membrane-bound PAR4 compared to soluble thrombin.

Methods: To observe cleavage and activation of PAR4 by various thrombin concentrations, plasmids containing PAR4 tagged with an N-terminal alkaline phosphatase (AP) were transfected with a thrombomodulin plasmid in HEK 293T cells. The cleavage of the AP-PAR4 construct was detected by an established assay measuring activity of the cell bound and the released AP.

Results: We found that PAR4 was cleaved only at high thrombin concentrations of 30 nM in absence of thrombomodulin, whereas in presence of thrombomodulin PAR4 was significantly cleaved at low thrombin concentrations of 1 nM.

Conclusions: For the first time, we showed a switch of PAR4 from high- to low-dose thrombin receptor, which was caused by the thrombin binding to the endothelial receptor thrombomodulin. Whether the enhanced efficiency of PAR4 cleavage by thrombin in presence of thrombomodulin results in increased PAR4 activation will be studied by an *in vitro*NF-kB luciferase reporter system. The clinical relevance of PAR4 activation by low concentrations of thrombomodulin-bound thrombin will be tested on vascular barrier integrity as well as platelet activation.

PB2033 | The Protective Effects of PAR1-agonists - Thrombin, Activated Protein C and Peptide 9 in the Model of the Neuroinflammation *in vitro*

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Background: Mast cells (MC), neurons and astrocytes are the main participants in neuroinflammation, which is a characteristic feature of neurodegenerative disorders. The activation of PAR1 is the key point in the coupling of coagulation and neuroinflammation processes in the brain.

Aims: To investigate the participation of PAR1-agonists - thrombin, APC and its functional analogue, the new peptide 9 (AP9), an analogue of the “tethered ligand” released from PAR1 by APC - in the model of the neuroinflammation in vitro.

Methods: To create the model of neuroinflammation in vitro, MC, astrocytes and neurons were used. The toxic effect of activated MC on neurons or astrocytes and effects of proinflammatory factors on cells were estimated by using a morphological and biochemical analysis of survival. The intracellular calcium was measured by FURA-2AM. The change in the cytoskeleton was carried out by immunocytochemical staining.

Results: AP9 by PAR1 prevented the neuronal death at excitotoxicity, similar to APC. APC and AP9 did not change the [Ca2+]i in neurons, but AP9, not APC, decreased Ca-influx in MC. APC and AP9 significantly increased the percentage of neurons that restored the basal level of calcium after the excitotoxicity. Activation of cells by thrombin(50 nM), like as proinflammatory factors, leads to disorganization and re-distribution of the actin cytoskeleton, however, pre-incubation of MC with the AP9 and APC promotes actin ordering, stabilize cell membranes and reducing secretion similar to control. Pre-treatment of MC with the AP9 significantly reduced the level of IL-6 secretion by cells during co-cultivation of astrocytes and MC. Also, AP9 by PAR1 leads to decrease toxic effect of MC on neurons and a decrease in the proportion of apoptotic cells, like APC.

Conclusions: PAR1-agonists-AP9 and APC exert an anti-inflammatory and protective actions, in the model of neuroinflammation in vitro.

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STEM CELLS AND VASCULAR CELL GROWTH

PB2034 | Pituitary Hormones Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Prolactin (PRL) Modulate Endothelial Colony Forming Cells Angiogenic and Thrombotic Properties

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Background: Pituitary sexual hormones (SexHs) follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) have been described to stimulate mobilization, migration or proliferation of several human hematopoietic, stem or cancer cells. Endothelial colony forming cells (ECFCs) have been identified as the human postnatal vasculogenic cells involved in vascular repair and thrombotic disorders.

Aims: Our aim was to determine how FSH, LH and PRL could contribute to ECFC commitment and modulate their angiogenic properties.

Methods: We investigated effect of SexHs on angiogenic properties and prothrombotic in ECFCs derived from cord blood.

Results: We showed that FSH, LH and PRL at the dose of 100ng/mL did not modify ECFC commitment from mononuclear cells. They were all CD31+ CD45- CD90- with a strict endothelial phenotype. However, FSH, LH and PRL significantly increased ECFC migration, by Transwell chemotaxis assay and wound healing assays without affecting proliferation properties. We showed also that hormones gave more sprouts than control in a three-dimensional fibrin gel assays with ECFC seeded onto Cytodex® 3 microcarrier beads and embedded in fibrin gel containing SexHs. Moreover, we observed an increase in pro thrombotic properties in vitro by measuring thrombin generation time (TGT) of poor platelet plasma with ECFCs pretreated with SexHs during 72 hours. LH and PRL pretreatment of ECFCs increased total thrombin formation measured by endogenous thrombin potential (ETP) and maximal peak. This effect was associated with a decreased expression of the median fluorescence index of the endothelial protein C receptor treated with LH and PRL ECFC. The thrombomodulin expression was not affected.

Conclusions: This study provides new evidence that SexHs improves angiogenic potential of ECFCs and their procoagulant status by modulating EPCR. Our data also emphasize potential endothelial dysfunction associated with thrombosis recurrence induced by SexHs.

PB2035 | Thrombopoietin Rescues Megakaryopoiesis, Reduces Hemorrhage via Protecting Bone Marrow Endothelial Function of Chemotherapy-Treated Hematological Malignancies Patients

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Background: Bone marrow (BM) endothelial progenitor cells (EPCs) play a crucial role in regulating megakaryopoiesis. BM-EPCs are shown to be impaired in patients treated with chemotherapy. Our previous studies suggested that thrombopoietin (TPO) has a protective effect on hypoxia-induced apoptosis in human endothelial cells.

Aims: To explore whether TPO can protect BM-EPCs in chemotherapy patients and its underlying mechanism.

Methods: Firstly, CCK8 was used to explore the proliferative effect of BM-EPCs isolated from hematological patients treated with chemotherapy. Cells were divided into two groups: TPO-treated (experimental) group and TPO-free (control) group. BM-EPCs were identified by positively stained with CD34, CD309 and CD133. DiL-Ac-LDL uptake and FITC-UEA-I binding assay was performed to evaluate the amount of BM-EPCs; Secondly, the function of BM-EPCs in two groups were evaluated and compared by using tube-formation and migration experiments respectively. Lastly, BM-EPCs of two different groups were co-cultured with human megakaryocytes, and the proliferation of megakaryocytes was detected by flow cytometry.

Results: CCK8 results showed that TPO enhanced the proliferation of BM-EPCs as compared to the control group, and the optimal concentration is 100ng/ml. Flow cytometry indicated that TPO-treated cells had high expression of CD34/CD133/CD309. Double immunofluorescence assay indicated that the number of BM-EPCs in the experimental group was significantly higher than that in the control group (n=10; p=0.03); The second part data showed that the tube-formation (n=10; p=0.02) and migration (n=10; p= 0.01) abilities of BM-EPCs in TPO-treated group were stronger than that of control group. The co-cultured experiment indicated that BM-EPCs in the

experimental group significantly enhanced the human megakaryocytes proliferation(n=6; p=0.04).

Conclusions: Our data indicates that TPO may rescue megakaryopoiesis and reduce hemorrhage via protecting the function of BM-EPCs.

PB2036 | Investigation of a Significant Factor to Improve an Establishment of Endothelial Colony Forming Cell

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Background: Endothelial colony forming cell (ECFC) is a primary endothelial cell (EC)-like cell derived from peripheral blood mononuclear cells (PBMCs). Since ECFCs express several EC markers and have angiogenetic function, ECFC is used as an in vitro model mimicking EC environment in several studies of vascular disease or von Willebrand disease (VWD). However, a current method culturing PBMCs-derived ECFCs is not efficient, and blood samples from some donors do not establish ECFCs.

Aims: To identify a factors associating an establishment of PBMCs-derived ECFC.

Methods: We included 23 Japanese cases including 12 cases of VWD (age, 15-78 years-old Japanese people; sex, 11 males and 12 females), and examined several donor-derived factors (age, sex, blood type, and VWD) and culture condition (density of seeding cells). Data were statistically analyzed with Fisher's exact test.

Results: We established ECFC in 15 cases of 23 cases (65.8%). The establishment rate of ECFC was not significantly different in comparisons among several factors, age, sex, VWD, and the seeding cell density.

Conclusions: In this study, an efficient factor relevant with PBMCs-derived ECFC was not found out. Further investigations are required to develop the highly efficient method of ECFC culture.

PB2037 | Transplantation of Fetal Liver Cells into Newborn Hemophilic Mice for Hemophilia A Cell Therapy

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Background: Hemophilia A cell therapy approaches in pediatric individuals require suitable FVIII-producing cell populations presenting stable engraftment potential. We previously showed that adult-derived FVIII-producing cells, i.e. liver sinusoidal endothelial cells (LSEC) and hematopoietic stem cells (HSC), can be used for the treatment of adult hemophilia A (HA) mice. However, after transplantation in busulfan-conditioned newborn mice, adult LSEC/HSC cannot efficiently engraft, while murine fetal liver (FL) hemato/vascular cells from day 11-13 of gestation (E11-E13) strongly reconstitute the hematopoietic compartment and showed multiorgan endothelial reconstitution potential while secreting FVIII.

Aims: To investigate the engraftment of FL cells in newborn HA mice as a new strategy for the treatment of paediatric HA patients.

Methods: We transplanted FL cells from E11-E13 GFP+ mice into newborn HA mice pre-treated with busulfan (FLE11-E13+BU). Control groups received FLE11-E13 GFP+ cells without busulfan pretreatment (FLE11-E13noBU) or PBS±BU. The engraftment level as well as the FVIII production and activity was assessed in recipients starting from 4 weeks and followed-up for 18 months after transplantation. Moreover, we evaluated the presence of anti-FVIII antibodies/inhibitors in plasma of transplanted mice.

Results: In FLE11-E13+BU group we observed >60% engraftment in peripheral blood with concomitant FVIII activity (up to 16%) which remained stable up to 18 months after transplantation, while engraftment and FVIII activity were lower in absence of preconditioning. Additionally, FLE11-E13+BU group showed the presence of up to 4% of GFP+ LSEC 18 months after transplantation, demonstrating that transplanted FL cells in BU-conditioned newborn mice contributed in reconstituting the hemato/vascular compartment. Mice of FLE11-E13+BU and E13noBU groups did not develop anti-FVIII antibodies, not even after FVIII immunization.

Conclusions: FL cells transplantation may provide a novel and highly promising preclinical model for HA treatment, paving the way for studies aiming at deriving long-term reconstituting “fetal-like” hemato/vascular progenitors from other sources (e.g. iPSC).

VENOUS THROMBOEMBOLISM AND CARDIOEMBOLISM

ARTIFICIAL VALVES

PB2039 | Standard Versus Higher Intensity Anticoagulation for Patients with Mechanical Aortic Valve Replacement and Additional Risk Factors for Thromboembolism: A Multi-center Registry Analysis

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Background: Current guidelines recommend targeting an INR of 3.0 (2.5-3.5) in warfarin-treated patients with mechanical aortic valve replacement (AVR) and additional risk factors for thromboembolic events (atrial fibrillation [AF], prior thromboembolism, left ventricular dysfunction, hypercoagulable conditions). A target INR of 2.5 (2.0-3.0) is recommended for mechanical AVR patients without thromboembolic risk factors. However, available literature supporting the higher intensity INR goal in patients with thromboembolic risk factors is lacking.
Aims: Evaluate the efficacy and safety of standard (2.5) versus higher (3.0) intensity anticoagulation in this patient population.
Methods: The six-center Michigan Anticoagulation Quality Improvement Initiative database was used to identify warfarin-treated patients with mechanical aortic valve and at least one additional risk factor. Patients enrolled into the database between November 2009 and March 2019 and followed through May 2019 were included in the analysis. Patients were classified into two groups based on INR goal: standard intensity (INR goal 2.5) versus high intensity (INR goal 3.0). Patients with non-bileaflet AVR or mitral valve replacement were excluded. Regression analysis was used to control for differences in baseline characteristics. The primary outcome was a composite of thromboembolic events, bleeding, or all-cause death.
Results: 373 patients with a mechanical AVR were screened of which 175 in the standard intensity group and 39 in the high intensity group met inclusion. Standard intensity patients were generally older and more likely to be on aspirin. AF was the most common additional risk factor for inclusion, and patients had a mean HAS-BLED score of 2.9 and 2.4 in the standard and high intensity groups, respectively. The primary composite outcome occurred less frequently in the standard intensity group (p=0.002).
Conclusions: Standard intensity anticoagulation resulted in a bleeding reduction while maintaining efficacy compared to higher intensity anticoagulation. These results challenge current guideline recommendations for management of mechanical AVR in patients with additional risk factors.

ATRIAL FIBRILLATION

PB2040 | Clinical Case: Reversal of Dabigatran Levels in Patients with Renal Insufficiency that Have Required the use of Idarucizumab

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Background: Dabigatran is the only DOA with a commercial reversor agent, which is Idarucizumab. It allows a quick and safe reversal of the anticoagulant effect. In the REVERSE-AD study, where its effect and safety were studied, 1.8% of patients required a second dose, all of them with decreased GFR. 51 of 91 patients (46.0%) with severe renal impairment had re-elevation of dabigatran levels to 20 ng/ml at either 12 or 24 h.
Potential explanations for re-elevation include delayed redistribution of the drug resulting in saturation of idarucizumab in patients with higher baseline levels of dabigatran, or progressive degradation

TABLE 1 Outcomes in standard vs high intensity INR target ranges

Outcomes (# per 100 pt-yr)	Standard Intensity N=175	High Intensity N=39	p-value	Event rate ratio (95% CI)
Primary Outcome	17.1	23.4	0.002	0.73 (0.60-0.89)
Ischemic/Embolic Stroke	1.0	1.1	1	0.91 (0.35-2.36)
ISTH Major Bleed	3.9	6.4	0.02	0.61 (0.40-0.92)
ISTH Clinically-Relevant Non-Major Bleed	8.3	8.5	0.94	0.98 (0.72-1.34)
Hospitalization (Any)	21.5	21.3	0.96	1.0 (0.83-1.23)
Death	2.9	3.2	0.8	0.91 (0.53-1.55)
RBC transfusion	3.9	7.4	0.001	0.53 (0.35-0.79)
Vitamin K administration	3.4	7.4	<0.001	0.46 (0.30-0.70)

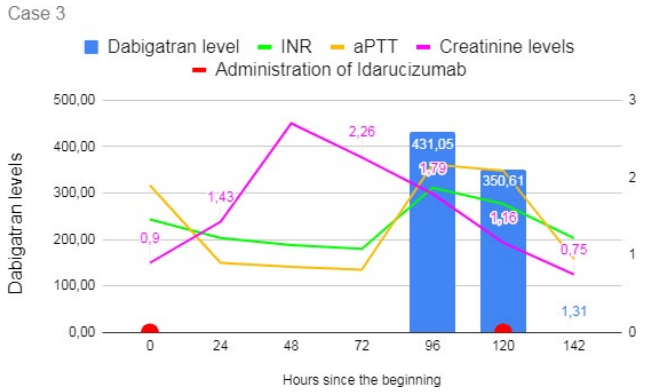

FIGURE 1 Case 3

of the dabigatran-idarucizumab complex in patients with impaired renal function, with corresponding release of dabigatran.

Aims: This study aims to analyze the relationship between dabigatran levels and acute renal failure with the need for a second dose of reversing agent.

Methods: We present the cases of three patients from our center with dabigatran treatment for atrial fibrillation that required a reversal of the anticoagulant effect. We track the evolution of their renal function, dabigatran levels and hemostatic parameters in relation of the administration of Idarucizumab. (Example: Case 3)

Results: In all the cases, acute renal insufficiency was observed and the levels of dabigatran reappeared after a successful initial reversion. All of them needed a new reversal dose for bleeding persistence.

Conclusions: Dabigatran allows a quick and safe reversal of the anticoagulant effect. But these cases alert us of the recurrence of levels of dabigatran more than 12 hours after the administration of Idarucizumab (5 days after in the last case) in patients with severe renal impairment and initial levels of Dabigatran >1000 (ng/ml). Therefore, more studies are needed to assess the relationship of acute renal insufficiency in the accumulation of dabigatran and its role in the reversal effect with Idarucizumab which its physiopathology isn't clear.

PB2041 | Reduced Bleeding After an Intervention to Limit Excess Aspirin Use Among Patients on Chronic Warfarin

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Background: For some warfarin-treated patients, the addition of aspirin (ASA) significantly increases bleeding without reducing thrombosis. We implemented a nurse/pharmacist-lead quality improvement intervention aimed at reducing inappropriate ASA use.

Aims: To assess changes in inappropriate ASA use, bleeding, and thrombosis event rates.

Methods: Within the six-center Michigan Anticoagulation Quality Improvement Initiative, we implemented an anticoagulation-clinic based quality improvement intervention that identified warfarin-treated patients with atrial fibrillation (Afib) or venous thromboembolism (VTE) without a clear indication for concomitant ASA (eg. coronary artery disease, mechanical heart valve). If the indication for adding ASA was unclear, the patient's care provider was contacted to advise if ongoing ASA+warfarin was recommended. ASA was discontinued if suggested by their managing doctor. Data was abstracted by trained abstractors, using predefined forms, and verified through chart audits. The intervention was implemented between Q4 2017 and Q2 2018. In December 2019, the rates of inappropriate ASA use, bleeding, and thrombotic outcomes were compared pre- and post-intervention using Poisson Regression with robust standard error. Site specific definitions of inappropriate ASA use and implementation dates were used.

TABLE 1 CASE 3

Hours since the beginning	Dabigatran levels (ng/ml)	INR	aPTT	Creatinine levels
0	-	1.46	1.9	0.9
24	-	1.22	0.9	1.43
48	-	1.13	0.85	2.7
72	-	1.08	0.81	2.26
96	431.05	1.87	2.17	1.79
120	350.61	1.66	2.09	1.16
140	1.31	1.22	0.95	0.75

TABLE 1 Event rates of patients on warfarin for AFib and/or VTE without an indication for ASA use pre and post intervention

	Pre-intervention* N=5,980	Post-intervention* N=1,634	P value†
Aspirin n (%)‡	1,541 (25.8)	323 (19.8)	<0.01
Patients in both groups n (%)	1,036 (17.3)	1,036 (63.4)	–
Event rate (95% CI) # of events/100 patient-years			
Any bleeding	26.2 (25.1 - 27.3)	17.1 (15.1 - 19.3)	<0.01
Major bleeding	3.9 (3.5 - 4.3)	3.2 (2.4 - 4.3)	0.023
CRNMB	8.5 (7.9 - 9.1)	5.2 (4.1 - 6.5)	<0.01
Admission for bleeding	6.3 (5.7 - 6.8)	4.5 (3.5 - 5.7)	<0.01
Thrombosis¶	2.4 (2.0 - 2.7)	2.0 (1.4 - 2.9)	0.76

*The pre-intervention group includes patients that were no longer followed by the Michigan Anticoagulation Quality Improvement Initiative prior to the intervention. The pre and post intervention groups were not mutually exclusive. Indications for ASA use were assessed at enrollment for the pre-intervention group and at the first follow-up visit post-intervention for the post-intervention group. †Comparisons of event rates are adjusted for ASA indication and HAS-BLED score/CHA2DS2-VASc score, as assessed for the pre-intervention group at enrollment and the post-intervention group at the time of the intervention or, if enrolled post-intervention, at the time of enrollment. ‡Aspirin use was assessed at enrollment for the pre-intervention group. Aspirin use for the post-intervention group was assessed at the first follow-up visit post-intervention. ¶Thrombosis includes any new ischemic or embolic stroke, transient ischemic attack, pulmonary embolism, deep vein thrombosis, intracardiac thrombus, or other/unknown clot. Abbreviations: ASA, aspirin; CRNMB, clinically relevant non-major bleeding.

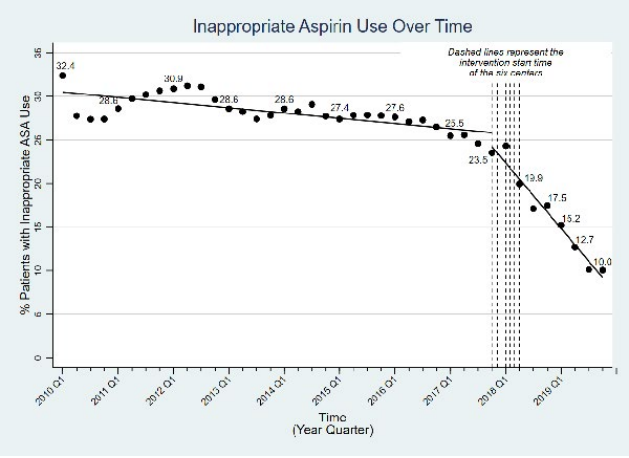

FIGURE 1 Inappropriate aspirin use by quarter pre- and post- intervention

Results: A total of 5,980 and 1,634 patients with AFib and/or VTE without an indication for ASA use were identified during the pre- and post-implementation periods, respectively. Inappropriate ASA use was reduced by over 50% from pre- to post-implementation (Figure). Patients were followed an average of 17.6 months pre-intervention and 11.3 months post-intervention. The rate of bleeding, major bleeding, emergency room visits, and hospitalizations related to bleeding were all significantly lower in the post- vs. pre-intervention period (Table). We did not observe a difference in thrombotic outcomes but data on myocardial infarction may not have been well captured.

TABLE 2 Event rates of patients on warfarin for AFib and/or VTE without an indication for ASA use pre and post intervention

	Pre-intervention* N=5,980	Post-intervention* N=1,634	P value†
Aspirin n (%)‡	1,541 (25.8)	323 (19.8)	<0.01
Patients in both groups n (%)	1,036 (17.3)	1,036 (63.4)	–
Event rate (95% CI) # of events/100 patient-years			
Any bleeding	26.2 (25.1 - 27.3)	17.1 (15.1 - 19.3)	<0.01
Major bleeding	3.9 (3.5 - 4.3)	3.2 (2.4 - 4.3)	0.023
CRNMB	8.5 (7.9 - 9.1)	5.2 (4.1 - 6.5)	<0.01
Admission for bleeding	6.3 (5.7 - 6.8)	4.5 (3.5 - 5.7)	<0.01
Thrombosis¶	2.4 (2.0 - 2.7)	2.0 (1.4 - 2.9)	0.76

*The pre-intervention group includes patients that were no longer followed by the Michigan Anticoagulation Quality Improvement Initiative prior to the intervention. The pre and post intervention groups were not mutually exclusive. Indications for ASA use were assessed at enrollment for the pre-intervention group and at the first follow-up visit post-intervention for the post-intervention group. †Comparisons of event rates are adjusted for ASA indication and HAS-BLED score/CHA2DS2-VASc score, as assessed for the pre-intervention group at enrollment and the post-intervention group at the time of the intervention or, if enrolled post-intervention, at the time of enrollment. ‡Aspirin use was assessed at enrollment for the pre-intervention group. Aspirin use for the post-intervention group was assessed at the first follow-up visit post-intervention. ¶Thrombosis includes any new ischemic or embolic stroke, transient ischemic attack, pulmonary embolism, deep vein thrombosis, intracardiac thrombus, or other/unknown clot. Abbreviations: ASA, aspirin; CRNMB, clinically relevant non-major bleeding.

Conclusions: Our multi-center quality improvement intervention was associated with a significant reduction in inappropriate ASA use and lower rates of bleeding among patients anticoagulated with warfarin for AFib and/or VTE.

PB2042 | Relationship between TTR and Daily Vitamin K Intake in Patients Taking Warfarin: The Difference among VKORC1 Genotype

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Background: The restriction of oral vitamin K (VK) intake is said to be essential for patients with warfarin anticoagulation. However, until now, the ideal daily intake of VK for each patient was not well established.

Aims: In this study, we evaluated the correlation between warfarin control and daily VK intake from the perspective of the VK epoxide reductase complex subunit 1 (VKORC1) genotype.

Methods: We enrolled 84 patients (29 females, average age 75.6) taking warfarin for more than one year. The serum VK concentration and VKORC1 genotype were evaluated with informed consent, and the original questionnaire about the average daily food menu was contrived to estimate the daily VK intake. The baseline characteristics of each patient were also extracted and analyzed.

Results: The VKORC1 genotype AA and AG were found in 81% and 19% respectively. None of our patients had GG genotype. All patients with AG took daily VK less than 250μg and the daily VK intake among AA patients distributed in a wide range (50-600μg). Both VK concentration and average warfarin dosage in patients with AG were significantly higher than those with AA ($p<0.001$). Whereas there existed a significant correlation between VK concentration and total VK intake in patients with AG ($p<0.001$), there was no correlation in patients with AA. Also, we found a significant correlation between VK intake and average warfarin dosage in patients with AG, no correlation was found in patients with AA. In spite of these results, the difference in average TTR in both genotypes was not significant.

Conclusions: The influence of daily oral VK intake for warfarin treatment was recognized only among patients with VKORC1 AG genotype. For patients with AG, the daily intake of VK up to 250μg is acceptable, furthermore, a strict restriction of VK may not need among those with AA.

PB2045 | Persistence to Direct Oral Anticoagulants among Non-Valvular Atrial Fibrillation Patients in the Netherlands: A Nationwide Cohort Study

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Background: Long-term oral anticoagulation is recommended in most atrial fibrillation (AF) patients to prevent thromboembolism. Direct oral anticoagulants (DOACs) are replacing vitamin K antagonists (VKAs) to be the main therapeutic option in AF due to their attractive pharmacokinetic profile, well-established efficacy and safety. However, poor persistence to DOACs remains a concern due to lack of regular control visits.

Aims: To assess the persistence pattern to DOACs and investigate predictive factors associated with non-persistence among non-valvular AF patients in the Netherlands.

Methods: The study used Dutch national statistics from January 1, 2013, to September 30 2017 for all non-valvular AF patients who started to use DOACs from 1 January 2013 onwards. Non-persistence was defined as a 100-day gap according to the last prescription during the follow-up, taking mortality into account and allowing for a switch to VKA. The cumulative incidence of persistence was estimated by Kaplan-Meier analysis. Risk factors for non-persistence were explored using Cox regression adjusted for age and sex.

Results: 70574 incident DOACs users with AF were included, among which 50741 (72%) had a baseline CHA_2DS_2-VASc score ≥2 and 20413 (29%) were switched from VKAs. The cumulative incidence of persistence to OAC was 86.8% (95%CI 86.5-87.1%), 80.5% (95%CI 80.2-80.9%), 74.6% (95%CI 74.1-75.1%), and 68.2% (95%CI 67.5-69.0%) at 1, 2, 3, and 4 years after the first DOACs prescription, respectively. Amongst 62 tested variables, 29 were associated with non-persistence, of which female sex, age 65-74 years, permanent AF, thromboembolic stroke history, BMI > 25 kg/m^2, baseline CHA_2DS_2-VASc score ≥2 and baseline HAS-BLED score ≥3 were most pronounced to reduce the risk of non-persistence (Table 1).

Conclusions: In this population based study, non-persistence to OAC was 20% after 2 years in non-valvular AF patients who initiated with DOAC. The many baseline characteristics we identified can be helpful to identify patients who tend to be non-persistent.

TABLE 1 Risk of being non-persistent to OAC according to clinical characteristics

	Observation years	Event no.	Adjusted HR (95%CI)	Adjusted HR (95%CI); restricted to CHA2DS2-VASc score ≥2 and first year of DOAC use
Female sex	41414	4264	1 (reference)	1 (reference)
Male sex	56254	7476	1.12 (1.08-1.17)	1.07 (1.01-1.14)
Age 55-64 years	19198	2868	1.92 (1.82-2.02)	1.25 (1.11-1.41)
Age 65-74 years	39298	3013	1 (reference)	1 (reference)
Age 75-84 years	25405	2356	1.17 (1.10-1.23)	1.12 (1.10-1.28)
Age ≥ 85 years	6775	1115	1.90 (1.77-2.04)	1.98 (1.82-2.16)
CHA2DS2-VASc score ≥ 2	67406	6255	1 (reference)	Not applicable
CHA2DS2-VASc score 1	20755	2581	1.25 (1.18-1.31)	Not applicable
CHA2DS2-VASc score 0	9507	2904	2.68 (2.52-2.86)	Not applicable
HAS-BLED score ≥3	16384	1429	1 (reference)	Not applicable
HAS-BLED score 0-2	81284	10311	1.16 (1.09-1.23)	Not applicable
Stroke history present	8839	735	1 (reference)	1 (reference)
Stroke history absent	88829	11005	1.34 (1.24-1.45)	1.23 (1.12-1.36)
Permanent AF	3453	518	1 (reference)	1 (reference)
Paroxysmal AF	9602	1645	1.19 (1.08-1.32)	1.08 (0.92-1.27)
BMI 18.5-20 (available for subpopulation)	196	23	1 (reference)	1 (reference)
BMI 25-30 (available for subpopulation)	4677	411	0.65 (0.43-1.00)	0.62 (0.40-0.97)
BMI ≥30 (available for subpopulation)	2240	162	0.51 (0.33-0.80)	0.60 (0.38-0.96)

PB2046 | Non-Valvular Atrial Fibrillation Patients Anticoagulated with Rivaroxaban Compared with Warfarin Exhibit Attenuated Plasma Platelet Activation Markers and Pro-Inflammatory Extracellular Vesicle Proteomic Signatures

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Background: Rivaroxaban is a direct oral anticoagulant with similar efficacy to vitamin K antagonists in non-valvular atrial fibrillation (NVAF). Recent studies, including the landmark COMPASS trial, demonstrate that Rivaroxaban possesses cardiovascular protective and anti-inflammatory properties, however, underlying mechanisms remain poorly understood. The effects of Rivaroxaban on circulating pro-inflammatory extracellular vesicles (EVs) are currently unknown. We hypothesize that EV profiles differ in NVAF patients treated with Rivaroxaban compared to those treated with warfarin.

Aims:

1. To characterize the effects of Rivaroxaban on circulating EV concentration and size in NVAF patients.
2. To perform comparative proteomic analysis of EVs from NVAF patients treated with Rivaroxaban and warfarin, respectively.

Methods: Plasma from Rivaroxaban (n=8) or matched warfarin (n=13) treated NVAF patients was obtained with informed consent. Vesicle-distribution profiles were acquired using Nanoparticle Tracking Analysis and flow cytometry. Label-free quantitative proteomic analysis was also performed, and platelet activation was assessed.

Results: We observed an overall reduction in circulating EV levels in Rivaroxaban-treated patients compared to warfarin (2.4-fold reduction; p=0.005). Quantitative proteomic analysis of enriched EVs revealed 103 differential proteins between the two patient cohorts. Strikingly, EVs from Rivaroxaban-treated patients exhibited a profound decrease in highly pro-inflammatory protein expression and complement factors, together with increased expression of negative regulators of inflammatory pathways. Moreover, we also found a reduction in circulating levels of platelet activation markers in

Rivaroxaban-treated patients which negatively correlated with the patient's time on treatment.

Conclusions: Collectively, these data demonstrate that NVAF patients anticoagulated with Rivaroxaban (compared with warfarin) exhibit both a reduced pro-inflammatory state and evidence of reduced platelet activation. These findings are of translational relevance towards characterising the cardioprotective and anti-inflammatory mechanisms associated with Rivaroxaban therapy.

PB2047 | Non-Persistence to Direct Oral Anticoagulants and Risk of Thromboembolic Stroke among Patients with Non-Valvular Atrial Fibrillation: A Dutch Nationwide Cohort Study

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Background: Direct oral anticoagulants (DOACs) are used for thromboembolic stroke prevention in patients with non-valvular atrial fibrillation (AF), but their effectiveness is expected to depend on drug persistence.

Aims: To study the association between non-persistence to DOACs and thromboembolic stroke risk among AF patients.

Methods: With nationwide statistics we identified and followed all AF patients who started DOAC treatment between January 2013-September 2017. Non-persistence was defined according to the last DOAC prescription during follow-up, using a 100-day gap and allowing a switch to vitamin K antagonists. With time-dependent Cox modelling, we estimated the risk of thromboembolic stroke/death due to ischemic stroke (composite outcome) associated with non-persistence. Adjustments were made for age, sex, VKA history, stroke history, AF type, and household income. In addition, a stratified analysis based on CHA2DS2-VASc >2 was performed. According to guidelines, AF patients with a score of < 2 who are converted to sinus rhythm may discontinue anticoagulation contrary to those with high CHA2DS2-VASc scores.

Results: 70574 incident DOAC users with AF were included; mean age was 71.4 years, 57%(n=40318) were men and 49%(n=34272) had a CHA2DS2-VASc >2. The cumulative incidence of non-persistence was 42% at 4 years follow-up and the overall incidence of the composite outcome was 1.08% (95%CI,1.01-1.14) per year. The adjusted hazard ratio [AHR] for the composite outcome was 1.69 (95%CI,1.31-2.18) for those who were non-persistent to DOAC (Table). Subgroup analyses suggested that those with CHA2DS2-VASc 0-2 who were non-persistent had a lower risk of the composite outcome (HR 0.71; 95%CI,0.48-1.04) compared to persistent patients. However, patients with CHA2DS2-VASc >2 who were non-persistent to DOAC had high risks of the composite outcome, compared to persistent patients (HR 2.51; 95%CI,1.87-3.35 and 2.46;1.76-3.42, for CHA2DS2-VASc score 3-4 and CHA2DS2-VASc score ≥5, respectively).

Conclusions: Non-persistence to DOAC was associated with an increased risk of thromboembolic stroke in Dutch non-valvular AF patients.

PB2048 | Long-term Mortality and Functional Status in Older Adults with Atrial Fibrillation After Stroke

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Background: Atrial fibrillation (AF) disproportionately affects older adults and increases risk of stroke. Short-term consequences of stroke are well-described, but less is known about how stroke affects long-term outcomes in AF patients who have other risk factors for death and functional decline.

Aims: Determine if, and to what degree, stroke affects mortality and functional disability over time in older AF patients.

Methods: We used data from 1992-2014 from the Health and Retirement Study, a longitudinal, nationally representative survey of Americans linked to Medicare claims. Subjects were ≥ 65 years with AF who did not require help with 6 activities of daily living (ADLs) (e.g. dressing, bathing). We matched incident stroke patients with 5 nonstroke patients using time from AF diagnosis. Outcomes were time to death and dependence in any of 6 ADLs assessed at 2-year intervals. We used a competing risks survival analysis framework.

Results: We identified 184 subjects with stroke and 935 controls. Two years after stroke, 52% (95% CI 45-60%) of stroke patients had died versus 24% (95% CI 21-26%) of controls. By 4 years, 72% (95% CI 64-79%) of stroke patients had died versus 40% (95% CI 37-44%)

TABLE 1 Non-persistence to DOAC in relation to the composite outcome (ischemic stroke and ischemic stroke-related mortality)

	Observation years	No. of events	adjusted HR (95%CI) (age and sex)	adjusted HR (95%CI) (all variables)	HR (95%CI); CHA2DS2-VASc 0-2	HR (95%CI); CHA2DS2-VASc 3-4	HR (95%CI); CHA2DS2-VASc ≥ 5
Persistent	96789	1037	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Non-persistent	10982	123	1.64 (1.36-1.98)	1.69 (1.31-2.18)	0.71 (0.48-1.04)	2.51 (1.87-3.35)	2.46 (1.76-3.42)

of controls, and by 8 years, 91% (95% CI 85-96%) versus 64% (95% CI 60-67%). Cumulative incidence of ADL dependence at 2 years was 52% (95% CI 45-59%) for stroke patients versus 23% (95% CI 21-27%) for controls, at 4 years 66% (95% CI 59-73%) versus 40% (95% CI 36-43%) and at 8 years 78% (95% CI 71-83%) versus 59% (95% CI 56-63%).

Conclusions: In older AF patients, half of stroke patients died after 2 years, double the risk of those without stroke. Stroke was associated with significantly greater loss of functional independence over time. These results inform decision-making and patient counseling on anticoagulation and advance care planning.

PB2049 | Characterization of VKA Control in Patients with Thrombotic and Major Hemorrhagic Events in Anticoagulation Hematology Units: Results from the EVENTHO Study

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Background: Despite the high risk of hemorrhagic and thrombotic complications in the prevention of Atrial Fibrillation (AF) and Venous Thromboembolism (VTE) in vitamin K antagonist (VKA) treated-patients, VKA anticoagulation is subject to major limitations, especially its narrow therapeutic range (given as the international normalized ratio, INR).

Aims: Characterize the profile of VKA-treated patients with severe hemorrhagic events (SHE) and thrombotic events (TE) and their relationship with time in therapeutic range (TTR) of INR prior to the event.

Methods: Post-authorization, observational, retrospective, multicenter study conducted in 22 anticoagulation Spanish Units. The study included patients ≥18 years, with AF and/or VTE, who have suffered SHE (n=441) or TE (n=214) during the whole 2016 and VKA-treated (acenocumarol (97.7% of patients) or warfarin). TTR was calculated according to the Rosendaal method based on INR values of the previous 6 months.

Results: The study assessed 655 patients (median [range] 82.2 [32.4-96.2] years; 51.8% men; mean [95% confidence interval, CI] CHA_2DS_2-VASc: 4.31 [4.19_4.43] and HASBLED: 2.17 [2.07_2.26]). The mean (95%CI) last value of the INR and INR at the time of admission was significantly higher in SHE patients than in TE patients (2.63 [2.51_2.74] and 3.90 [3.50_4.31] versus 2.40 [2.25_2.55] and 2.22 [2.02_2.42], respectively) (Table_1). Inadequate INR control (TTR< 65%) was higher in SHE patients (57.0%) than in TE patients (47.9%) and VKA treatment was maintained in 62.9% and 49.8% patients after the event, respectively.

Conclusions: INR control was in range at the time of SHE or TE, nevertheless TTR was low, indicating a poor VKA anticoagulation control in more than half of those patients. Despite this low TTR, VKA was restarted in at least the half of these patients after the event. These results highlight the need for better and safer therapeutic alternatives in AF and VTE patients with low TTR instead of restarting VKA treatment.

PB2050 | Ratio of Inhibited Thrombin Generation Based on Dilute Prothrombin Time May Predict the Major Bleeding or Thrombotic Tendency in NVAF Patients Treated with Anti-Xa DOAC

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Background: During treatment with direct oral anticoagulants (DOAC) in nonvalvular atrial fibrillation (NVAF) patients, measuring

TABLE 1 Characterization of thrombotic and hemorrhagic events and anticoagulation control grade

	Thrombotic event (n=214)	Severe hemorrhagic event (n=441)	P-value*
Last INR value (n)	209	430	
Number missing	5	11	
Mean (95% CI)	2.40 (2.25, 2.55)	2.63 (2.51, 2.74)	0.0070
INR at admission (n)	211	431	
Number missing	3	10	
Mean (95% CI)	2.22 (2.02, 2.42)	3.90 (3.50, 4.31)	< 0.0001
TTR control (>65%)**	209	430	
No	100 (47.9%)	245 (57.0%)	0.0298¥

(*) Severe hemorrhagic event versus thrombotic event. All values reported as P-value Wilcoxon unless otherwise indicated. (**) No missing patients. (¥) P-value Chi-square. CI, confidence interval; INR, international normalized ratio; n, number; TTR, time in the therapeutic range.

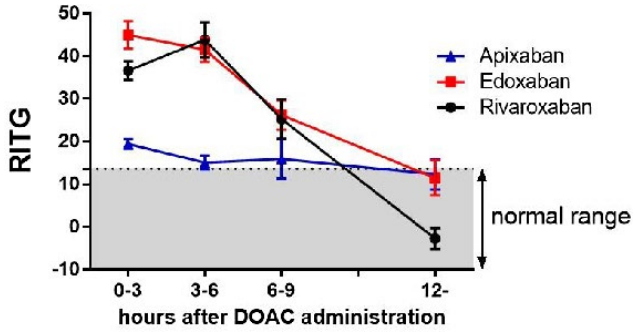

FIGURE 1 Time corse of RITG (mean ± SE) among three DOAC

their anticoagulant effects may be needed in certain circumstances to prevent major bleeding and/or refractory thrombosis.

Aims: The aim of this study is to investigate the association between major bleeding or thrombotic tendency and the laboratory tests including the clinical parameters, coagulant markers, the drug concentrations and Ratio of Inhibited Thrombin Generation (RITG) calculated using dilute prothrombin time (dPT).

Methods: Citrated plasma samples were obtained from the patients with receiving rivaroxaban (n=514), edoxaban (n=443) and apixaban (n=1081) at three sites in Japan between January 2012 and December 2019. Clinical characteristics were obtained from medical records retrospectively. The plasma concentrations of DOAC were measured by anti-Xa assay, specially calibrated for each drug. First, we compared the parameters including drug concentrations and RITG at the last visit and events between the patients with major bleeding and those without any adverse clinical event. Second, we compared RITG between the patients with or without positivity for coagulant markers such as D-dimer and fibrin monomer complex.

Results: A total of 31 major bleeding occurred, 8 in rivaroxaban, 14 in edoxaban and 9 in apixaban. In those patients, PT was longer ($p < 0.001$) and RITG was higher ($p=0.03$) compared to control group. RITG showed unique patterns of time dependent change among each DOAC (Figure1). In apixaban, RITG levels were decreasing at a slower rate over time compared to its drug concentrations and kept to some extent. Temporal RITG during treatment were higher in patients with major bleeding compared to control group in apixaban, but not in other drugs. RITG below normal range indicated higher positivity for D-dimer in apixaban.

Conclusions: RITG, calculated using dPT may indicate the tendencies for major bleeding or thrombosis by reflecting their anticoagulant effects in NVAF patients treated with DOAC, especially apixaban.

PB2051 | Differences in Treatment and Outcomes in Recently Diagnosed Atrial Fibrillation between the Netherlands and Belgium: Results from the GARFIELD-AF Registry

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Background: The uptake rate of non-vitamin K oral anticoagulants (NOAC) for the treatment of non-valvular atrial fibrillation (AF) differed greatly between the Netherlands (NL) and Belgium (BE).

Aims: To explore the effect of these differences on thromboembolism (TE) and bleeding.

Methods: Dutch and Belgian data from the worldwide GARFIELD-AF registry was used. Patients with new-onset AF and at least one investigator-determined risk factor for stroke were included

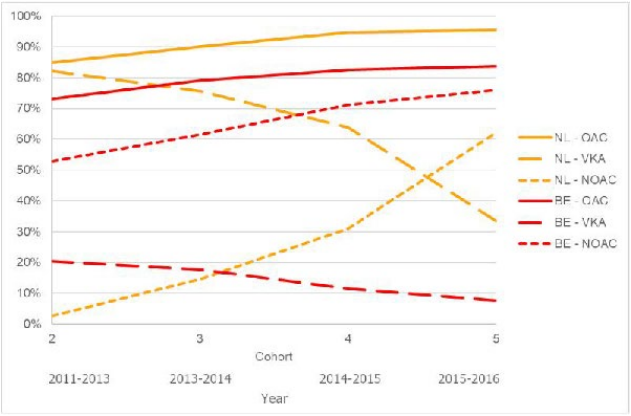

FIGURE 1 Changes in oral anticoagulation (OAC) in NL and BE

between 2010-2016. Event rates from two years of follow-up were used.

Results: In total, 1186 and 1705 patients were included in NL and BE, respectively. Female sex (42.3% vs 42.2%), mean age (70.7 vs 71.3 years), CHA_2DS_2-VASc (3.1 vs 3.1) and HAS-BLED score (1.4 vs 1.5) were comparable between NL and BE, respectively. In NL and BE, mean INR was 2.9 (±1.0) and 2.4 (±1.0), respectively. Overall, at diagnosis in NL vs BE 72.1% vs 14.6% received vitamin K antagonists (VKA), 17.8% vs 65.5% NOACs and 4.8% vs 10.6% antiplatelet monotherapy, varying across cohorts (Figure 1).

Event rates per 100 patient-years in NL and BE, respectively, of all-cause mortality (3.38 vs 3.90; hazard ratio (HR) 0.86 [0.65-1.15]), ischemic stroke/TE (0.82 vs 0.72; HR 1.14 [0.62-2.11]) and major bleeding (2.06 vs 1.54; HR 1.33 [0.89-1.99]) did not differ significantly.

Conclusions: In GARFIELD-AF, despite similar characteristics, patients were treated distinctly different with predominantly VKA vs NOAC in NL and BE, respectively. Although the rate of major bleeding was 33% higher in NL, variations in bleeding, mortality and TE rates were not statistically significant.

PB2052 | Factor V Leiden Is Associated with Atrial Fibrillation, but Not Ischemic Stroke in Atrial Fibrillation. The Tromsø Study

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Background: Factor V Leiden (FVL) is a prevalent genetic mutation associated with venous thromboembolism, but also with arterial disease and adverse pregnancy outcomes. While atrial fibrillation (AF) is a prothrombotic condition, it is scarcely known to what extent FVL is associated with AF development and subsequent ischemic stroke.

Aims: To investigate whether the presence of FVL was associated with incident AF and ischemic stroke in a cohort recruited from a general population.

Methods: A randomly selected sub-cohort (n=3663) was sampled from the fourth survey of the Tromsø study (1994/1995) and incident cases of AF and ischemic stroke were identified through December 31, 2012. DNA isolated from blood of all participants was genotyped for FVL, and Cox proportional hazards regression models were used to calculate hazard ratios (HR) with 95% confidence intervals (CI) for AF and ischemic stroke by FVL. The regional committee for research ethics approved the study and all study participants provided informed written consent.

Results: 235 participants (6.4%) were carriers of ≥ 1 risk allele in FVL. During follow-up, 545 participants were diagnosed with AF, and 314 participants experienced ischemic stroke. Carriers of FVL had 1.4-fold increased risk of AF (HR 1.37, 95% CI 1.00 - 1.85), and the risk was more pronounced in men (HR 1.52, 95% CI 1.04-2.24) than in women (HR 1.09, 95% CI 0.66 - 1.81). Carriers of ≥ 1 risk allele in FVL did not have significantly increased risk of ischemic stroke (HR 1.22, 95% CI 0.80-1.87). Moreover, while subjects who developed AF had higher risk of subsequent stroke (HR 3.22, 95% CI 2.44-4.25), the presence of FVL did not add to these risk estimates.

Conclusions: FVL was associated with increased risk of incident AF, especially in men. FVL was nevertheless not an important risk factor for ischemic stroke in general, nor in AF patients in particular.

PB2053 | Predictors of Left Atrial Thrombus before Catheter Ablation or Cardioversion in Patients with Non-Valvular Atrial Fibrillation

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Background: In patients with non-valvular atrial fibrillation (AF) left atrial thrombus (LAT) may be revealed despite of oral anticoagulants use and low risk of stroke according to modern clinical scores.

Aims: To assess frequency and predictors of LAT before catheter ablation or cardioversion in patients with non-valvular AF.

Methods: In a retrospective single center study medical records of 1994 patients with non-valvular AF subjected to the transthoracic echocardiography before catheter ablation or cardioversion between 2014 and 2019 were analyzed. LAT was revealed in 33 (1.6%) of them. For comparison 167 patients without LAT were randomly selected from the database. Logistic regression analysis was used for searching associations between different variables and LAT.

Results: Patients with LAT were older (64.8±10.6 vs 59.4±10.8 respectively; p=0.01), more often had persistent or permanent AF, hypertension, chronic heart failure, had lower EF and eGFR and larger left atrial dimension from parasternal long-axis view. They also were more symptomatic according to modified EHRA score. All these features were univariate predictors of LAT.

CHA_2DS_2-VASc score was higher in patients with LAT (3.9±1.9 vs 2.3±1.6 respectively; p=0.0001) and was univariate predictor of LAT (OR 1.6, 95% CI 1.3-2.0; p=0.001). None of patient with CHA_2DS_2-VASc score 0 had LAT while LAT was revealed in 4 patients (all men) with CHA_2DS_2-VASc 1. Oral anticoagulants used 88% of patients and it was not associated with LAT.

Only EHRA score (OR 5.4; 95% CI 1.98-14.96; p=0.001) and EF (OR 0.87; 95% CI 0.80-0.95; p=0.002) were independently associated with LAT. Multivariate OR of LAT in patients with EF < 48% (cut-off value obtained at ROC analysis) was 7.4 (95% CI 1.2-46.7; p=0.03).

Conclusions: In this group of patients with non-valvular AF and low incidence of LAT symptomatic arrhythmia and reduced EF but not CHA_2DS_2-VASc score or oral anticoagulants use were independently associated with LAT.

PB2054 | Effect of Patient and Surgery Characteristics on Clinical Outcomes in the Perioperative Anticoagulation Use for Surgery Evaluation (PAUSE) Study

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Background: In the Perioperative Anticoagulant Use for Surgery Evaluation (PAUSE) Study, patients taking a direct oral anticoagulant (DOAC) for atrial fibrillation (AF) followed a simple, standardized perioperative management strategy prior to an elective surgery/procedure.

Aims: To analyze how patient and surgery characteristics affect clinical outcomes.

Methods: We categorized patients based on 3 pre-surgical variables: (i) surgical bleed risk (high vs. low); (ii) DOAC dose, and (iii) patient renal function (creatinine clearance [CrCl] < 50 or ≥50 mL/min). Clinical outcomes analyzed included major bleeding (MB), arterial thromboembolism, any bleeding (composite of MB and clinically-relevant non-major bleeding (CRNMB)), and any thromboembolism (TE) (composite of arterial and venous thromboembolism). We used descriptive statistics to summarize clinical outcomes, where the frequency, proportion and 95% confidence interval were reported. Fisher's exact tests were used for testing the null hypothesis of independence between the clinical outcome and patient characteristic, where the test p values were reported.

Results: There were 3,007 patients with AF requiring perioperative DOAC management. The most important pre-surgical variable was surgical bleed risk where the rate of MB was higher after high bleeding risk procedures (Table 1). The excess bleeding occurred despite a doubling in the duration of DOAC interruption before and an additional 24-48-hour delay in DOAC resumption after high risk procedures. Surgical bleeding risk did not influence the risk of TE outcomes (Table 2). There were no significant differences in bleeding or TE outcomes when comparing DOAC dose and patient renal

TABLE 1 Effect of surgical bleed risk on major bleeding and any bleeding

	DOAC Cohort and Surgical Bleed Risk					
	Apixaban		Dabigatran Etexilate		Rivaroxaban	
Outcome	High bleed-risk (n=406)	Low bleed risk (n=851)	High bleed-risk (n=228)	Low bleed-risk (n=440)	High bleed-risk (n=373)	Low bleed-risk (n=709)
Major Bleeding						
n, % (95% CI)	12, 2.96 (1.7-5.09)	5, 0.59 (0.25-1.37)	2, 0.88 (0.24-3.14)	4, 0.91 (0.35-2.31)	11, 2.95 (1.65-5.2)	9, 1.27 (0.67-2.39)
P-value	P=0.001		P=1.000		P=0.059	
Any bleed (MB+CRNB)						
n, % (95% CI)	23, 5.67 (3.8-8.36)	15, 1.76 (1.07-2.89)	7, 3.07 (1.4-6.2)	12, 2.73 (1.57-4.71)	25, 6.70 (4.58-9.71)	20, 2.82 (1.83-4.32)
P-value	P<0.001		P=0.809		P=0.004	

TABLE 2 Effect of Surgical Bleed Risk on Arterial Thromboembolism and Any Thromboembolism

	DOAC Cohort and Surgical Bleed Risk					
	Apixaban		Dabigatran Etexilate		Rivaroxaban	
Outcome	High bleed-risk (n=406)	Low bleed risk (n=851)	High bleed-risk (n=228)	Low bleed-risk (n=440)	High bleed-risk (n=373)	Low bleed-risk (n=709)
Arterial Thromboembolism						
n, % (95%CI)	1, 0.25 (0.04-1.38)	1, 0.12 (0.02-0.66)	1, 0.44 (0.08-2.44)	3, 0.68 (0.23-1.99)	2, 0.54 (0.15-1.93)	2, 0.28 (0.08-1.02)
P-value	P=0.542		P=1.000		P=0.612	
Any TE						
n, % (95% CI)	7, 1.72 (0.84-3.52)	2, 0.24 (0.06-0.85)	3, 1.32 (0.45-3.8)	4, 0.91 (0.35- 2.31)	3, 0.80 (0.27-2.34)	2, 0.28 (0.08-1.02)
P-value	P=0.007		P=0.695		P=0.347	

function (p=NS for apixaban, dabigatran, rivaroxaban, data not shown).

Conclusions: Our results suggest that surgical bleed risk is an important determinant of bleeding but not thromboembolic outcomes. DOAC dose and renal function appeared to have no effect on these outcomes. Further study is needed to investigate independent determinants of bleeding and thromboembolism in patients who need to interrupt DOAC therapy for an elective surgery/procedure.

PB2055 | Anticoagulation in Non-Valvular Atrial Fibrillation: Old or New Friends?

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Background: Chronic anticoagulation is recommended in patients with non-valvular atrial fibrillation (AF) and a CHA_2DS_2-VASc score ≥2. Direct oral anticoagulants (DOAC) have changed tremendously the management of AF and begin to steadily replace vitamin K antagonists (VKA).

Aims: To investigate the prescription patterns of DOAC and VKA in AF patients.

Methods: We conducted a retrospective analysis of the medical charts of AF patients hospitalized in the Internal Medicine Clinic, Clinical Emergency Hospital of Bucharest, Romania, during two months in 2018. Written informed consent was collected from all subjects. The hospital's ethics council approved the study (approval number 4263/13.05.2019). We respected the regulations of the national law and Helsinki Declaration (1975, revised in 5/2008).

Results: We recruited 31 AF patients (mean age=76.72±7.87 years): 16 males (51.61%) and 15 males (48.39%). All patients had a CHA_2DS_2-VASc score ≥2 and were on long term anticoagulation. DOAC were just slightly chosen over VKA to anticoagulate AF patients (51.61% vs. 48.39%). Acenocoumarol was the most common anticoagulant prescribed (n=15, 48.39%), followed by apixaban (n=9, 29.03%) and dabigatran (n=7, 22.58%). There were no differences in terms of age between patients who received acenocoumarol vs. dabigatran (mean age: 75.40±7.57 vs. 78.11±6.94, P=0.3907) or vs. apixaban (mean age: 75.40±7.57 vs. 78.43±5.80, P=0.3614) or dabigatran vs. apixaban (mean age: 78.11±6.94 vs. 78.43±5.80, P=0.9239). Also, we found no differences in terms of mean number of comorbidities in patients receiving acenocoumarol vs. dabigatran (11.38±2.94 vs. 12.11±4.08, P=0.6061) or apixaban (11.38±2.94 vs. 9.14±1.95, P=0.0819), or dabigatran vs. apixaban (12.11±4.08 vs. 9.14±1.95, P=0.0992). Also, we found no differences in terms of sex (male vs. female) in the prescription patterns of acenocoumarol (P=0.4795), dabigatran (P=0.22), apixaban (P=1.000) or VKA vs. DOAC (P=0.4795).

Conclusions: The popularity of DOAC is increasing in Romania, with more AF patients receiving DOAC vs. VKA.

PB2056 | Management of DOACs in Elective Surgery Procedures: A Measurement Based Approach

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Background: EHRA guidelines recommend pre-operative DOAC therapy interruption based on pharmacokinetics, procedure-associated bleeding risk, and estimated creatinine clearance (ECrCl). In our Institution, 24h measurement of DOACs plasma levels is available.

Aims: Managing pre-operative interruption of DOACs based on trough plasma levels and estimated creatinine clearance.

Methods: From January 2014 to December 2019, 324 patients (86% with NVAF, 65.4% men, mean age 77±10 years, mean ECrCl 71±30 ml/min) on dabigatran etexilate (n=96, 29.6%), apixaban (n=100, 30.9%), rivaroxaban (n=97, 29.9%), and edoxaban (ED, n=31, 9.6%) underwent elective invasive maneuvers (n=44, 13.6%), minor (n=71, 21.9%) and, importantly, major surgery (n=209, 64.5%). DOACs trough levels (ng/ml), measured a median of 15 days before intervention, were 116±75, 123±73, 42±47, and 44±43, respectively. Seventy-two patients (22.2%, 18 on dabigatran etexilate) had ECrCl < 50 ml/min. Timing of last DOACs intake was decided based on trough levels and the expected half-life of the DOAC, aiming at levels ≤30 ng/ml on the morning of the intervention, when all patients had DOACs' levels measured. Hemoglobin loss was taken as a measure of bleeding.

Results: The average time of last DOAC intake before the morning of intervention was 38±21 hours. On the morning of the intervention, DOACs levels (ng/ml) were 13±13 (dabigatran),15±18 (apixaban), 6±9 (rivaroxaban), and 7±8 (edoxaban), with 90% of measurements ≤30 ng/ml. Levels exceeding 50 ng/ml were observed in 7 patients (2.2%), with peak levels (ng/ml) of 76 (dabigatran), 69 (apixaban), 39 (rivaroxaban), and 32 (edoxaban). There was no instance of unexpected bleeding.

Conclusions: Compared to EHRA guidelines, timing of interruption of DOACS based on trough measurements is safe and may avoid early interruptions, potentially dangerous in patients at high thromboembolic risk.

PB2057 | Assessment and Predictors of Inappropriate Dose of Direct Oral Anticoagulants

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Background: Direct oral anticoagulants (DOACs) are widely used medications in the last decade for different indications including venous thromboembolism (VTE) treatment and prevention as well as stroke and systemic embolization prevention in patients with non-valvular atrial fibrillation (NVAF)(1). Dosing of DOACs is based on indication, age, weight or body mass index (BMI), and concurrent medications and/or renal function. Recent studies have shown that a notable proportion of patients were receiving inappropriate doses of DOACs (2).

Aims: We aim to evaluate DOACs dosing prescription according to the recommendations of the drug label and predictors of inappropriate dosing in clinical practice within a community teaching hospital.

Methods: We performed a retrospective analysis of patients managed with DOACs for VTE treatment or AF from June 2016 to January 2018 at Prince Mohammed Bin Abdulaziz Hospital, Riyadh, Saudi Arabia.

Results: Direct oral anticoagulants were prescribed in 253 patients, 99 patients (40%) were on rivaroxaban, 110 patients (43%) were on apixaban, and 44 patients (17%) were on dabigatran. The majority of patients were on DOACs for NVAF 172 patients (86%), while 81 patients (32%) for VTE treatment. The mean age was 79.7±19 years. Of the total number of patients, one third were prescribed an inappropriate dose of DOACs, 65 patients (26%) received a lower dose and 15 patients (6%) received a higher dose (Table 1). In multivariate analysis, the prescription of inappropriate dose was associated with younger age (< 75), and high creatinine clearance (>50 mL/min) (Table 2).

Conclusions: About one third of patients treated with DOACs received inappropriate dose. Age and creatinine clearance are important predictors for inappropriate doses.

PB2058 | Vitamin K Antagonists Dosing According to Pharmacogenetics

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Background: Genetic factors can explain a great deal of the inter-individual variability to Vitamin K antagonists (VKA) treatment. Genetic variations affecting both hepatic cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex subunit I (VCORC1) are associated with a decrease in the VKA dose requirement and an increased risk of bleeding as well as with high dose requirement and VKA resistance.

Aims: To evaluate the efficacy of the targeted VKA dose according to pharmacogenetics in achieving the therapeutic INR range (2-3).

Methods: CYP2C9 (*2 and *3 alleles; rs1799853 and rs1057910) and VCORC1 (1639G A; rs9923231) variant genotyping was performed in 74 AF patients treated with acenocoumarol in which TTR was above or below the targeted TTR of 60%. At least ten consecutive INR results per patient were compared to the VKA dose.

Results: According to the genetic testing, 23 (31%) patients were classified as having a slow VKA metabolism with significant risk of bleeding with main targeted dose od acenocoumarol of 0.75 mg/day. In about 50% of them only 0.25/day was enough to maintain the therapeutic INR. In 51 (69%) patients without genetic mutations the targeted VKA dose of 3.25mg/day did not correspond with the dose with which the therapeutic INR was achieved; being 56% higher (in 29 patients) and 44% lower (in 22 patients) than the main therapeutic INR dose of 2.0mg/day and 4.5 mg/day respectively.

However, the best correlation between the genetically targeted dose and achived therapeutic INR dose was observed in patients with high dosage requirement of 4.5mg/day.

Conclusions: The pharmacogenetic targeted VKA dose showed poor correlation with the actual mutation especially in patients with lower targeted dose. Although further and larger studies are required, this may indicate that patients do not athere to the dose especially when higher dose is necessary being afraid of bleeding complications.

PB2059 | Rates, Management and Outcome of Bleeding Complications during Edoxaban Therapy in Daily Care - Results from the Dresden NOAC Registry

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Background: Edoxaban is licensed in many countries around the world, following successful phase-III trials in stroke prevention in atrial fibrillation (SPAF) and treatment of venous thromboembolism (VTE) but, at present, little is known about edoxaban-related bleeding complications in daily care.

Aims: We aimed to evaluate bleeding complications during edoxaban therapy.

Methods: Using data from a prospective, non-interventional oral anticoagulation registry, we analysed rates, management and outcome of edoxaban-related bleeding.

Results: Between 1 October 2011 and 28 February 2019, 996 patients were enrolled in the edoxaban cohort and a total of 891 bleeding events were observed (53.2% ISTH minor, 41.9% clinically relevant non-major and 4.9% major bleeding events). In case of major bleeding, surgical or interventional treatment was performed

TABLE 1 Severity and management strategies of edoxaban-related bleeding complications in the as-treated population

891 bleeding events in 530 patients	Conservative (no treatment/ compression / tamponade / transfusion)	Intervention	Surgery	RBC	Vit K	FFP	PCC	rFVII
Minor 474/891 (53.2)	474/474 (100)	0	0	0	0	0	0	0
NMCR 373/891 (41.9)	334/373 (89.5)	36/373 (9.7)	3/373 (0.8)	0	0	0	0	0
Major 44/891 (4.9)	33/44 (75)	9/44 (20.5)	2/44 (4.5)	25/44 (56.8)	0	0	2/44 (4.5)	0
TOTAL	841/891 (94.4)	45/891 (5.1)	5/891 (0.6)	25/891 (2.8)	0	0	2/891 (0.2)	0

FFP, fresh frozen plasma transfusion; CRNM, non-major clinically relevant; PCC, prothrombin complex concentrate; RBC, red blood cell transfusion; rFVII= recombinant Factor VII; Vit K, vitamin K supplementation.

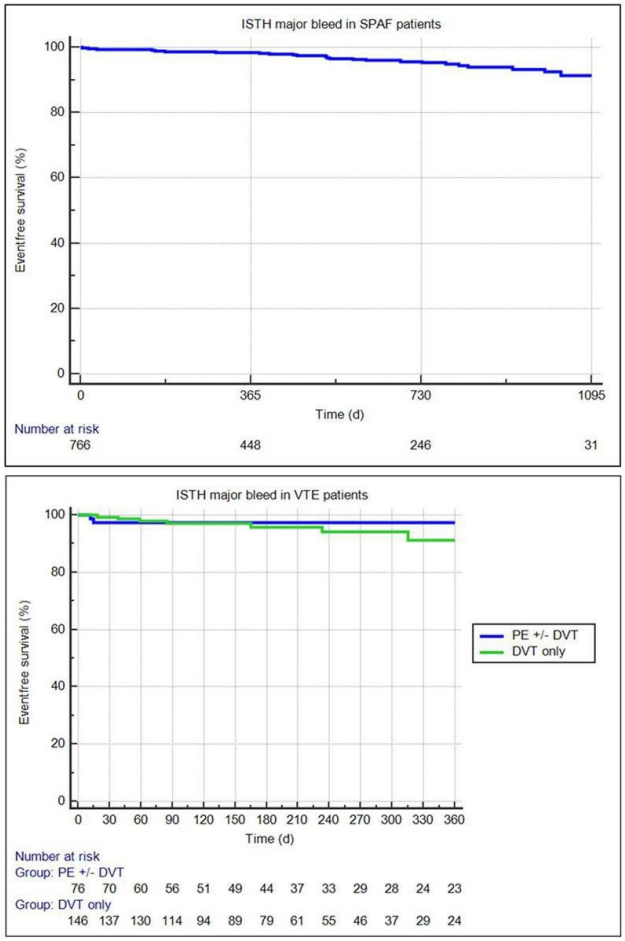

FIGURE 1 Kaplan-Meier time-to-first-event curves for SPAF (left panel; mean edoxaban exposure time 527.6 days) and VTE patients (right panel; mean edoxaban exp

in 25.0% and prothrombin complex concentrate was given in 2 cases (Fig. 1). In the time-to-first-event analysis, 100-patient-year rates of major bleeding were 3.1/100 patient-years (95% CI 2.2-4.2; Fig. 2). In the as-exposed analysis, case-fatality rates of edoxaban-associated bleeding leading to hospitalizations were 7.5% and 9.0% at days 30 and 90 post bleeding, respectively.

Conclusions: Taken together, our data indicate that, in real life, rates of edoxaban-related major bleeding in line with rates observed in phase III trials and that bleeding pattern, management and outcome of these events are not different from those reported for other direct factor Xa inhibitors.

PB2060 | Association of Clinical Risk Factors and Biomarkers with Thromboembolism, Major Bleeding, and All-Cause Death in Patients with Atrial Fibrillation in a Tertiary Care Setting

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Background: Current paradigm on stroke prevention in atrial fibrillation (AF) calls for anticoagulation of all AF patients unless assumed to be at low risk of stroke, which, in routine clinical care, is assessed using the CHA_2DS_2-VASc score. Current guidelines do not issue a strong recommendation for use of biomarkers in risk assessment.

Aims: To investigate the association of clinical risk factors and biomarkers (troponin T, NT-proBNP, D-dimer, von Willebrand factor, and factor VIII) with the occurrence of thromboembolic events (TEE), ISTH major bleeding, and all-cause death.

Methods: We enrolled 316 patients (median age [IQR]: 72 years [65-77]; 39.6% women) with non-valvular AF into a hospital-based registry and prospectively recorded occurrence of TEE, bleeding, and all-cause death. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated using the Cox-proportional hazard model.

Results: Over a median observation period of 308 days (244-560), we recorded 10 (3.3%) TEE and 11 (3.7%) bleedings. Until November 7, 2018, a total of 54 study participants (18.0%) had died from all causes. The CHA_2DS_2-VASc score (HR 2.99 [per 1 point increase], 95% CI 1.7-5.25) and two of its individual risk factors, female sex (HR 15.7, 95% CI 2.5-101) and history of stroke (HR 21.0, 95% CI 3.6-123), were independently associated with increased TEE risk. None of the biomarkers were associated with risk of TEE or bleeding. NT-proBNP (HR 1.09 [per 1000 pg/mL increase], 95% CI 1.03-1.14), D-dimer (HR 1.28 [per 1 μg/mL increase], 95% CI 1.01-1.63), and age (HR 1.05 [per 1 year increase], 95% CI 1.01-1.1) were independently associated with increased risk for all-cause death.

Conclusions: In our cohort of predominately anticoagulated AF patients, history of stroke, female sex, and the CHA_2DS_2-VASc score were independent predictors for TEE. While no biomarker was associated with TEE or bleeding risk, NT-proBNP and D-dimer were associated with increased risk for all-cause death.

PB2061 | The Role of Hemodialysis in Patients with Re-Elevation of Dabigatran Levels After Idarucizumab Treatment: A Case Series

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Background: Idarucizumab is a monoclonal antibody that effectively reverses coagulation activity of dabigatran, regardless of renal function. However, in patients with renal insufficiency, re-elevation of dabigatran levels may occur within 12 to 24h.

Aims: We performed a case-series study with objective to evaluate the efficacy of idarucizumab and the role of hemodialysis for reversal of dabigatran activity in patients with acute renal insufficiency.

Methods: We are describing case-series of adult patients with acute renal insufficiency that needed urgent reversal of dabigatran treatment. Three patients, two men and a female, age 69, 70 and 71 years, presented in the emergency unit with acute abdomen, severe hemorrhage and need for placement of central venous catheter, respectively. The indication for dabigatran was atrial fibrillation in all patients, and none of them had previous renal insufficiency.

Results: The patient with a need of urgent surgical procedure and patient with active severe gastrointestinal bleeding received idarucizumab in a dose of 2x2.5 mg iv, which enabled laparotomy and endoscopy with local hemostasis in the rectum, respectively. Both procedures were successful without excessive bleeding. However, within 12h from the idarucizumab treatment, bleeding diathesis was observed, and dabigatran concentrations were measured again at supra-therapeutical levels. Hemodialysis effectively reduced the dabigatran levels, and no additional bleeding symptoms occurred. The third patient had indication for an urgent hemodialysis due to acute renal failure. Immediately before placement of the catheter the patient received idarucizumab 2.5 g iv. The procedure went uneventful. Patient continued with everyday hemodialysis and had no bleeding complications.

Conclusions: In patients with re-elevation of dabigatran levels after idarucizumab treatment hemodialysis can be considered as consequential therapy. Initial idarucizumab would provide a safe period for the urgent management of patient, but intensive treatment with repetitive idarucizumab doses can be thus avoided and replaced with hemodyalisis.

PB2062 | Differential Effect of Direct Oral Anticoagulants (DOACs) on Thrombin Generation and Clot Lysis in Patients with Non Valvular Atrial Fibrillation (AF) and Venous Thromboembolism (VTE)

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Background: Recent reports suggest that DOACs may induce different anticoagulant and profibrinolytic responses.

Aims: Head-to-head comparison of the changes in thrombin generation parameters and t-PA-induced clot lysis induced by different DOACs.

Methods: We studied 137 patients (72 AF, 65 VTE) under treatment with apixaban (n=38), edoxaban (n=29), rivaroxaban (n=39), or dabigatran (n=31). Plasma samples were taken at C-trough and C-peak. Thrombin generation was evaluated by calibrated automated thrombogram (CAT). For dabigatran samples, the internal calibrator was supplemented with idarucizumab to neutralize dabigatran. Lysis time of clots exposed to 40 ng/ml t-PA was assessed by turbidimetry.

Results: C-peak drug levels were 2-fold (apixaban and dabigatran) to 6-8 fold higher than C-trough levels. C-peak samples displayed longer lagtime and lower peak thrombin than C-trough samples for all drugs, whereas C-peak ETP was reduced only in dabigatran and rivaroxaban samples. Moreover, ETP was lower and peak thrombin higher in dabigatran samples as compared to all "xabans". Rank correlation showed that the levels of each xaban were strongly correlated with lagtime and peak thrombin (rho>0.7) whereas dabigatran levels were best correlated with lagtime and ETP (rho=>0.62). Clot lysis time was significantly shorter in C-peak than in C-trough in all groups but apixaban. Intergroup comparison showed that clot lysis time of dabigatran samples was significantly shorter than that of xabans, at both C-trough and C-peak. Moreover, a significant correlation between drug level and clot lysis changes was only observed in dabigatran group (rho=0.53). Finally, a strong correlation between clot lysis time and both peak thrombin and ETP was only seen in dabigatran group (rho ≥ 0.51).

Conclusions: Thrombin generation may represent a valid assay to assess the anticoagulant effect of DOACs, provided that the right CAT parameters are considered for each type of drug. The greater profibrinolytic activity of dabigatran may improve its antithrombotic efficacy.

PB2063 | Plasma from Patients under Direct Oral Anticoagulants (DOACs) Displays a Strong Resistance to Heparin Compared to Patients under Vitamin K Antagonists (VKA)

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Background: Patients anticoagulated with DOACs undergoing atrial fibrillation (AF) ablation have been reported to require higher doses of heparin (UFH) than patients on VKA therapy.

Aims: To evaluate the anticoagulant and profibrinolytic responses to UFH added to plasma from patients under DOACs or VKA.

Methods: One hundred-thirty-six patients (71 AF, 65 venous thromboembolism, VTE) treated with dabigatran (n=30), apixaban (38), edoxaban (29) or rivaroxaban (39) were studied at both C-trough and C-peak. Twenty-seven patients under VKA and 20 healthy subjects served as control groups. Thrombin generation and t-PA-induced clot lysis, without and with 0.1 IU/ml UFH, were evaluated by calibrated automated thrombogram (CAT) and turbidimetry, respectively.

Results: There were no differences between AF and VTE patients nor were there differences in antithrombin level between DOAC and VKA. UFH displayed a markedly more pronounced anticoagulant effect on VKA samples than on DOAC samples, as witnessed by the greater changes in lag-time, peak thrombin and ETP (table). Notably, the reduced response of DOAC samples to UFH was evident, for all CAT parameters but ETP, at both C-trough and C-peak. Rather surprisingly, a greater refractoriness of DOAC samples to heparin was also detected by the thrombin time test, suggesting the presence of factor(s) interfering with UFH activity. Concerning fibrinolysis, clot lysis time shortening by heparin was significantly greater in VKA than in C-trough, but not C-peak, DOAC samples. Qualitatively similar differences were observed when DOAC samples were compared to plasma from healthy subjects (not shown). Intergroup comparison showed that dabigatran displays a greater response to the anticoagulant activity of UFH than xabans, at either C-trough or C-peak or both (i.e. lagtime).

Conclusions: Using global assays that better mimic the clotting and fibrinolytic processes, we show that patients under DOAC display a marked heparin-resistance, whose mechanism(s) and clinical relevance warrant further investigation.

TABLE 1 Changes in CAT parameters and clot lysis time induced by heparin addition to plasma samples from patients under DOACs or VKA

Variable	VKA	All DOACs		Dabigatran		Xabans	
		Trough	Peak	Trough	Peak	Trough	Peak
Concentration (ng/ml) or INR	2.6 (2.1-3.0)	47 (29-88)	193 (133-278)	63 (45-119)	152 (92-230)	41 (28-80)	216 (147-312)
Lagtime (%)	660 (290-2188)	120* (98-163)	134* (103-362)	155† (119-296)	306† (179-960)	115 (95-137)	127 (102-219)
Peak Thombin (%)	3.0 (1.0-8)	24* (18-37)	33* (19-52)	21 (8-36)	4.0† (2-16)	24 (19-37)	38 (27-56)
ETP (%)	0.0 (0.0-19)	79* (55-110)	0.0 (0.0-74)	70† (39-81)	0.0 (0.0-65)	86 (61-122)	6.5 (0.0-8)
Clot lysis time (%)	69 (62-82)	90* (80-100)	75 (60-88)	90 (60-100)	64 (55-89)	90 (80-100)	79 (62-88)

Data (median, IQR) are expressed as percent of the value obtained without heparin. *, statistically different from VKA; †, statistically different from corresponding values of Xabans.

PB2064 | Clinical Outcomes for Patients of High Body Weight on Apixaban or Rivaroxaban

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Background: Direct-acting oral anticoagulants (DOACs) are licensed for stroke prevention in non-valvular atrial fibrillation and prevention and treatment of venous thromboembolism (VTE). There is uncertainty about the efficacy and safety of the licensed doses for these indications in the obese population, particularly those with a weight greater than 120kgs[1].

Our previous audit conducted in 2017, which looked at whether high body weight reduced drug exposure to apixaban and rivaroxaban using anti-Xa levels, found no evidence of reduced drug exposure in this patient group.

Aims: To identify whether high body weight affects clinical outcomes for patients on apixaban and rivaroxaban.

Methods: The patient cohort from the 2017 audit were followed up in January 2020. Each patient was contacted via telephone to ask if they had experienced: VTE recurrence, CVA (cerebrovascular accident), a major bleeding event or a non-major bleeding event.

Results: Of the 30 patients contacted to ask about clinical outcomes, 2 patients were uncontactable and therefore excluded and 5 patients had died. For 4 of the 5 patients who died, the cause of death was not a result of VTE, CVA or a bleed. The cause of death for the

TABLE 1 Baseline characteristics and findings

Age, mean +/- SD (range) years	61 +/- 12 (23-78)
Gender	25 male, 5 female
Weight, mean +/- SD (range) kg	137 +/- 22 (120-230)
Indication	26 stroke prevention in atrial fibrillation 3 secondary prevention of VTE 1 treatment of VTE
DOAC type	19 rivaroxaban, 11 apixaban

TABLE 2 Clinical outcomes

Exclusions	2 uncontactable, 5 deceased
VTE event	0/23
CVA event	0/23
Major bleeding	0/23
Non-major bleeding	2/23

remaining patient remains unknown. For the remaining 23 patients, there were no VTE recurrence events, CVA events or major bleeding. There were 2 episodes of non-major bleeding reported, both of these being nosebleeds. The follow up period was 2 years.

Conclusions: In this prospective cohort study, we found few adverse clinical outcomes for high body weight patients receiving DOACs over a 2 year follow-up period. However, this study is limited by the lack of a comparative group of "normal" weights. More studies in this important area are required.

PB2065 | Fatal Intracerebral Hemorrhage in a Patient on Warfarin: A Case Report

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Background: Warfarin remains an important therapeutic alternative in low and middle-income countries. However, it increases the risk of intracerebral hemorrhage (ICH) which is associated with significant morbidity and mortality.

Aims: To present a case of fatal ICH in a patient on warfarin.

Methods: The patient had been recruited for the control arm of a clinical trial (NCT01006486-ClinicalTrials.gov) designed to assess the impact of an anticoagulation clinic in Brazil. She provided a signed informed consent.

Results: A 42-year-old non-white woman was on warfarin for stroke prevention for five years due to atrial fibrillation and prosthetic heart valves. Target INR was 2.5-3.0. There was no history of major bleeding, but she had several episodes of minor bleedings, including hematomas, nose and gum bleeding. Comorbidities included systemic arterial hypertension, hypothyroidism, heart failure, rheumatic cardiomyopathy, dyslipidemia, pulmonary thromboembolism and depression. She was taking chronically nine medications and acetaminophen was eventually used. Vitamin K intake was regular. There was no report of alcohol consumption or smoking. Time in therapeutic range was 33.7%. The patient began a progressive holocranial headache. She was admitted in an emergency hospital in the very next day with nausea, vomits and consciousness impairment. Warfarin dose was 70mg/week. There were no signs of hemodynamic impairment or infection. Spontaneous hematomas began to appear in her right thigh. She was scored with 3 points by the Glasgow Coma Scale. A computed cranial tomography revealed a rounded image sized 4.0cm per 2.8cm in the left cerebellar lobar area, with liquid interface in its interior. Additionally, there was a spontaneous subarachnoid bleeding invading ventricular space and cerebral edema. INR value at admission was 5.25. The patient died two days later presenting an irreversible asystole despite the prompt assistance.

Conclusions: This case reinforces the need of close monitoring and educational interventions to prevent complications in a patient with a complex clinical condition.

PB2066 | Relationship of Health Literacy and Adherence to Treatment with Oral Anticoagulants in Patients with Atrial Fibrillation in Brazil

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Background: Atrial fibrillation (AF) is the most frequent arrhythmia in clinical practice. The use of oral anticoagulants is useful to prevent thromboembolism. Patient adherence is essential to achieve effectiveness and safety of drug therapy. Health literacy (HL) is one of the major obstacles, which can impact on adherence to drug therapy.

Aims: To investigate the relationship between HL and adherence to treatment with oral anticoagulants in patients with FA.

Methods: Patients were recruited at a public cardiology clinic in Brazil between February and September, 2019. Inclusion criteria were: age ≥18 years, AF diagnosis by electrocardiogram and/or Holter, indication of full oral anticoagulation, use of vitamin K inhibitors or direct anticoagulants. Data collection involved the review of medical records and patient interviews. We collected sociodemographic and clinical data. Validated instruments were used to measure the HL

and adherence to drug therapy. All participants signed an informed consent form (ethics approval CAAE:00193018.9.0000.5149).

Results: Overall, 100 patients were included in the study, presenting the mean age of 68.8 years old and 54% of women. Most patients (74%) had completed elementary school. The average family monthly income was US $ 348.34. A total of 69% of participants were taking chronically ≥five drugs. HL was classified as adequate for 21% of patients and 34% of them were considered adherent to oral anticoagulation. There was no statistically significant relationship between adequate HL and adherence to drug therapy (Odds ratio (OR) 1.25; confidence interval (CI) 95% 0.45-3.37; p=0.66).

Conclusions: We identified low rates of adequate HL and adherence to drug therapy, and there was no statistical relationship between these variables. Our results suggest the need of further studies to investigate factors associated with adherence to oral anticoagulation in vulnerable populations, and the design of strategies for its improvement in public health.

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PB2068 | Emergency Room Access of Patients on DOACs: A Six Years Single Center Experience

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Background: Reports about Emergency Room (ER) access of patients on DOACs show that bleeding is a critical issue also in these patients.

Aims: To analyze reasons for ER access of patients on treatment with DOACs from 2014 to 2019, based on testing of DOACs′ plasma concentrations.

Methods: Based on test requests, we analyzed ER records for age, gender, type of DOAC, hour of admission to the ER, polypharmacy, reason for access, diagnosis, treatment and outcome. Time of last DOAC intake was only available for few patients.

Results: 975 patients (mean age 78±11 years, 47.9% women, 87.6% with NVAF) accessed the ER, increasing from 34 in 2014 to 406 in 2019. Dabigatran etexilate, apixaban, rivaroxaban and edoxaban were prescribed in 26%, 43%, 21%, and 10% of patients. Once-daily treatments were 1.73-fold more frequent in VTE patients (p=0.004), who were on average 10 years younger than NVAF patients (p< 0.00001). Bleeding was responsible for ER access in 36% of cases in 2014 and for 21% of cases in 2019, while incidence of embolism/thrombosis was 20% and 5%, respectively (p< 0.0005). Bleeding and embolic/thrombotic complications had similar frequency irrespective of gender (p=0.53) and DOAC (p=0.18). Patients' age was not different according to the reason for ER access (p=0.06). Intracerebral hemorrhage and gastrointestinal bleeding accounted for 20% and 41% of bleeding episodes. Co-administration of antiplatelet agents increased the bleeding risk (OR =2.16; 1.74-2.68 95%CI). In patients on twice-daily treatment, and with diagnoses other than bleeding or embolism/thrombosis, DOAC plasma concentrations showed a circadian pattern (nadir levels at 7.00-8.00 am and 8.00-9.00 pm). There was a trend for DOAC concentrations to be 25% lower in patients with embolism/thrombosis than in the remaining patients.

Conclusions: These data show that bleeding represents a major reason for ER access of patients on DOACs, especially for those who have co-administration of antiplatelet agents.

PB2069 | Anticoagulation Resumption After Intracerebral Hemorrhage in Two Cohorts of Patients Treated with Vitamin K Antagonists and Direct Oral Anticoagulants

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Background: Intracerebral hemorrhage (ICH) is associated with severe prognosis and recurrent risk. This risk impacts on the decision to resume anticoagulation in patients with atrial fibrillation (AF) or venous thromboembolism (VTE).

Aims: To evaluate the incidence rate of recurrent ICH in patients with AF or VTE resuming anticoagulation after a first episode of ICH.

Methods: We report data of two cohorts of patients who started anticoagulants for AF or VTE after the occurrence of a first ICH. The VKA cohort of 166 patients derives from the CHIRONE Study and includes AF and VTE patients on VKAs, the DOAC cohort of 178 patients derives from the START2-Register and includes AF and VTE patients on DOACs.

Results: The clinical characteristics of the two cohort are similar with the exception of more prevalence of history of previous stroke/TIA in DOAC patients in DOAC patients with respect to VKA patients (p=0.02)) and of levels of serum creatinine >1.5 mg/dL in VKA patients with respect to DOAC patients (p=0.0001).

The index ICH was spontaneous in 66.4% among DOAC cohort and in 33.7% among VKAs cohort (p=0.0001). During follow-up, 14 recurrent ICH were recorded; 9 (rate 2.5 x 100 patient-years) in VKA

cohort and 5 (rate 1.3 x 100 patient-years) in DOAC cohort (Relative Risk 1.9; 95% CI 0.6-7.4; p=0.2).

The univariate logistic regression analysis including both cohorts showed that patients with recurrent ICH were more frequently males, hypertensive, with a history of previous Stroke/TIA and older than patients without recurrence. None of these differences reached statistical significance. VKA patients showed a higher (but not statistically significant) risk of recurrence with respect to DOAC patients (OR 1.9) (95% CI 0.7-6.7; p=0.2).

Conclusions: A trend toward fewer ICH recurrences was detected among patients who resumed DOACs after ICH in comparison to the previously reported rate of patients on warfarin.

PB2071 | More Precise Dosing of Acenocoumarol for Better Control in Patients Aged above 80 Years, a Randomized Controlled Pilot Study

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Background: Many elderly patients are confined to treatment with vitamin K antagonists (VKA) instead of direct oral anticoagulants (DOACs). However, quality of VKA treatment declines with age. At the same time, the average VKA dose requirement decreases with age. This increases the day-to-day variation in VKA dose.

Aims: To obtain information on the effect size for, and feasibility of a full scale trial to assess whether more precise dosing of acenocoumarol increases the quality of VKA treatment in elderly patients.

Methods: In this pilot study we randomized 80 patients aged 80 and above who used 0.5-2.0 mg acenocoumarol daily to either regular dosing in 1.0 mg acenocoumarol increments, or more precise dosing with 0.5 mg increments. We compared changes in time in therapeutic range (TTR) and International Normalised Ratio (INR) variability (VGR) between the two treatment groups, in the six month before and six months during study period. In addition changes in anticoagulation-related quality of life were assessed using PACT-Q.

Results: Patients were aged 84±3 years. Overall baseline TTR was 61% (± 19.2). TTR had improved in both groups, but somewhat more in the intervention group (3.4%, 9.0 vs 5.6; 95% CI -3.7 to 10.5). The INR variability also improved, slightly more in the 0.5 mg group. PACT-Q convenience remained constant in time and did not differ between groups. PACT-Q satisfaction decreased, almost equally, in both groups. Four dosing errors related to dose increments occurred, three in the intervention and one in the control group.

Conclusions: Although more precise dosing of acenocoumarol leads to a slightly better quality of anticoagulation, this effect is too small to convey a relevant clinical benefit. We will not proceed with a full scale RCT. Notably, the overall decrease in treatment satisfaction should be considered when performing future anticoagulation studies in this population.

PB2072 | Time in Therapeutic Range During Therapy with Vitamin K Antagonists Is Lower in Women than in Men and Is Not Explained by Differences in Age

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Background: Adherence and efficacy of anticoagulant therapy with vitamin K antagonists (VKA) is evaluated by Time in Therapeutic Range (TTR). Percentage TTR above 65% is considered appropriate. A few reports suggest that TTR in women might be lower than in men, and hypothesize that women on VKA, being usually older than men, are more sick and thus less adherent. Since not all patients are amenable to direct oral anticoagulants, persistence on VKA for these patients is a necessity for preventing embolism. Thus, understanding what affects TTR is relevant since it could promote preventive measures to ensure effective anticoagulation.

Aims: To ascertain whether women have a lower TTR than men and to study which factors affect TTR among patients with atrial fibrillation and mechanical valves undergoing chronic VKA therapy at a University Hospital Anticoagulant Clinic.

Methods: We retrospectively studied 2428 patients on VKA (1168 women and 1260 men). Differences were analyzed by ANOVA and t-test or non parametric tests, as appropriate. Influence of variables on TTR was evaluated by multiple regression analysis.

Results: The Table shows main characteristics of patients. Women were older than men (81±11.2 vs 78±12.3 years, p< 0.0001), had a lower TTR (65±20.3 vs 69±19.8%, p< 0.0001), but did not differ by extent of comorbidity (Charlson Comorbidity Index): median score was 5 in both groups. In multiple regression analysis, sex, type of VKA and indication for anticoagulation (atrial fibrillation or mechanical valves) weighted slightly on TTR in all patients, while duration of anticoagulation and age did not.

TABLE 1 Characteristics of patients on VKAs by sex

Sex	Number	TTR % Mean (SD)	Age Years Mean (SD)	CCI index Median (range)	Time on VKAs Days Mean (SD)	Type of VKAs % on warfarin	Indication for anticoagulation % atrial fibrillation
Men	1260	69 (19.8)	78 (12.3)	5 (0-14)	2676 (1888.9)	88	73
Women	1168	65 (20.3)	81 (11.2)	5 (0-10)	2792 (1948.5)	88	70

Conclusions: We confirm that women have lower TTR than men. Age did not concur to determine TTR values, nor did comorbidity. We could not identify a variable among those studied that could extensively explain the observed difference in TTR between men and women. Further studies should perhaps focus on dietary habits and/or genetics.

PB2073 | Towards Individualizing Apixaban Therapy in Elderly Patients Through Thrombin Generation

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Background: Apixaban treatment in elderly patients is challenging because health conditions and hypercoagulability status modify its safety profile. As monitoring apixaban levels could be insufficient, thrombin generation assay (TGA) might better predict the anticoagulant effect.
Aims: To determine correlation between apixaban plasma concentration (AXa) and TGA in two groups: under (≤*70y*) and over 70 years old (>*70y*), and estimate inter-individual variability (CVi) in patients >*70y*.
Methods: Patients with atrial fibrillation taking apixaban 2.5 or 5 mg BID were enrolled after obtaining ethical approval and informed consent. Blood was taken at peak and trough. AXa was determined with apixaban calibrated chromogenic anti-FXa assay. TGA parameters: peak height (PH), endogenous thrombin potential (ETP) were assessed by calibrated automated thrombogram activated with tissue factor 5 pM (Thrombinoscope). Statistics: Spearman's correlation, Shapiro Wilks, Levene, Wilcoxon, Student tests.
Results: 39 patients (27 >*70y*) received 2.5 mg and 42 patients (27 >*70y*) received 5 mg. Patients on 2.5 mg: >*70y* achieved higher AXa at trough and peak compared with ≤*70y* (p< 0.001); ETP and PH were significantly lower in >*70y* compared with ≤*70y*, at trough and peak. Patients on 5 mg: in >*70y* AXa was higher at trough and peak, compared with ≤*70y* (p< 0.002). ETP and PH were lower in >*70y*, without significant difference, comparing with ≤*70y*. In patients>*70y* CVi for AXa with 2.5 mg was higher than for 5 mg (peak 46/41%, trough 60/51%). CVi for ETP with 5 mg was lower than for 2.5 mg (peak 33/41%, trough 35/38%).
Conclusions: Elderly patients achieved higher apixaban levels and lower ETP, compared with the younger age group at both doses. Nevertheless, at 5 mg ETP showed no difference between age groups reaching similar values irrespective of apixaban's plasma level. TGA reflects the anticoagulant effect depending on each patient's coagulation status. It could help individualizing apixaban treatment with moderate inter-individual variation.

PB2074 | Anticoagulant Management Strategies in Cancer Patients with Atrial Fibrillation in Daily Clinical Practice

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Background: Few small studies have evaluated the optimal anticoagulant management of atrial fibrillation (AF) in patients with cancer and direct comparative studies between different oral

TABLE 1 Primary anticoagulant strategy of cancer patients with AF

		2012-2013	2013-2014	2014-2015	2015-2016	2016-2017	2017-2018	Total	No anticoagulants
Cohort 1 N=181	No anticoagulants	3	1	13	7	13	11	48	27%
	LMWH	0	0	4	3	6	6	19	
	VKA	3	11	21	24	13	12	84	
	DOAC	0	0	0	3	9	18	30	
Cohort 2 N=361	No anticoagulants	11	3	9	9	9	14	55	15%
	LMWH	1	0	3	1	4	1	10	
	VKA	27	36	53	44	50	58	268	
	DOAC	0	0	1	1	7	19	28	

TABLE 2 Reasons for withholding therapeutic anticoagulant (VKA/DOAC/LMWH) treatment

	Cohort 1 (n=49)	Cohort 2 (n=55)
Cancer-related		
Perceived increased bleeding risk or active bleeding from tumour site	20	17
Recent or planned oncological surgery	4	6
Oral administration not possible	1	0
Non cancer-related		
Perceived increased bleeding risk or active bleeding	3	5
CHA_2DS_2-VASc	10	2
No reason specified	11	25

anticoagulants are lacking. Moreover, cancer-specific conditions such as (intermittent) thrombocytopenia, interaction between oral anticoagulants and systemic anti-cancer treatment, and frequent (surgical) interventions contribute to the clinical challenge of optimal antithrombotic care in this patient category.

Aims: To assess anticoagulant management strategies of cancer patients with AF in the daily practice of a tertiary oncological referral centre.

Methods: Patients with co-existing cancer and AF diagnoses between 01-01-2012 and 31-12-2017 were identified. The choice for initial anticoagulant treatment was extracted from the medical charts, as were cancer-specific circumstances.

Results: We identified 181 cancer patients that subsequently developed AF after 01-01-2012 (cohort 1), and 361 AF patients that subsequently developed cancer after 01-01-2012 (cohort 2). In both cohorts, the majority of patients was treated with vitamin K antagonists, although DOACs were increasingly prescribed from 2016 on (Table 1). LMWH was described in a small number of patients as well (5.3% of all patients). Overall, 104/543 (19%) of patients did not start with therapeutic doses of anticoagulants after the "AF and cancer" diagnosis (Table 2), of whom 12% because of a CHA2DS2-VASc score of 0. In 48 of 104 cases (46%), cancer-specific conditions were the main reason for withholding anticoagulant treatment. Untreated patients were at higher risk of stroke during 2-year follow-up than those treated with anticoagulant therapy (Hazard ratio 4.7; 95%CI 1.6-14).

Conclusions: The majority of patients with AF and cancer was treated with vitamin K antagonists, although a gradual increase in DOAC prescription was seen. A considerable number of patients remained untreated, often because of cancer-specific conditions. Untreated patients were at increased risk for stroke.

PB2075 | Heparin Resistance and a Probable Dabigatran Non Response in a Case of AFib and Left Auricular Atrium Clot

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Background: Unfractionated Heparin (UFH) and Direct oral anticoagulants (DOACs) are indicated for use in atrial fibrillation and thromboembolic disease.

Aims: We report a challenging clinical case of atrial fibrillation with left auricular atrium clot complicated with a possible heparin resistance and non response to Dabigatran.

Methods: A 37 year-old male previously healthy and autonomous for his daily activities with no previous medical and familiar history presented with sharp, stabbing chest pain in the left side of the chest and dyspnea. In the emergency room was reported tachyarrithmia with atrial fibrillation and complete left block with the need for ventilatory and medical support at intensive care unit. Imaging showed extensive cloth in the left auricular atrium and moderate pericardial effusion. Anticoagulation treatment was initiated with UFH with the eventual need to perform periocardiocentesis. At his pick heparin dose he was receiving 50400 UI/24h. He was diagnosed with heparin resistance with the inability to reach the target thromboplastin time and anti-Xa. With a high thrombogenic risk and the eventual need to perform a high risk technique the collective decision was to switch UFH to Dabigatran due to the immediate availability of Dabigatran antidote and effectiveness of continuous monitoring. Despite this, after four therapy days with Dabigatran (150mg bid) there was no dilute thrombin time (DTT) improvement.

Results: Since there was clinical improvement and no need for invasive techniques he was started on acenocumarol. After two days on acenocumarol, target levels were reached with an INR of 2.1. The patient remained symptom-free and was discharged from the hospital.

Conclusions: This case illustrates the challenge to overcome heparin resistance especially in the setting of critical illness. Dabigatran has a predictable pharmacokinetics with no need for continuous monitoring therefore the lack of evidence in the literature to confirm its resistance.

PB2076 | Single Centre Daily Practice Evaluation of Bleeding and Thromboembolic Complications in Cancer Patients with Atrial Fibrillation

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Background: Cancer is suspected to impose an increased thromboembolic and bleeding risk in patients with atrial fibrillation (AF). Detailed data on thromboembolic and bleeding complication rates in practice based conditions are however scarce.

Aims: To determine the incidence rates of bleeding and thromboembolic complications in cancer patients with AF.

Methods: Patients with co-existing cancer and AF diagnoses between 01-01-2012 and 31-12-2017 in our hospital (a tertiary oncological referral centre) were identified. Bleeding and thromboembolic complications were assessed during 2 years of follow-up. We calculated incidence rates per 100 patient years for ISTH major bleeding (MB) and/or clinically relevant non-major bleeding (CRNMB), arterial thromboembolism (ATE; e.g. ischemic stroke, myocardial infarction) and venous thromboembolism (VTE). All complications were adjudicated by independent experts.

Results: We identified 181 cancer patients who developed AF (cohort 1), and 361 AF patients who were newly diagnosed with cancer (cohort 2; Figure 1). Of all 542 patients, 161 had a complete 2-year follow-up, 185 patients died, 171 had a shorter follow-up because of complete remission of cancer or AF, and 25 were lost to follow-up. The incidence rates of MB were 7.0 and 5.8 for cohort 1 and 2 respectively (Table 1). Notably, 41% of all bleedings had a cancer-related predisposing factor such as chemotherapy induced thrombocytopenia < $50*10^9$/L, cancer surgery/interventions or bleeding from cancer site. The incidence rates of ATE and VTE were 4 and 2/100PY, and 4.8 and 2/100PY, for cohort 1 and 2, respectively.

Conclusions: The incidence rates of both bleeding and thromboembolic complications were high in our patients. These data suggest that cancer should be an important determinant in the assessment of risk for AF-related complications.

Figure 1. Patient characteristics of patients with atrial fibrillation and cancer between 1st Jan 2012 and 31st Dec 2017

	Cohort 1 First Cancer, then AF diagnosis after 01-01-2012	Cohort 2 First AF, then cancer diagnosis after 01-01-2012
N =	181	361
Age, mean (SD)	70.4 (9.5)	72.8 (8.2)
Male, n (%)	107 (59.1%)	236 (65.4)
Cancer status at index		
Hematological	55 (30.4)	40 (11.1)
Lung or pleura	28 (15.5)	46 (12.7)
Reproductive (male)	12 (6.6)	32 (8.9)
Renal or Urological	12 (6.6)	29 (8.0)
Lower GI	12 (6.6)	47 (13.0)
Breast	10 (5.5)	27 (7.5)
Metastasized, n (%)	67 (37.0)	86 (23.8)
Medical History		
Hypertension, n (%)	87 (48.1)	220 (60.9)
Diabetes Mellitus, n (%)	31 (17.1)	73 (20.2)
Ischemic heart disease, n (%)	23 (13.3)	87 (24.1)
Peripheral arterial disease, n (%)	14 (7.7)	21 (5.8)
Ischemic stroke/ Transient ischemic attack, n (%)	22 (12.2)	61 (16.9)
Intracranial bleeding, n (%)	4 (2.2)	7 (1.9)
CHA_2DS_2-VASc score, median (IQR)	2 (2-4)	3 (2-4)
CHA_2DS_2-VASc score ≥ 1, n (%)	165 (91.2%)	349 (96.7)

FIGURE 1 Patient characteristics of patients with atrial fibrillation and cancer between 1st Jan 2012 and 31st Dec 2017

TABLE 1 Incidence rates per 100 patient years of thrombo-embolic and bleeding events in AF patients with cancer

	Cohort 1 *First Cancer, then AF diagnosis after 01-01-2012*		Cohort 2 *First AF, then cancer diagnosis after 01-01-2012*	
Number	**181**		**361**	
Total follow-up (years)	**170**		**379**	
	Number	**Incidence rate/100 patient years**	**Number**	**Incidence rate/100 patient years**
Bleeding events	23	13	39	10
- MB	12	7.0	22	5.8
- CRNMB	11	6.5	17	4.5
Arterial thromboembolism	7	4	18	4.8
Venous thromboembolism	3	2	7	2
Death	54	32	79	21

PB2077 | Stroke, Major Bleeding, and Net-clinical Benefit of Anticoagulation in Patients with Atrial Fibrillation on Hemodialysis

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Background: The benefit of anticoagulation in hemodialysis (HD) patients with atrial fibrillation (AF) is uncertain because of increased risk of both stroke and bleeding.

Aims: We aimed to evaluate anticoagulation in AF patients on HD.

Methods: In a prospective cohort of HD patients in Vienna, Austria, we selected patients with a diagnosis of AF at baseline or de-novo AF during follow-up (N=238, baseline characteristics given in table 1). During a maximum follow-up of 45 months, we recorded the occurrence of stroke, major bleeding, and for net-clinical benefit we analyzed a composite outcome of stroke, major bleeding and cardiovascular death. Exposure to anticoagulation included vitamin-K-antagonist (VKA) and long-term low-molecular-weight heparin (LMWH).

Risks for outcome occurrence for patients on anticoagulation were analyzed treating AF as a time-dependent variable and death as a competing event.

Results: During follow-up 25 (10.5%) patients suffered strokes, 42 (17.6%) patients had major bleeding, and 90 (39.4%) patients reached the net-clinical benefit endpoint. Anticoagulation was received by 99 patients (41.6%), including 61 (25.6%) patients on VKA and 38 (16.0%) on LMWH.

Treatment with anticoagulant agents did not result in a significantly reduced risk of stroke (subdistribution hazard ratio (SHR) 0.79, 95%CI 0.35-1.79, p=0.565), but a significantly increased risk of major bleeding after adjustment for prior stroke and antiplatelet co-medication (SHR 2.07, 95%CI 1.07-4.01, p=0.031) compared to no anticoagulation.

Patients on anticoagulation were further at significantly increased risk for occurrence of the net-clinical benefit outcome (SHR 1.55, 95%CI 1.03-2.34, p=0.037) which remained statistically significant after multivariable adjustment for prior stroke and antiplatelet co-medication (SHR 1.71, 95%CI 1.09-2.70, p=0.020). Especially patients with antiplatelet comedication were at increased risk for the net-clinical benefit outcome (table 2).

Conclusions: In HD patients with AF treated with anticoagulant agents, we found increased risk of major bleeding and no reduced risk of stroke, resulting in no net-clinical benefit of anticoagulation.

TABLE 1 Baseline patient characteristics according to anticoagulation treatment

Characteristic	No anticoaguatlion N = 139 (58.4%)	With anticoagulation N = 99 (41.6%)	p-value
Female sex	50 (36.0%)	70 (70.7%)	0.329
Age, median (25th to 75th percentile)	73 (69 - 76)	71 (70 - 74)	0.594
CHA2DS2-Vasc Score, median (25th to 75th percentile)	4 (3 - 5)	4 (3 - 4)	0.546
Prior stroke	25 (18.0%)	38 (38.4%)	0.001
Cardiovascular disease	84 (60.4%)	65 (65.7%)	0.419
Congestive heart failure	49 (35.3%)	43 (43.3%)	0.225
Diabetes	63 (45.7%)	39 (39.4%)	0.355
Smokers	64 (46.7%)	50 (51.5%)	0.508
Antiplatelet comedication	98 (70.5%)	39 (39.4%)	<0.001

TABLE 2 Multivariable subdistribution hazard model for net-clinical benefit

Covariable	Subdistribution hazard ratio	95% CI	p-value
No antithrombotic treatment	1	-	-
LMWH mono-therapy	1.57	0.52 - 4.74	0.420
VKA mono-therapy	2.80	1.19 - 6.56	0.018
Antiplatelet mono-therapy	2.05	0.90 - 4.64	0.087
LMWH + antiplatelet therapy	3.03	1.15 - 7.94	0.025
VKA + antiplatelet therapy	2.94	1.05 - 8.26	0.041
History of stroke	1.16	0.73 - 1.86	0.524

PB2078 | Study of Real Clinical Practice to Evaluate the Efficacy and Safety of Edoxaban in Prevention of Stroke in Patients with Non-valvular Atrial Fibrillation. Differences between Results by Age and Creatinine Clearance Greater than 95 ml/min

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Background: Edoxaban is a well-established treatment for the prevention of stroke in NVAF and Systemic Embolism. The older people (>75 y-o) represent a growing group, and have a high risk of complications. The FDA considered that, in patients with AF and a Creatinine Clearance > 95 ml/min, the use of edoxaban is not recommended.

Aims: Primary Objective: To evaluate the efficacy and safety of Edoxaban.

Secondary Objectives: To study the subpopulation of older people (> 75 y-o). To study the subpopulation of hyper-filters.

Methods: This is a descriptive study. A total of 672 patients with NVAF (97%) and DVT (3%) were included. The mean follow-up was 21 months with a median of 23 months. 47.9% women and 52.1% men. The mean age was 75 y-o, being 58.2% over 75 y-o. 16% of patients were hyper-filters. Mean CHA_2DS_2-VASc was 3.65. Mean HAS-BLED was 3.01.

Results: 0.5% of patients presented stroke. 4 patients presented severe bleeding (3 intracranial-bleeding), dying two of them. In older people, the average age was 81.7 y-o. Average CHA_2DS_2-VASc of 4.2 and average HAS-BLED of 3.19. 88% of deaths occurred in this group. Among hyper-filter patients, only one stroke occurred, representing 0.5% of this population. 35 patients finished the treatment and the anticoagulant treatment was changed in 4.3% of them.

Conclusions: This is a large sample with more than 23 months of follow-up. The good results of Edoxaban in normal clinical practice may be confirmed for general population, the older population and those with a creatinine clearance of more than 95 ml/min. These data reinforce and even improve the data obtained in the ETNA-AF Europe study with a percentage of Stroke/Systemic Embolism of 0.82% and 1.05% of Major Bleeding with 0.23% of Intracranial Bleeding.

PB2079 | Beyond the Registry: A Nuanced Evaluation of Real World Anticoagulation for Nonvalvular Atrial Fibrillation Patients at a Tertiary Care Center

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Background: Oral anticoagulant (OAC) therapy is a cornerstone of thromboembolic (TE) prevention in patients with atrial fibrillation (AF). A significant lack of OAC prescription to reduce TE risk across the U.S. has been described in the PINNACLE and ORBIT AF registries. Both registries use data from electronic medical records (EMR).

Aims: Understand if EMR diagnostic code data reflects the true clinical appropriateness of OAC therapy prescribing practice for nonvalvular AF patients.

Methods: A health data warehouse at our institution provided a list of patients ≥ 18 years old, enrolled in two primary care clinics from January 2012 until June 2019 who had a diagnostic code for

TABLE 1 Iterative percentage of AF patients prescribed anticoagulation following relevant exclusions

Documented Reasons Excluded	# Excluded	N	# Not Prescribed OAC	% Prescribed OAC
		Iterative Calculation		
		1,204	441	63.4%
Incorrect diagnosis	80	1,124	361	67.9%
No longer receives care at our institution	65	1,059	296	72%
Has left atrial occlusion device (WATCHMAN), or a documented discussion about implantation	32	1,027	264	74.3%
Major event or severely elevated bleeding risk (CNS or gastrointestinal bleed, HASBLED ≥ 5)	31	996	233	76.6%
Resolved AF (single episode > 1 yr ago, reversible cause, post ablation & followed by EP, post-operative AF, or heart transplant)	74	922	159	82.7%
Low CHA_2DS_2-VASc Score (female ≤ 2, male ≤ 1)	86	836	73	91.3%
Patient preference	22	814	51	93.7%

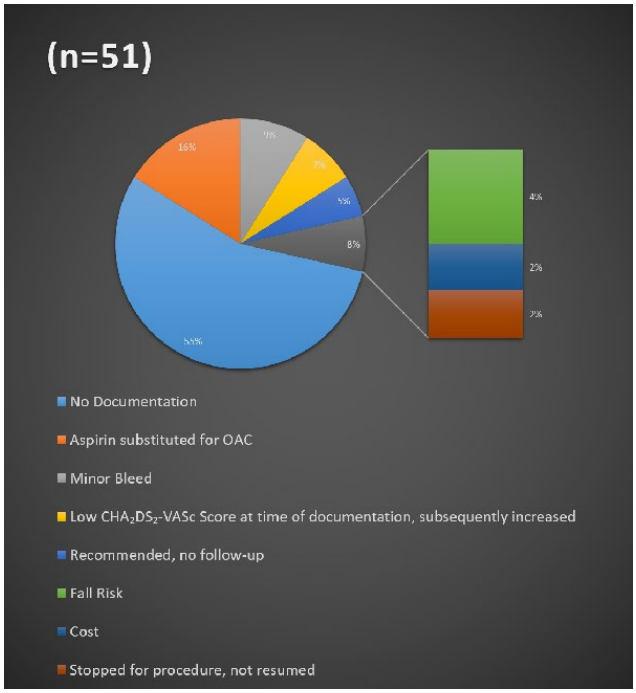

FIGURE 1 Documented Reasons AF Patients With a Class I Indication Were Not Prescribed Anticoagulation

nonvalvular AF in our EMR. A retrospective chart review was performed for each patient. Based on available documentation, iterative exclusions were made, which left a cohort of AF patients with a Class I indication (2019 AHA/ACC/HRS Focused Update) for anticoagulation (AC). Descriptive statistics (number and percentage) were reported for the baseline and Class I cohorts.

Results: The baseline cohort included 1,204 AF patients for review. Only 63% (n=763) were prescribed OAC therapy. Seven clinically relevant exclusions were made (n=390), which left 814 AF patients with a Class I indication for anticoagulation (see table). After exclusions, 93.7% of AF patients were prescribed OAC therapy. Documented reasons for withholding anticoagulation in AF patients with a Class I indication were aspirin substituted for anticoagulation (n=9), minor bleeding (n=5), OAC therapy recommended but not prescribed (n=4), and a CHA_2DS_2-VASc score that increased following time of documentation (n=4) (see figure).

Conclusions: Registries that use EMR data may underestimate the true performance of health system adherence to guideline recommended anticoagulation prescribing practice for patients with nonvalvular AF.

PB2080 | Association of Body Mass Index (BMI) with Bleeding Events in Patients Taking Oral Anticoagulation for Atrial Fibrillation (AF) or Venous Thromboembolism (VTE)

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Background: To decrease the risk of thrombotic events, patients with AF and VTE are treated with warfarin or a direct oral anticoagulant (DOAC), which can cause bleeding. The association between bleeding and patient weight in these patients is uncertain.

Aims: To compare bleeding rates in patients taking oral anticoagulants across various levels of Body Mass Index (BMI).

Methods: Patients within the Michigan Anticoagulation Quality Improvement Initiative ($MAQI^2$) database taking either warfarin or a DOAC for AF or VTE between June 2015 to September 2019 were separated into six groups based on BMI. Minor, major, and life-threatening bleeding events were identified within each group. Event rates (# per 100 pt-yr) of the normal weight patients were compared to patients in the underweight group and each of the overweight groups using Poisson test.

Results: With warfarin, underweight patients had significantly higher rates of major bleeding compared to normal weight patients (9.8 vs. 6.0, p=0.003) (Table). There was no significant difference in major bleeding rates between normal weight and each of the higher BMI groups. With DOACs, there was no significant difference in major bleed rates in underweight compared to normal weight patients (9.6 vs. 7.8, p=0.2). However, patients in each of the higher BMI groups had significantly lower rates of major bleeding.

Conclusions: In a large cohort of patients treated with warfarin or DOACs for AF and VTE, we observed associations between BMI and bleeding rates. Underweight warfarin patients had more major bleeds while higher BMI patients had similar major bleeds compared to normal weight patients. Higher BMI patients taking DOACs had fewer major bleeds compared to normal weight patients. These findings may be related to lower drug levels in higher BMI patients taking DOACs which is not an issue for warfarin patients. Further studies looking at the correlation with DOAC drug levels and BMI are warranted.

TABLE 1 Bleeding Outcomes

Warfarin Patients (N=3,354)	BMI <19.9 Underweight (n=157)	20-24.9 Normal (n=579)	25-29.9 Overweight (n=966)	30-34.9 Class 1 Obesity (n=756)	35-39.9 Class II obesity (n=391)	>40 Morbidly Obese (n=505)
Minor Bleeds /100 pt-yr (p-value compared to normal BMI group)	24.5 (0.03)	29.6	24.5 (0.03)	26.3 (0.18)	23.7 (0.01)	24.9 (0.05)
Major Bleeds /100 pt-yr (p-value compared to normal BMI group)	9.8 (0.003)	6.0	5.7 (0.85)	4.6 (0.21)	6.0 (1)	5.4 (0.64)
Life-threatening Bleeds /100 pt-yr (p-value compared to normal BMI group)	4.1 (0.09)	2.6	2.0 (0.46)	1.8 (0.29)	2.7 (1)	2.8 (0.89)
DOAC patients (N=2,977)	<19.9 Underweight (n=97)	20-24.9 Normal (n=537)	25-29.9 Overweight (n=860)	30-34.9 Class 1 Obesity (n=760)	35-39.9 Class II obesity (n=353)	>40 Morbidly Obese (n=370
Minor Bleeds /100 pt-yr (p-value compared to normal BMI group)	33.0 (0.02)	39.5	33.1 (0.02)	29.3 (<0.001)	23.9 (<0.001)	24.4 (<0.001)
Major Bleeds /100 pt-yr (p-value compared to normal BMI group)	9.6 (0.02)	7.8	3.6 (<0.001)	3.4 (<0.001)	2.6 (<0.001)	4.9 (0.01)
Life-threatening Bleeds /100 pt-yr (p-value compared to normal BMI group)	3.2 (0.35)	2.4	0.8 (0.01)	1.0 (0.02)	0.9 (0.01)	1.6 (0.27)

PB2081 | The Association between Neighborhood Socioeconomic Status, Geographical Area and Non-persistence to Using DOACs

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Background: Previous studies have described suboptimal persistence to direct oral anticoagulant (DOAC) use for which the reasons are not well known.

Aims: To study whether urbanization, neighborhood socioeconomic status or regional policy of DOAC prescribing (identified by geographical area) is associated with DOAC non-persistence.

Methods: Dispensing data from the Dutch Foundation of Pharmaceutical Statistics were used to monitor non-persistence to DOAC in atrial fibrillation (AF) patients from January 2012-April 2016. AF patients who could be followed for at least 1 year were included. Non-persistence was defined as the cumulative incidence

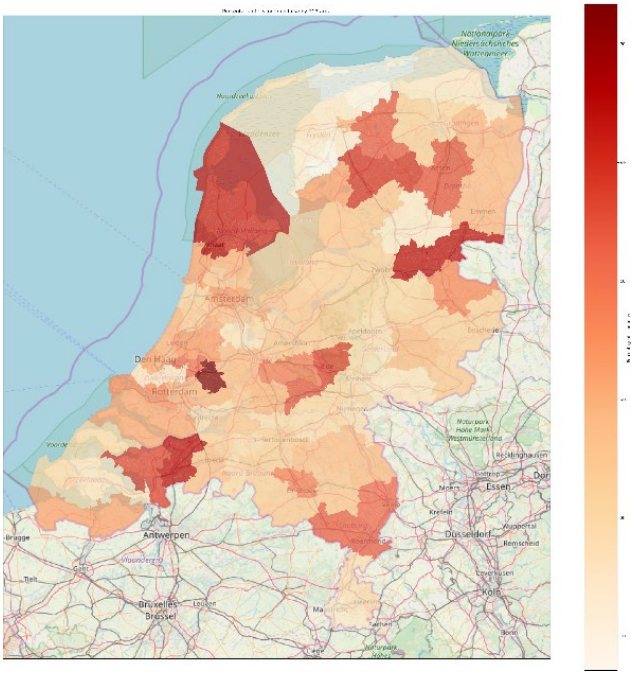

FIGURE 1 Geographical distribution of non-persistence to DOAC in the Netherlands (red is highest)

of patients who discontinued their DOAC within 1 year. The association between non-persistence and neighborhood socioeconomic status was evaluated by considering property value, status-score (which combines educational level, income and unemployment) and urbanization (all stratified by quintiles). In addition, we studied non-persistence to DOAC across geographic areas.

Results: 43670 AF patients were included (mean age 70 years), 20130 (46%) were men, 4951 (11%) used apixaban, 18387 (42%) dabigatran, and 20332 (47%) rivaroxaban. The overall non-persistence rate was 41% within 12 months. Non-persistence was not associated with urbanization, status-score or property value (first vs other quintiles, risk ratios ranged between 0.91-1.02). Geographical rates of non-persistence ranged between 17-73% (Figure 1). Of the lowest scoring regions (bottom five), all prescribed rivaroxaban most often, showing that non-persistence may be related to rivaroxaban use. However, in the highest scoring regions (top five) two also prescribed rivaroxaban most often, suggesting that non-persistence is also related with prescribing patterns (e.g., instructions, monitoring).

Conclusions: In the Netherlands, non-persistence to DOAC was not associated with urbanization, neighborhood socioeconomic status or neighborhood property value. However, DOAC non-persistence varied substantially across areas, suggesting that local policy, for example monitoring, may be relevant for DOAC persistence.

PB2082 | Inappropriate Dosing of Direct Oral Anticoagulants in Older Patients with Non-Valvular Atrial Fibrillation: Impact on Plasma Drug Levels

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Background: Direct oral anticoagulants (DOAC) are increasingly prescribed in older adults with non-valvular atrial fibrillation (NVAF). However, inadequate low doses are frequently used. In Belgium, 43% of DOAC patients are treated with a reduced dose, much more than observed in phase III trials.

Aims: This study aimed at investigating the impact of inadequate low DOAC doses on plasma drug levels in older NVAF patients.

Methods: We performed a retrospective analysis of electronic medical records (2012-2018). Older NVAF patients (≥ 75 years) treated with apixaban, rivaroxaban or dabigatran etexilate and who underwent DOAC measurement at peak or trough were included. DOAC plasma levels were compared between 2 groups (appropriate dose vs inappropriate low dose), and to the on-therapy ranges observed in pivotal trials.

Results: This analysis included 80 patients (median age [IQR] 81 [77-85]), treated with apixaban (n=33), dabigatran etexilate (n=18) and rivaroxaban (n=29). DOAC dosing was appropriate for 54 patients (68%). Eighteen patients (23%) were receiving an inappropriate low dose (Table 1).

In total, 152 DOAC measurements were performed (figure 1). At peak, mean apixaban and rivaroxaban levels were significantly lower for "inappropriate low dose" patients compared to "appropriate dose" patients (p=0.0092 and 0.0090 respectively). At trough, mean DOAC plasma levels did not differ between groups.

Four (16%) peak and one (7%) trough measurements were under the expected range for "inappropriate low dose" patients. On the contrary, 24 (39%) peak and 7 (18%) trough measurements were above the expected range for "appropriate dose" patients.

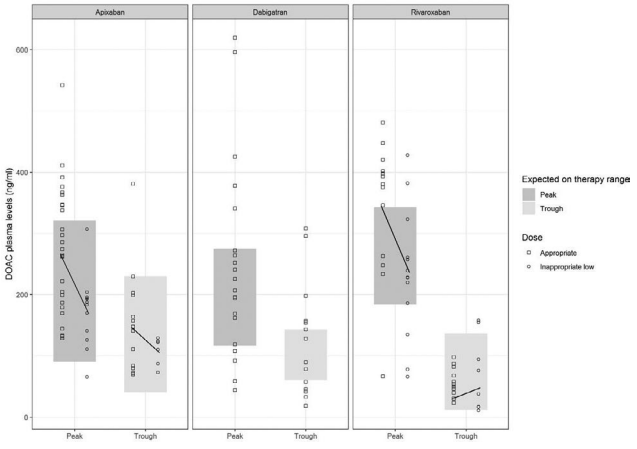

FIGURE 1 DOAC plasma levels according to the appropriateness of dosing

TABLE 1 Appropriateness of dosing according to DOAC

	Appropriate dose (n [%])	Inappropriate low dose (n [%])	Inappropriate high dose (n [%])	TOTAL (n)
Apixaban	21 [64]	9 [27]	3 [9]	33
Dabigatran etexilate	18 [100]	0 [0]	0 [0]	18
Rivaroxaban	15 [52]	9 [31]	5 [17]	29
All DOACs	54 [68]	18 [23]	8 [10]	80

Conclusions: Apixaban and rivaroxaban were frequently prescribed at an inappropriate low dose in older NVAF patients. However, most underdosed patients had DOAC levels within the expected on-therapy range. Moreover, a high proportion of patients with an appropriate dose had higher-than-expected peak levels. This raises questions about the optimal DOAC regimen in real-life older patients.

PB2084 | The Impact of Soluble Thrombomodulin and Circulating miRNAs on Catheter Ablation in Patients with Atrial Fibrillation

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Background: Atrial fibrillation (AF) is the common cardiac arrhythmia and has a high risk of thromboembolism. The most effective approach, catheter ablation (CA), requires evaluation by electrocardiography.

Aims: The aim of present study was to investigate novel clinical markers that predict restoration of sinus rhythm (SR) after CA.

Methods: We studied 78 consecutive patients who provided written informed consent for all procedures. Patients with AF underwent CA were separated into 2 groups: restored SR and recurrent AF. The levels of 4 blood proteins (serum or plasma) and 3 mature microRNAs (miRNAs) and their primary miRNAs (pri-miRNAs) in serum were measured before and after CA, and the associations between each parameter were analyzed statistically. The protocol of this study was approved by the Institutional Ethics Committee of (Receipt number: 26-96).

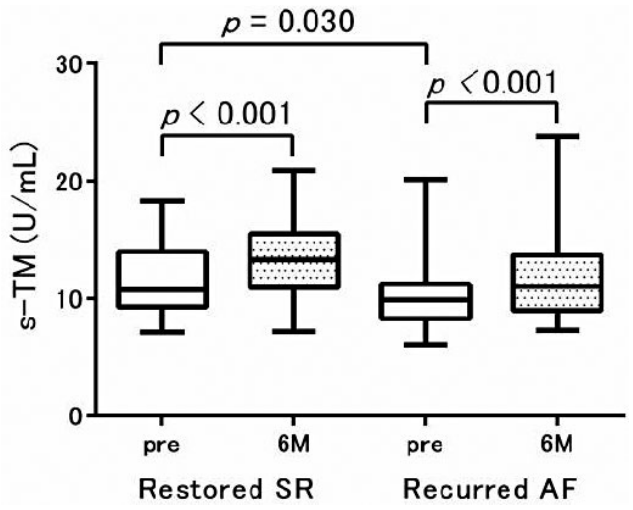

FIGURE 1 The level of s-TM at baseline was higher in the restored SR group than the recurrent AF group

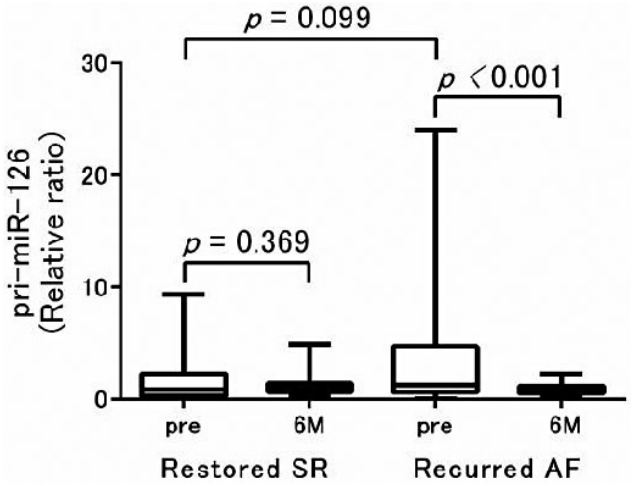

FIGURE 2 Pri-miR-126 Decreased after Ablation in the Recurrent AF Group

Results: Thirty-two (41%) patients restored sinus rhythm (SR) and 46 (59%) patients recurred AF at 6-month after CA. Soluble thrombomodulin (s-TM) and plasminogen activator inhibitor-1 (PAI-1) levels increased above baseline after ablation in both the restored SR (s-TM 11.55 [2.92] vs 13.75 [3.38], P < .001; PAI-1 25.74 [15.25] vs 37.79 [19.56], P < .001) and recurrent AF (s-TM 10.28 [2.78] vs 11.67 [3.37], P < .001; PAI-1 26.16 [15.70] vs 40.74 [22.55], P < .001) groups. Levels of high-sensitive C-reactive protein (hs-CRP) and asymmetric dimethylarginine (ADMA) were not significantly changed. Pri-miR-126 levels significantly decreased after ablation in the recurrent AF group, but the other miRNAs and pri-miRNAs did not.

Conclusions: The measurement of s-TM and pri-miR-126 in blood was a useful tool to reflect the condition of AF patients with CA.

PB2085 | Concomitant Use of Direct Oral Anticoagulants and Antiepileptic Drugs: A Prospective Cohort Study in Patients with Atrial Fibrillation

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Background: European guidelines do not recommend the use of carmazepime, levetiracetam, phenobarbital, phenytoin, topiramate and valproic acid in patients taking direct oral anticoagulants (DOACs). The clinical value of the interaction between DOACs and antiepileptic drugs is currently unexplored.

Aims: To evaluate the incidence of thromboembolic and bleeding events in patients with non-valvular atrial fibrillation (AF) treated with DOACs and antiepileptic drugs.

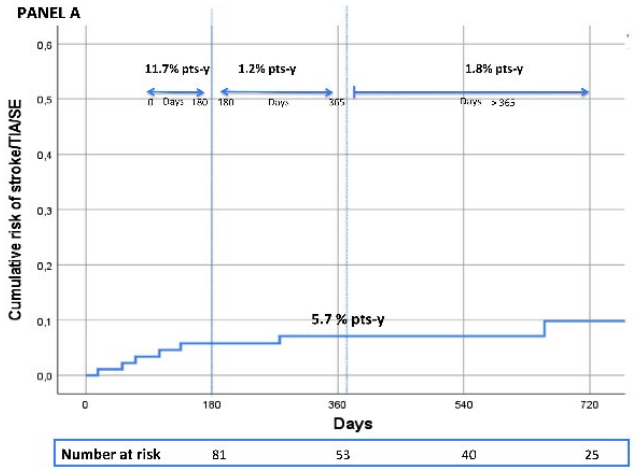

FIGURE 1 Cumulative incidence rates of ischemic stroke/TIA/SE

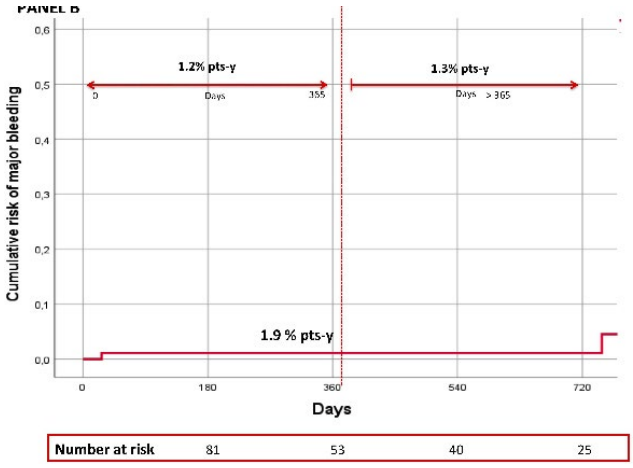

FIGURE 2 Cumulative incidence rates of major bleeding

Methods: Prospective multicentre cohort study of patients with non-valvular AF concurrently treated with DOACs and antiepileptic drugs. The primary outcome was ischemic stroke/transient ischaemic attack (TIA)/systemic embolism (SE). Secondary outcome was major bleeding (MB).

Results: Overall, 91 patients were included. Mean age was 78±9.5 years, 49.5% were female. Mean CHA_2DS_2-VASc was 4.76±1.59 and mean HAS-BLED was 2.67±1.26. Overall, 41, 20, 11, 10 and 9 out of 91 patients were treated with levetiracetam, valproic acid, phenobarbital, carbamazepine and other antiepileptic drugs, respectively. During a median follow-up of 17.5±14.5 months, stroke/TIA/SE occurred in 9 patients (5.7% patient-year) and MB in 3 patients (1.9% patient-year). Ischemic stroke was fatal in 3 patients (1.9% patient-year) and MB in one patient (0.6% patient-year). The figure shows the risk and incidence rates of ischemic stroke/TIA/SE (Panel A) and of major bleeding (Panel B) over time.

Conclusions: This is the first study reporting on the incidence of ischemic stroke/TIA/SE in a cohort of AF patients treated with DOACs and antiepileptic drugs. A relatively high rate of stroke/TIA/SE and a low rate of MB were observed in our cohort.

PB2086 | An observational Study of INR Control and Bleeding Events, According to European Society of Cardiology (ESC) Guidelines, in Non-valvular Atrial Fibrillation (NVAF) Stratified by Male and Female Sex

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Background: The ESC AF guidelines recommend patients treated with vitamin-K antagonists (e.g. warfarin), have their time in therapeutic range (TTR) kept as "high as possible" e.g. ≥ 70%. The CHADS2D2Vasc risk assessment tool has replaced CHADS2 score for identifying patients with NVAF who should receive oral anticoagulation (OAC). Female sex was identified as an additional stroke risk factor. HASBLED bleeding risk score does not discriminate between sexes.

Aims: High TTR is associated with reduced stroke risk. We aimed to determine whether poor TTR (i.e. ≤ 70%) was associated with bleeding outcomes and explore potential relationships with important covariates including sex.

Methods: Patient-level, population-scale, linked, anonymised data for warfarin-treated AF patients in Wales (UK). Descriptive statistics and Cox multiple regression estimates of hazard ratio for bleeding with TTR 70% ≤ and association with sex.

Results: Total 35,036 AF patients met inclusion criteria; mean age 74 years, 43% female sex, mean CHA2DS2Vasc 3.5 (SD1.7), of whom 15,074 (43%) had TTR ≤ 70%. A total of 5,809 bleeds occurred in 5,302 patients (table 1).

Multivariate models adjusted for relevant covariates (age, sex, prior bleeding, hypertension, diabetes, heart failure, ischaemic heart disease, stroke, peripheral vascular disease (PVD), thromboembolism,

TABLE 1 Bleeding events by site and sex

	GI	Urinary	ICH	Respiratory	Gynaecological	Ocular	Other
Total	2224 (38.3%)	1492 (25.7%)	601 (10.3%)	581 (10.0%)	334 (5.7%)	138 (2.4%)	439 (7.6%)
Male	1313 (59%)	1121 (75.1%)	332 (55.2%)	375 (64.5%)	N/A	76 (55.1%)	271 (61.7%)
Female	911 (41%)	371 (24.9%)	269 (44.8%)	206 (35.5%)	334 (100%)	62 (44.9%)	168 (38.3%)

liver disease, chronic kidney disease, and alcohol), showed TTR < 70% was associated with an overall increased hazard ratio of bleeding (1.45 [95%CI 1.37-1.53]) p< 0.001 with female sex associated with fewer bleeds (HR 0.84 [95%CI 0.80-0.89]) p< 0.001. These relationships were similar when considering other guideline criteria for poor INR control (e.g. UK NICE).

Conclusions: Periods of poor INR control are associated with increased bleeding event rates, independently of commonly recognised stroke and bleeding risk factors. Of note, bleeding risk is independently lower in females, emphasising the importance of exploring further the relationships between sex and site-specific bleeding risk.

PB2087 | Intracardiac Levels of Soluble P Selectin Is Associated with Ischemic Stroke Risk in Patients with Atrial Fibrillation

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Background: Atrial fibrillation (AF) is the most prevalent sustained arrhythmia that associates with an increased risk of stroke. Although in AF local, intracardiac hemostasis changes are thought to occur, limited data exists on soluble markers of platelet and endothel activation reflecting the local environment of the left atrium and their association with the risk of ischemic stroke.

Aims: Here we aimed to test whether levels of soluble P selectin (sPsel) and soluble E selectin (sEsel) differ in intracardiac blood samples of patients with AF as compared to a peripheral site. Also, we aimed to determine the association of intracardiac sPsel and sEsel levels with ischemic stroke risk.

Methods: Patient group consisted of 90 patients with paroxismal/persistent AF undergoing elective transcatheter ablation procedure. All medications influencing platelet activity were discontinued at least 10 days prior to procedure. Blood samples were drawn from the femoral vein and the left atrium before the initiation of ablation. Levels of sPsel and sEsel were determined from platelet-free plasma using ELISA method. Patients were grouped according to their estimated stroke risk based on their CHA2DS2-VASc score (0-1 points: "low-risk": group A, n=49 and 2-6 points: "high-risk": group B, n=41). All patients provided written informed consent.

Results: In the whole cohort, elevated levels of sPsel was found in intracardiac and peripheral samples as well. Levels of sEsel showed slight, but significant increase in intracardiac vs. peripheral samples in the whole cohort (mean±SD: 28.0±12.1 ng/mL vs. 26.3±11.5 ng/mL; p< 0.001). As compared to the group with low-risk, intracardiac sPsel was significantly elevated in the high-risk stroke group (group A: median: 35.6; IQR: 26.6-46.7 ng/mL vs group B: 44.6; IQR: 32.3-60.1 ng/mL; p=0.01). Intracardiac levels of sEsel did not differ between groups.

Conclusions: Intracardiac levels of sPsel is associated with the estimated ischemic stroke risk in patients with AF.

PB2088 | Atrial Fibrillation Treated with Warfarin at an Academic Medical Center: The Potential Impact of Direct Oral Anticoagulants on Future Management

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Background: Direct oral anticoagulants (DOACs) are the standard of care for stroke prevention in atrial fibrillation (AF). There are many patients on warfarin who may be eligible for DOAC therapy but have not been transitioned. The Brigham and Women's Hospital Anticoagulation Management Clinic (BWH AMS) pharmacists manage patients with AF on either warfarin or a DOAC.

Aims: To describe a systematic process for transitioning our eligible AF patients on warfarin to a DOAC.

Methods: After identifying patients eligible for DOAC transition based on previously described criteria (Figure 1), patients were evaluated in a multidisciplinary discussion between the BWH AMS clinician and referring physician. Patients approved for transition by both providers were contacted and offered the opportunity to switch anticoagulants. Selection of the specific DOAC was driven by the AMS clinician accounting for patient characteristics, preferences, and cost. Reasons for not transitioning eligible patients were documented to identify real-world barriers.

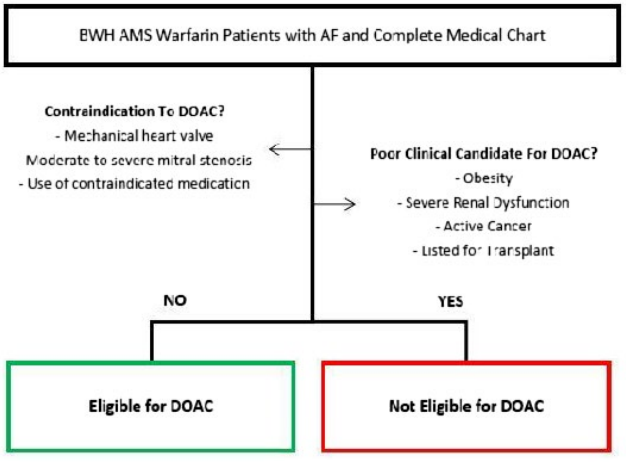

FIGURE 1 BWH AMS Criteria for Warfarin to DOAC Transition for Patients with Atrial Fibrillation

Total Patients Eligible for DOAC	438 (%)
Exclusions	
Death	5 (1.1%)
Loss to Follow Up	6 (1.4%)
Lack of Physician Response	15 (3.4%)
Identified Barriers to Transition	
AMS Clinician Preference	47 (10.7%)
Physician Preference	74 (16.9%)
Patient Preference	155 (35.4%)
Cost	54 (12.3%)
Previous Intolerance	15 (3.4%)
Total Patients Successfully Transitioned	**67 (15.3%)**

FIGURE 2 Exclusions and Barriers to Transition from Warfarin to DOAC

Results: Of the 438 eligible warfarin patients, 67 warfarin patients were transitioned to a DOAC as part of this initiative (Figure 2). There were 47 patients excluded due to AMS clinician preference and 74 by physician preference. Other reasons for exclusion included death (n=5, 1.1%), loss to follow up (n=6, 1.4%), and lack of physician response (n=15, 3.4%). For the patients who were contacted regarding DOAC therapy and declined, the most common reasons were comfort with warfarin therapy (n=155, 35.4%), cost (n=54, 12.3%), or previous intolerance to a DOAC (n=15, 3.4%). All successfully transitioned patients were initiated on either apixaban (80.6%) or rivaroxaban (19.4%).

Conclusions: Every institution or provider network that manages AF patients on warfarin should periodically assess their population to identify potential candidates for DOAC therapy. Our initiative outlines a process for transitioning eligible AF patients on warfarin to a DOAC and identifies the most common barriers that we encountered.

PB2090 | Trends in Direct Oral Anticoagulant Use in Renal Failure

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Background: In patients with atrial fibrillation and end-stage chronic kidney disease, the use of warfarin or direct oral anticoagulants (DOACs) for stroke prevention is controversial. Observational studies have shown a steady increase in the use of apixaban versus warfarin in atrial fibrillation (AF) patients on dialysis, and the American College of Cardiology guidelines state it might be reasonable to prescribe warfarin or apixaban in these patients while dabigatran, rivaroxaban and edoxaban are not recommended. The American College of Chest Physicians state DOACs are contraindicated for treatment of venous thromboembolism (VTE) in patients with severe renal impairment.

Aims: To evaluate if the proportion of patients with end-stage renal disease is increasing among patients prescribed different oral anticoagulants for AF or VTE.

Methods: The Michigan Anticoagulation Quality Improvement Initiative (MAQI2) is an ongoing registry of six anticoagulation clinics in the United Sates. Trained staff abstract a random sample of charts from patients initiating oral anticoagulation. The proportion of patients with end-stage renal disease (CrCl $\leq$ 15mL/min) within each year was calculated from the beginning of 2015 to end of 2019. Test for trend was done with chi-square and linear regression.

Results: In warfarin patients, the proportion of patients with end-stage renal disease ranged from 2.1-3.4% and increased slightly (P=0.001) over time (table). End-stage renal disease among apixaban patients ranged from 0-0.9%, and there was no trend (P=0.25) for increasing prevalence. Only 2 patients were treated with rivaroxaban and no patients on dabigatran or edoxaban had CrCl $\leq$ 15mL/min.

Conclusions: End-stage renal failure is uncommon in our six anticoagulation clinics, and we have not observed an increased prevalence in apixaban treated patients. A slight increase over time in warfarin patients may be indirect evidence of its preference over DOACs in patients with advanced kidney disease.

TABLE 1 Prevalence of end-stage renal disease

	2015	2016	2017	2018	2019	p-value
Warfarin patients	3702	3732	3364	3204	2989	–
CrCl≤15 mL/min, N (%)	76 (2.1)	79 (2.1)	88 (2.6)	103 (3.2)	100 (3.4)	0.001
Apixaban patients	30	766	1032	1104	1396	–
CrCl≤15 mL/min, N (%)	0	7 (0.9)	3 (0.3)	8 (0.7)	12 (0.9)	0.25
Rivaroxaban patients	8	509	654	588	530	–
CrCl≤15 mL/min, N (%)	0	1 (0.2)	0	0	1 (0.2)	–

PB2091 | Preliminary Results from EGINA Registry: A New Model in the Management of Anticoagulation with DOACs in Southern Italy

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Background: During the last 40 years oral anticoagulant therapy has been managed in Italy in patients with atrial fibrillation (AF) and venous thromboembolism (VTE) by a network of Anticoagulation Centers (ACs). From 2013 onwards, direct oral anticoagulants (DOACs) have been made available. In patients taking DOACs, ACs have no longer performed periodical clinical and laboratory evaluations because of a different follow-up system, not requiring frequent blood withdrawal for the modification of the drug dosage. Since 2018 EGINA (Excellence model for the Integrated Management of New Anticoagulants) model has been used in 19 southern Italy ACs in order to improve the clinical management of patients taking DOACs.
Aims: The purpose of this study was to evaluate the incidence of ischemic and hemorrhagic events in patients taking DOACs, followed by ACs according to EGINA model.
Methods: 8 southern Italy ACs provided data on patients anticoagulated with DOACs. Patients with nonvalvular AF in anticoagulant prophylaxis with a DOAC, with a minimum observation time of 12 months, were included. Data were collected retrospectively.
Results: Overall 372 subjects with AF has been assessed. Mean age was 76.01 (ranging from 35 to 98 years old) and 179 patients were female (48.1%). On average CHA2D2-VASC score was 3.23 (SD= 1.23) and HAS-BLED 1.79 (SD= 0.95). Rivaroxaban was the most prescribed DOACs (36.8% of patients), followed by apixaban (25.8%), edoxaban (22.3%) and dabigatran (15.1%). The 36.6% of included subjects were naïve for anticoagulant therapy. Over 12-month follow-up, we observed an overall incidence of 2.7% for minor bleedings and of 1.3% for major bleedings. In our cohort, no ischemic event was observed.
Conclusions: EGINA model propose as a new protocol to be used by ACs for the clinical management of patients taking DOACs. Preliminary data from our registry show promising results with a low incidence of bleeding events.

PB2092 | Using Baseline Characteristics to Differentiate Atrial Fibrillation Prevalence and Incidence in Pulmonary Embolism

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Background: Pulmonary embolism (PE) has been associated with increased prevalence and incidence of atrial fibrillation (AF).
Aims: We sought to investigate the differences in baseline characteristics that may differentiate patients with preexisting versus new onset atrial fibrillation after presenting with an acute PE episode.
Methods: Study participants were enrolled from a Pulmonary Embolism Response Team (PERT) registry between March 2016 and March 2019 at two hospital health systems. Baseline characteristics like BMI, PESI, and CHA2DS2-VASc were analyzed to differentiate these three groups. Kruskal Wallis ANOVA tests, Dunn's multiple comparison tests, and Mann-Whitney U-tests were done using GraphPad Prism software.
Results: Out of 398 PE patients, the prevalence of AF was 10.6% (42 patients) while the incidence of AF was 9.0% (36 patients). PESI scores were overall significantly different between the PE patients with a prior diagnosis of AF, PE patients with no diagnoses of AF, and PE patients with a subsequent diagnosis of AF (130.7±0.5 vs. 115.0±3.3 vs. 119.4±17.0, p=0.0328). PESI score was significantly higher in PE patients with a prior diagnosis of AF compared to PE patients who did not develop AF (p=0.0341). There were no significant differences in BMI between the three groups and in CHA2DS2-VASc scores between the two groups (p>0.05).
Conclusions: In our cohort, 20% of PE patients also had a diagnosis of AF. PE Patients with a prior diagnosis of AF had an elevated PESI score, suggesting that AF may increase the risk of mortality and morbidity after a PE. CHA2DS2-VASc scores were also not found to be significantly different in patients with a prior diagnosis of AF patients and patients who developed AF after their PE episode. This data suggests that there is a high prevalence and incidence of AF in patients with PE and that AF increases the severity of a PE episode.

PB2093 | Predictive Value of CBC Indices in Determining Risk of Atrial Fibrillation After Pulmonary Embolism

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Background: Pulmonary embolism (PE) has been demonstrated to increase the incidence and prevalence of atrial fibrillation (AF) through right sided cardiac pressure overload and elevated cytokines.
Aims: We sought to investigate predictive accuracy of complete blood count (CBC) indices and ratios in differentiating patients with

preexisting versus new onset atrial fibrillation after presenting acute PE episode.

Methods: Acute PE patients were divided into 3 groups: prior diagnosis of AF (n=26), patients who developed AF after PE (n=26), and patients who do not develop AF (n=247). CBC indices as well as neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), lymphocyte to monocyte ratio (LMR), and platelet to neutrophil ratio (PNR) were compared in these three groups.

Results: Hemoglobin were overall significantly different between the PE patients with a prior diagnosis of AF, PE patients who did not develop AF, and PE patients with a subsequent diagnosis of AF (10.7±0.5 vs. 12.5±0.2 vs. 12.5±0.4, p=0.0028). Hemoglobin was significantly lower in PE patients with a prior diagnosis of AF compared to PE patients who did not develop AF (p=0.0019) and PE patients who did develop AF (p=.0361). Hemoglobin was significantly lower in PE patients with a prior diagnosis of AF compared to PE patients who did not develop AF (p=0.0032). RDW was overall significantly different a prior diagnosis of AF, PE patients who did not develop AF, and PE patients with a subsequent diagnosis of AF (17.0±0.7 vs. 15.4±0.2 vs. 15.0±0.3, p=0.0491). RDW was significantly higher in PE patients with a prior diagnosis of AF compared to PE patients who did not develop AF (p=0.0432).

Conclusions: Decreased levels of Hgb and hematocrit as well as increased RDW indicates that patients with preexisting AF may have an increased amount of inflammation that necessitates bone marrow compensation.

PB2094 | Profile of Patients Using Oral Anticoagulants in Primary Care: *'Anticoagula Divinópolis"* Project

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Background: Warfarin is the most widely used anticoagulant in low and middle income countries. Adherence to treatment, knowledge concerning warfarin therapy and health literacy are directed related to the success of patient control. The "*Anticoagula Divinópolis*" Project aims to implement an anticoagulation system of care in a medium income city in Minas Gerais, Southeast Brazil, to improve rates of control and reduce the incidence of thromboembolic and hemorrhagic events.

Aims: To assess the profile of primary care patients on warfarin therapy followed up in primary care units (PCU), prior to the implementation of the program.

Methods: Retrospective study, which assessed adult patients using warfarin in 20 PCU from May/18 to May/19. Clinical, sociodemographic, and other features involving warfarin were evaluated, as well as the following tests: Oral Anticoagulation Knowledge (OAK) Test; measurement of adherence to treatment (adapted MAT test) and evaluation of health literacy (Short Assessment of Health Literacy For Portuguese-Speaking Adults - SAHLPA-18).

Results: The 117 patients included were 65±12 years old on average, and 56.4% were women. The main indications for anticoagulation were atrial fibrillation (AF - 33.0%), venous thromboembolism (25.0%) and mechanical valve prosthesis (17.0%). In 15.9% of the cases, the reason for anticoagulation was not registered. In all AF cases, the CHADS2Vasc or HAS-BLED scores were not registered in the patient records. By the adapted MAT, 85.1% of the patients are adherent to therapy. There were 52.0% of correct answers in the OAK Test, reflecting patients' insufficient knowledge about warfarin therapy, and 71.8% had insufficient health literacy by the SAHLPA-18 score.

Conclusions: Patients have inadequate health literacy and insufficient knowledge about warfarin therapy, and the medical team is unaware of the importance of assessing thromboembolic risk. These data are essential for developing strategies to improve anticoagulation control.

CANCER ASSOCIATED THROMBOSIS

PB2095 | Efficacy of Primary Thromboprophylaxis Agents (PTA) in Multiple Myeloma (MM) Treated with Immunomodulators (IMiD): A Network Meta-Analysis

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Background: MM patients are at an increased risk of venous thromboembolic events (VTE). This risk is further augmented with the use of current induction chemotherapy regimens that incorporate IMiDs. Studies of frontline IMiD-containing regimens have reported VTE rates up to 30%. Several trials evaluated the benefits of thromboprophylaxis with aspirin (ASA), low-dose warfarin (WAR), low molecular weight heparin (LMWH) and direct oral anticoagulants (DOAC). Some studies compared these agents to no intervention and some to one another with conflicting results. There has been no trial comparing all four classes in a head-to-head manner.

Aims: To compare the relative efficacy of commonly used PTA in MM, this network meta-analysis was conducted.

Methods: A review of the medical literature was conducted using online databases. Inclusion criteria consisted of English language, diagnosis of multiple myeloma, treatment with IMiD-containing regimens, comparative studies using thromboprophylaxis with ASA, WAR, LMWH, &/or DOAC, and studies that reported the incident of VTE in the compared arms. Studies that used a non-IMiD-based regimen as comparator were excluded. A frequentist network meta-analysis was conducted using the netmeta package and random effects model.

Results: Eleven studies involving 4 PTA classes and comprising a total of 2776 participants were included. ASA, LMWH, and WAR were each significantly superior to no intervention in lowering the relative risk of VTE. Based on the pair-wise and network meta-analyses, LMWH was ranked as the most effective PTA followed by ASA, WAR, and DOAC in a decreasing order. Inconsistency analysis did not reveal any significant differences between direct and indirect estimates.

Conclusions: This is the first network meta-analysis to compare all commonly utilized PTA in MM revealing LMWH to be the most effective. While DOAC ranked as the least effective PTA, it may be the result of small numbers and wide confidence intervals. Adequately powered randomized trials are warranted.

PB2096 | L-Asparaginase Induced Hyperlipidemia, Pseudohyponatremia and Cerebral Venous Thrombosis during Acute Lymphoblastic Leukemia Remission Induction Therapy

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Background: L-Asparaginase (L-ASP) is a chemotherapeutic agent used to induce disease remission in children with acute lymphoblastic leukemia (ALL). Common side effects such as allergy and pancreatitis, as well as coagulation and lipid disturbances may ocur within L-ASP treatment. Hypertriglyceridemia and low antithrombin 3 levels are the most important laboratory abnormalities. Low antithrombin 3 activity is the most important cause of thrombosis.

Aims: L-ASP is a critical component of ALL treatment but is associated with multiple potential toxicities, including hypertriglyceridemia.We present a case with hypertriglyceridemia presenting as pseudohyponatremia and cerebral venous thrombosis.

Methods: –

Results: We present a 15 year old male patient diagnosed with ALL followed up with treatment of ALLIC-BFM 2009 moderate chemotherapy risk group. Eighth dose of L-ASP therapy was applied on the 33rd day of chemotherapy regimen. Then, three days later (on 36rd day) patients admitted with complaints of abdominal pain, nausea and severe headache. Stupor was detected on physical examination. Initial laboratory findings were as follows; Serum sodium: 125 mmol/L, Aspartate aminotransferase: 88 U/L, Alanine aminotransferase:123 U/L, Total bilirubine:0.3 mg/dL, Triglyceride:1506 mg/dL, Total cholesterol :349 mg/dL, HDL: 92.3 mg/dL, LDL:154.7 mg/dL, Antithrombin 3 activity 35.6%, Fibrinogen:101.1 mg/dL, Amylase:60/L, Lipase:18 U/L. Confluens sinus, left transverse sinus trombosis and a filling defect compatible with cerebral venous thrombosis was observed on brain magnetic resonance imaging scans. Low molecular weight heparin, gemfibrozil, diet and supportive treatment were initiated in the patient. Clinical improvement was seen after 2 weeks. Improvement in the laboratory parameters were found at the end of the first month. In addition, recanalized venous sinuses were seen on the control MRI examinations.

Conclusions: In this case, we wanted to emphasize that hypertriglyceridemia as a complication of L-ASP therapy may be caused by pseudohyponatremia and cerebral venous thrombosis. Early diagnosis and initiation of proper dietary and medical treatment is thus essential.

PB2097 | Amelioration of the Pathologic Changes in the Hepatocyte-Specific *BRAF*V600E Mutated Mice by Administration of Aspirin

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Background: Previously, we have reported that the *Braf*V637E mutation, corresponding to the human *BRAF*V600E mutation, plays a pivotal role in hepatocarcinogenesis induced by diethylnitrosamine (DEN) in B6C3F1 mice. The livers of transgenic mice with a hepatocyte-specific human *BRAF*V600E mutation weighed 4.5 times greater than that of normal mice and was entirely consisted of hepatocytes resembling the DEN-induced preneoplastic hepatocytes. However, these transgenic mice spontaneously died seven weeks after birth due to thrombotic microangiopathy in the lung and kidney.

Aims: In this study, we aim to identify the role of platelets in hepatocarcinogenesis.

Methods: The *Alb-Cre/BRAF*V600E transgenic and control *BRAF*V600E mice were given aspirin (Asp) by oral intubation from 4 to 8 weeks after birth.

Results: The liver showed thrombopoietin (TPO) overexpression, which is associated with eventual megakaryocytosis and thrombocytosis, and activated platelets were deposited in hepatic sinusoids. TPO was also overexpressed in the DEN-induced hepatic tumors, and sinusoidal platelet deposition was observed in hepatic tumors of humans and mice. In the transgenic mice, Asp administration prevented platelet activation, reduced the liver/body weight ratio, decreased the platelet deposition in the liver, kidney and lung, prevented the erythrocyte dyscrasia and ameliorated the renal/pulmonary changes. Additionally, Asp administration partially inhibited the neoplastic morphology of hepatocyte.

Conclusions: These results indicate that the macroenvironment that results from TPO overproduction by the neoplastic hepatocytes, the eventual megakaryocytosis/thrombocytosis, and the platelet activation and interaction with hepatic sinusoidal cells may contribute to hepatocarcinogenesis from a very early stage. Also, platelet inhibition may have therapeutic effect on hepatocarcinogenesis.

PB2098 | Use of Direct Oral Anticoagulants (DOACs) as Primary or Secondary Prophylaxis of Venous Thromboembolism in Patients with Multiple Myeloma on Immunomodulatory Drugs

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Background: Recent reports support the potential role of DOACs for venous thromboembolism (VTE) prophylaxis in patients with multiple myeloma (MM) on any immunomodulatory agent (IMA).

Aims: To evaluate efficacy and safety of DOACs as thromboprofylactic agents in MM patients treated with some IM.

Methods: Between July 2018 and January 2020 all our patients diagnosed with MM and receiving some IMA-containing scheme were proposed to start VTE prophylaxis with a DOAC.

Results: Fourteen MM patients fulfilling mentioned criteria accepted to receive a DOAC. Median age was 67 years (range 53-86). Patients were receiving 1st (7 cases), 2nd (2 cases), 3rd line (1 case) or maintenance therapy following autologous stem cell transplantation (lenalidomide 2 cases; ixazomib-lenamidomide-dexamethasone 2 cases). All but one (on a pomalidomide-containing regimen) were receiving a lenalidomide-containing regimen (Rd- 5 cases; VRD 4 cases; lenalidomide or IRd maintenance 4 cases). Median duration of treatment was 339 days (range 23-526). Rivaroxaban (20 mg od; 5 cases) was the most commonly prescribed DOAC in patients with a prior history of VTE (edoxaban 60 mg; 1 case) whereas apixaban (2.5 mg bd) was prescribed off-label for primary VTE prophylaxis purposes (7 patients). One individual was receiving rivaroxaban due to non valvular atrial fibrillation when anti-MM treatment was started; in other 2 cases a DOAC replaced acenocumarol. No patient developed any VTE event. Two minor bleeding events (rectorrhagia, epistaxis) and no major or clinically relevant non-major bleeds were reported. Five subjects had stopped DOAC treatment at the end of the follow-up period due to death or lenalidomide discontinuation (median treatment duration 112 days; range 23-292).

Conclusions: Use of DOACs as a primary or secondary VTE prophylaxis in MM patients on IMA may be regarded as a useful and safe therapeutic tool to be considered in such cases.

PB2099 | JAK2 Mutation: A Predictor of Thrombotic Events in Myeloproliferative Neoplasia?

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Background: The major complications of non-chronic myeloid leukemia myeloproliferative neoplasms (non CML-MPN) are arterio-venous thrombosis, hemorrhage and risk of leukemic transformation. Mutations in JAK2 are seen in this group of MPNs in varied proportions -95% of polycythemia vera (PV), 55% of essential thrombocythemia (ET) and 65% of primary myelofibrosis (PMF). Risk factors for thrombosis are older age, previous thrombotic event, increased hematocrit and spontaneous in vitro megakaryocyte colony formation. Effect of mutated JAK2 on risk of thrombotic events has not been studied in Southern India.

Aims: To study the association of thrombotic events with JAK2 mutation in patients of non CML-MPNs.

Methods : Institutional ethics committee approval was obtained. Single centre retrospective descriptive study of 73 cases of bone marrow confirmed MPNs (from June 2013 to July 2018) was conducted. Age, gender, complete blood counts at diagnosis, occurrence of thrombotic events and JAK2 mutation status by real time polymerase chain reaction were recorded at a minimum follow-up period of 6 months. Continuous data was expressed in mean + SD. Association between CBC, mutated JAK2 and thrombosis was studied by Fisher exact test.

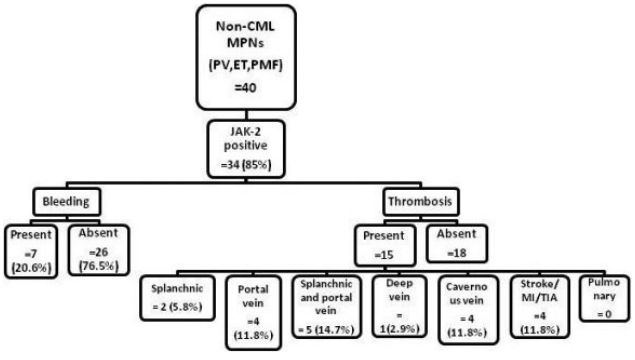

FIGURE 1 Summary of case distribution

TABLE 1 Association of thrombosis with age, gender and CBC parameters

	Polycythemia vera	Essential thrombocythemia	Primary myelofibrosis	p value
Age in years	55.10 (11.47)	56.71 (14.13)	66.00 (7.07)	0.453
Males	21 (70.0)	7 (87.5)	0	0.127
Females	9 (30.0)	1 (12.5)	2 (100.0)	
Hemoglobin/dL	20.51 (10.86)	20.73 (11.09)	36.70 (22.20)	0.161
Hematocrit	16.03 (2.71)	11.61 (3.36)	9.75 (0.35)	<0.001
Mean platelet volume in fL	8.37 (0.81)	8.89 (1.20)	6.80 (0.57)	0.02
Platelet count X1000/μL	590.27 (335.43)	624.38 (316.77)	78.05 (101.75)	0.103
WBC count X1000/μL	7.10 (1.53)	4.58 (0.92)	3.26 (0.16)	<0.001

Results: Forty cases were included the study as remaining 33 cases did not undergo testing for JAK2 mutation. Figure 1 depicts the case distribution. Summary of association of thrombosis with age, gender and CBC parameters are given in table 1. Thrombosis occurred in 67.6% of MPN with JAK2 mutation (p value: 0.03).

Conclusions: Previous studies imply that JAK-2 mutational status might represent a novel disease-associated risk factor that should ideally be incorporate into risk stratification risk factor. However, we did not find significant association of thrombosis with JAK2 mutation. However, high hematocrit and total WBC count particularly in PV is significantly associated with risk of thrombosis.

PB2100 | Development of Predictive Score for Venous Thromboembolism in Hematologic Malignancies

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Background: Venous thromboembolism (VTE) remains a major cause of morbidity and mortality in patients with cancer. Cancer is the leading cause of death in Cuba, in the age group between 15 and 65 years. In 2018, 1 603 deaths were due to hematologic malignancies (HM). Although several well validated scores stratify the risk for VTE in solid tumors, HM are underrepresented in these models.

Aims: To design a predictive score for VTE risk stratification in patients with HM.

Methods: A case-control study was carried out at the University Hospital Arnaldo Milián Castro in Santa Clara, Cuba from January 2016 to January 2019. The study population was 285 patients (case group: 94 patients with VTE / control group: 191 without VTE), hospitalized with a diagnosis of HM. For the univariate analysis chi-square test was applied, Odds Ratio for risk estimation, Cramer's V for strength of association. The logistic regression and the ROC curve were applied in the multivariate analysis. Power for each predictor were determined by their associated coefficients. Informed consent was obtained and the study was approved by a medical ethics committee complying with the Declaration of Helsinki.

TABLE 2 Evaluation of the VTE predictive score according to basic performance indicators

Predictive scores	Observed thrombotic events		
	Yes	No	Total
High risk (>=4 points)	56	15	71
Low risk (<4 points)	38	176	214
Total	94	191	285
Indicators	Value	CI (95%)	
Sensitivity (%)	59.57	49.12-70.03	
Specificity (%)	92.12	88.07-96.22	
Positive predictive value (%)	78.87	68.67-89.07	
Negative predictive value (%)	82.84	76.87-87.6	

Results: The predictive score for VTE includes 5 predictive factors: hypercholesterolemia, tumoral activity, bedding, use of thrombogenic drugs and diabetes mellitus. Patients obtaining four points or more was defined as high risk, which corresponded with the 75th percentile of the empirical distribution of the prognostic score values. Those who reached less than four points was defined as low risk. The score correctly classified 81.1% of the patients, with positive and negative predictive values of 78.8% and 82.2% respectively. The sensitivity was 59.5% and the specificity of a 92.1%.

Conclusions: The VTE risk score represented a specific and effective tool for the prediction of VTE in the patient with HM.

PB2101 | Risks and Benefits of Antithrombotic Therapy in Polycythemia Vera: A Systematic Review

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Background: Thrombosis is a major cause of morbidity and mortality in patients with polycythemia vera (PV). Aspirin and hematocrit control are key components of PV management to reduce thrombotic

TABLE 1 Predictive factors of VTE according to logistic regression analysis

Factors	Coefficient	Points	Wald	p	OR	CI 95% to OR
Bedding	2.28	2	28.01	0.000	9.80	4.21-22.81
Diabetes Mellitus	1.05	1	3.99	0.0458	2.86	1.02-8.04
Hypercholesterolemia	2.48	2	18.21	0.0000	11.97	3.83-37.41
Use of thrombogenic drugs	1.59	2	13.51	0.0002	4.90	2.10-11.43
Tumoral activity	2.41	2	33.59	0.0000	11.09	4.92-25.03
Constant	-3.41	-	64.62	0.0000	0.03	-

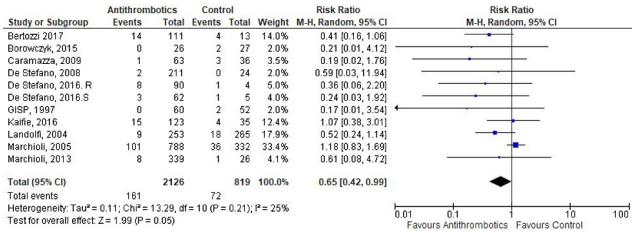

FIGURE 1 Forest plot of the association between antithrombotic therapy and thrombotic events in patients with polycythemia vera

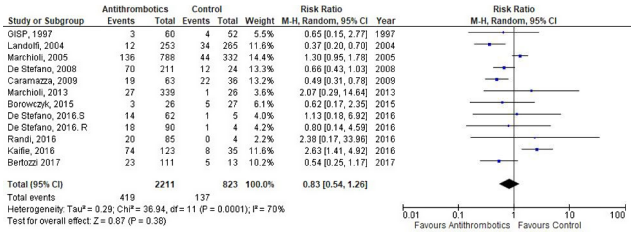

FIGURE 2 Forest plot of the association between antithrombotic therapy and all cause mortality in patients with polycythemia vera

events. However, patients with PV are also at risk for hemorrhagic complications, especially in the context of acquired Von Willebrand syndrome.

A systematic review conducted in 2013 identified 2 randomized control trials (RCTs) and concluded that low-dose aspirin does not increase the risk of major bleeding and is associated with a statistically non-significant reduction in fatal thrombotic events. The risks and benefits of other antithrombotics and the optimal management of thrombosis in patients with PV remains uncertain.

Aims: To examine the association between antithrombotic therapy and outcomes such as bleeding, thrombosis, and mortality in patients with PV. To summarize all available data, observational studies were included.

Methods: Databases including MEDLINE, CENTRAL, and Pubmed were searched. Articles examining antithrombotic use in patients with PV were included. Screening, full text review, and data extraction and risk of bias assessment were completed by 2 independent reviewers. The protocol was registered on PROSPERO (CRD42017079688).

Results: 4523 studies were identified and 12 studies (9 observational studies and 3 RCTs) were included. Antithrombotics (antiplatelets + anticoagulants) were associated with a lower risk of thrombosis (RR 0.83, CI 0.54 - 1.26, I^2 70%), increased bleeding (RR 1.07, CI 0.77 - 1.50, I^2 5%) and decreased all cause mortality (RR 0.65, CI 0.42 - 0.99, I^2 25%). Most studies were observational and at high risk of bias. There were insufficient data for subgroup analyses of direct oral anticoagulants.

Conclusions: In PV patients, use of antithrombotics is associated with reductions in all-cause mortality (35%) and thrombosis (17%), and a statistically non-significant increase in bleeding (7%). Further prospective studies regarding antithrombotics in patients with PV are required given the uncertainty in the evidence.

PB2102 | Clinical Outcome of Anticoagulation for Acute Venous Thromboembolism Associated with Different Types of Cancer

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Background: Anticoagulation (AC) of patients with venous thromboembolism (VTE) accompanied by cancer is associated with a higher rate of anticoagulation failure and higher risk of bleeding complications compared to VTE patients without cancer.

Aims: To compare VTE recurrence, major and clinically relevant non-major bleeding (CRNMB), and mortality amongst different types of cancer.

Methods: Consecutive patients enrolled in the Mayo Clinic VTE Registry between March 2013 and November 2019 with acute VTE were followed prospectively. With the exception of brain cancer, all other cancers were grouped separately if there were more than a hundred patients. Patient outcomes were assessed in person, by mailed questionnaire, or scripted phone interview.

Cancer type (Outcomes: rates 100-person years)	VTE recurrence	Mortality	Major Bleeding	CRNMB
Gastrointestinal	5.86	51.26	6.78	7.25*
GU/OV	2.02*	55.91	11.18	10.12*
Pancreatic	9.70	79.64	3.47*	6.01*
Hematologic	7.24	26.80	2.08*	1.35^
Lung	6.74*	77.48	11.44	13.84
Brain	No events	55.23	8.05	5.35*
Other cancers	5.99	41.71	9.06	6.40*
No Cancer	2.95	8.44	2.62	7.08

Outcomes by cancer type (* No difference with NoCa, ^ lower than NoCa).

Results: Of the 2798 patients enrolled, 1256 (44.9%) had active cancer including: gastrointestinal (n=244), genitourinary and ovarian (GU/OV, n=201), pancreatic (n=154), hematologic (n=136), lung (n=104), brain (n=47), and other cancers (n=370). Proportion of cancer patients treated with direct oral anticoagulants varied from 63% (pancreatic) to 47% (hematologic). The highest rate of VTE recurrence was in pancreatic (9.70) followed by hematologic (7.24) groups compared to patients with no cancer (NoCa) (2.95) but no difference in lung cancer (6.74, p=0.06) and GU/OV (2.02, p=0.55) (Table). Mortality rates were higher in all types of cancer compared to NoCa. Major bleeding rates were significantly higher in all groups except pancreatic and hematological cancers. Patients with lung cancer had higher, and patients with hematological malignancies lower, rates of CRNMB compared to NoCa patients.

Conclusions: Significant differences in clinical outcomes of anticoagulation for VTE exist amongst patients with different cancers. Pancreatic cancer is associated with the highest mortality and VTE recurrence while major bleeding and CRNMB are not different compared to NoCa patients. Lung cancer has the highest rate of major bleeding and CRNMB while VTE recurrence is not significantly different.

PB2103 | Survival after Cancer-related Venous Thrombosis: The Scandinavian Thrombosis and Cancer Study (STAC)

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Background: Cancer patients have an increased risk of developing a venous thromboembolism (VTE) and this combination is reported to result in poorer survival compared to cancer alone.

Aims: To investigate the impact of VTE on the survival of cancer patients in a general population.

Methods: The Scandinavian Thrombosis and Cancer (STAC) cohort, a population-based cohort including 144,952 subjects without previous VTE or cancer was used. During follow-up, cancer and VTE incidences were registered. 'Cancer-related VTE' was defined as VTE diagnosed in patients with overt or occult cancer. Survival of subjects without cancer or VTE ('disease-free subjects') was compared with survival of subjects diagnosed with cancer and cancer-related VTE. Cox-regression models with cancer and VTE as time-varying exposures were performed to calculate hazard ratios for death. Subanalyses were performed across different cancer types and stages.

Results: During follow-up (mean 11.7 years) 14,621 subjects developed cancer and 2,444 developed VTE, of which 1241 where cancer-related. The mortality rates (per 100 person-years) for disease-free subjects, VTE only, cancer only and cancer-related VTE were 0.63 (95% CI 0.62-0.65), 5.0 (95% CI 4.6-5.5), 9.2 (95% CI 9.0-9.5) and 45.3 (95% CI 41.1-50.0), respectively. Compared with cancer patients, the risk of death for cancer-related VTE patients was increased 3.4-fold (95% CI 3.1-3.8). Among all cancer types, the occurrence of a VTE increased the mortality risk 2.8 to 14.7-fold.

Conclusions: In a general population, cancer patients with a VTE had a 3.4-fold increased mortality risk compared to cancer patients without VTE, this effect was seen within all cancer types and stages studied.

PB2105 | Risk of venous Thromboembolism among Subgroups of Cancer Patients Undergoing Chemotherapy: Post-Hoc Analysis of the AVERT Trial

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Background: Patients with active cancer have an increased risk of venous thromboembolism (VTE) complications which is associated with morbidity, mortality and significant health care costs. The AVERT trial demonstrated that prophylactic doses of apixaban (2.5

TABLE 1 Incidence of venous thromboembolism during the study period

Table 1: Incidence of venous thromboembolism during the study period

	Apixaban (N=288)	Placebo (N=275)	HR (95% Confidence Interval)	p-value
Age (yrs)				
<75	10 (3.9%)	25 (10.3%)	0.37 (0.24-0.59)	<0.0001
≥75	2 (6.7%)	3 (9.4%)	0.79 (0.56-1.12)	0.19
Sex				
Male	4 (3.4%)	16 (13.7%)	0.25 (0.12-0.48)	<0.0001
Female	8 (4.7%)	12 (7.6%)	0.64 (0.28-1.43)	0.27
Weight (kg)				
<60	2 (3.7%)	3 (8.1%)	0.49 (0.12-1.94)	0.31
60-90	8 (5.4%)	13 (9%)	0.61 (0.36-1.01)	0.06
>90	2 (2.4%)	12 (12.9%)	0.18 (0.06-0.52)	0.002
Prior VTE				
Yes	1 (11.1%)	2 (25%)	0.46 (0.12-1.80)	0.26
No	11 (3.9%)	26 (9.7%)	0.41 (0.26-0.64)	0.0001
Antiplatelet therapy	1 (1.5%)	5 (8.1%)	0.18 (0.10-0.33)	<0.0001
Type of cancer				
Solid	7 (3.8%)	23 (12.7%)	0.30 (0.19-0.47)	<0.0001
Hematological	5 (6.1%)	4 (5.4%)	1.15 (0.51-2.61)	0.74
Metastatic disease	4 (5.5%)	8 (12.3%)	0.45 (0.26-0.78)	0.004
Bevacizumab use	2 (3.3%)	4 (5.6%)	0.60 (0.23-1.58)	0.30

mg twice-daily) significantly decreased the rate of VTE when compared to placebo in intermediate-to-high risk (Khorana score ≥2) patients starting chemotherapy. The efficacy of apixaban in different subgroups of cancer patients remains unclear.

Aims: We sought to evaluate the risk of VTE in different subgroups of ambulatory patients initiating chemotherapy.

Methods: This is a pre-planned post-hoc analysis of the AVERT trial, which was a randomized, placebo-controlled, double-blind clinical trial. Hazard ratios with 95% confidence intervals (CI) were calculated using the Cox proportional hazards model to compare the treatment effect accounting for clustering at center level. Subgroup analyses were based pre-defined subgroups including: age, gender, race, weight, prior VTE, creatinine clearance, antiplatelet therapy, cancer characteristics, bevacizumab use, and baseline ECOG status.

Results: During the study period, VTE events occurred in 40 (7.1%) patients: 4.2% and 10.2% of the apixaban and placebo groups, respectively [HR 0.41; 95%CI, 0.26-0.65]. Patient characteristics associated with decreased VTE rates included: age < 75 [HR 0.37; 95%CI, 0.24-0.59]; male sex [HR 0.25, 95%CI 0.12-0.48], weight >90Kg [HR 0.18, 95%CI, 0.06-0.52]; and no prior history of prior VTE [HR 0.41, 95%CI 0.26-0.64]. Cancer characteristics associated with decreased rates of VTE with apixaban included solid cancers [HR 0.30; 95%CI, 0.19-0.47] and metastatic disease [HR 0.45; 95%CI, 0.26-0.78]. Concurrent use of antiplatelet therapy was also associated with decreased VTE rates [HR 0.18, 95%CI 0.10-0.33).

Conclusions: In the AVERT trial, apixaban thromboprophylaxis provided significantly decreased risk of VTE in patients with age< 75, male sex, weight >90Kg, no prior VTE, solid cancer, and metastatic disease. (Funded by the Canadian Institutes of Health Research and Bristol-Myers Squibb-Pfizer Alliance; NCT02048865.)

PB2106 | Thrombosis in Hematologic Malignancies: A Five Year Single-Center Experience in Mexico

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Background: Thrombotic risk occurs in all hemato-oncological entities. It's well known that this patients have an increased risk of venous thromboembolism (VTE), but it has also been recognized that may be an increased risk of arterial thrombosis.

Aims: To characterize the thrombotic events in patients with hematological malignancies in a second level Hematology Service in Mexico City.

Methods: We conducted a cohort of consecutive patients diagnosed with: lymphoma, myeloma, chronic myeloproliferative syndromes (CMPN) and acute leukemia, who presented at least one thrombotic event (arterial or venous) corroborated by image studies from January 1, 2014 to December 31, 2018.

Results: Forty two cases of thrombosis were identified, 50% were women. Mean age 59 years (26-92 years). VTE ocurred in lower limbs in 57% of cases,in thoracic limbs (30.8%). PT in 14%, portal vein thrombosis in 4.7%, renal thrombosis in 2.3% and thrombosis of suprahepatic veins 2.3%.

The most frequent site of arterial events was cerebral (14% of all thrombotic events).

Thrombosis occurred most frequently in patients with Non Hodgkin Lymphoma: DBCL 35%, FL 4.8%, plasmablastic 4.8%, mantle cell 2.4% and peripheral T 2.4%. Followed by CMPN: essential thrombocythemia 21%, myelofibrosis 2.4%, polycythemia vera 4.8%, chronic myeloid leukemia 2.4%, Hodgkin's lymphoma 9.5%, multiple myeloma 7.1%, acute myeloid leukemia 2.4%.

The treatment of choice for VTE was low molecular weight heparins. Currently 65% of cases continue with treatment: 32.7% of patients receive low molecular weight heparins, 9.5% rivaroxaban, 11.9% acenocoumarin and 14.3% with ASA for arterial thrombosis.

Conclusions: VTE is the most frequent cause of thrombosis in patients with hematologic malignancies. There is no gender predominance. Venous thrombosis was more frequent in patients with lymphoma and arterial in patients with CMPN. Long-term follow-up is required to determine recurrence and long-term mortality.

PB2107 | Risk Factors of Venous Thromboembolism in Patients Undergoing Systemic Cancer Therapy: A Systematic Review and Meta-Analysis

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Background: Patients undergoing systemic cancer therapy are susceptible to developing venous thromboembolism (VTE). The most pertinent risk factors for the development of VTE in this population remain unclear.

Aims: This systematic review and meta-analysis aims to summarize the risk factors associated with VTE in patients with general cancers receiving systemic therapy.

Methods: Medline, Embase, and Cochrane Central Register of Controlled Trials (CENTRAL) databases were searched for observational or randomized studies that used multivariate analysis adjusted for tumour type and/or metastatic disease to model the risk of VTE. Risk of bias was assessed using the Quality in Prognostic Factor Studies (QUIPS) tool and the quality of evidence was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) tool. Random-effect meta-analysis was used to pool the risk of VTE for each risk factor.

Results: From 5,988 citations, 15 eligible studies were identified. The final cohort included a total of 14,078 patients of whom 734 (5.2%) developed a VTE. The search identified 47 risk factors. The pooled hazard ratio (HR) was 1.21 (95% Confidence Interval (CI): 0.98 to 1.43, 95% Prediction Interval (PI): 0.89 to 1.52) for presence of metastatic disease, 0.83 (95% CI: 0.49 to 1.16, 95% PI: 0.28 to 1.37) for females, and 2.88 (95% CI: 1.84 to 3.91, 95% PI: 1.20 to 4.55) for Eastern Cooperative Oncology Group (ECOG) Performance Status of 2 or more points. Commonly used chemotherapy agents, bevacizumab and platinum-based compounds had a pooled HR of 1.77 (95% CI: 0.56 to 2.97, 95% PI: 0.00 to 3.73) and 1.28 (95% CI: 0.63 to 1.92, 95% PI: 0.23 to 2.32), respectively.

Conclusions: Our review identified many risk factors, however, the evidence for association with development of VTE for most of the factors is inconclusive. ECOG performance and metastases are associated with increased risk of VTE.

PB2108 | Elevated Von Willebrand Factor Antigen and Activity Levels Are Risk Factors for Predicting Thrombosis in Cancer

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Background: Von Willebrand factor (VWF) acts as a bridge between platelet and the subendothelial matrix, and plays a vital role during thrombosis. Cancer associated thrombosis is serious complication and major cause of death in cancer patients. Plasma VWF antigen (VWF:Ag) in cancer is increased, while VWF activity is unclear.

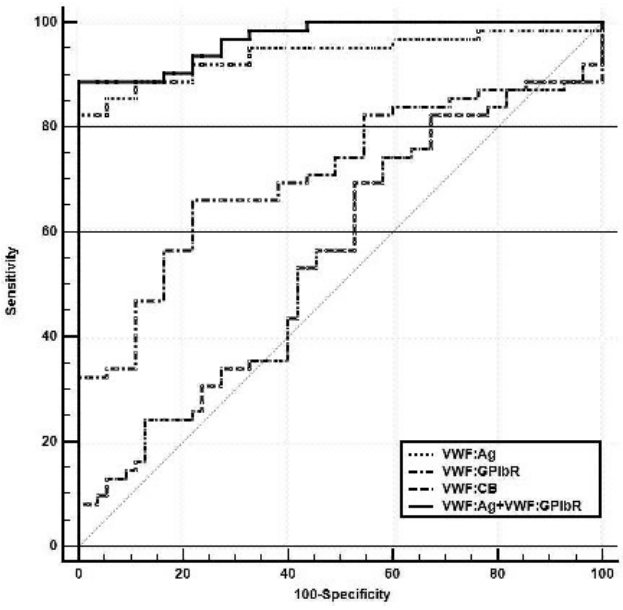

FIGURE 1 Diagnostic value of VWF on cancer by ROC

Aims: To investigate plasma level of VWF:Ag, activity (VWF Ristocetin-triggered GPIb binding assay, VWF:GPIbR; VWF collagen binding assay, VWF:CB) in cancer patients, and analyze the clinical value of VWF activity in the development of cancer.

Methods: A total of 124 patients with cancer including respiratory cancers, genitourinary cancers, and gastrointestinal cancers were retrospectively analyzed. 110 healthy volunteers who matched with gender and age were selected as the control group. Plasma levels of VWF:Ag, VWF:GPIbR and VWF:CB were measured by ELISA. The correlation between VWF antigen and activity was analyzed by Spearman correlation. The predictive value of VWF index for cancer was evaluated by ROC curve.

Results: Plasma VWF:Ag and VWF:GPIbR levels in cancer patients were significantly higher than those in control group (mean±SD, 261.63%±1.78 vs 56.39%±15.84, 182.39%±1.04 vs 111.60%±47.65, respectively, all $P< 0.001$), and this difference also existed in the comparison of cancer subgroups with the control group, while there was no difference for VWF:CB between cancer patients and control. VWF:Ag level was positively correlated with VWF:GPIbR and VWF:CB levels (r=0.896, 0.434, respectively, $P< 0.001$), and VWF:GPIbR was also positive correlation with VWF:CB (r=0.542, $P< 0.001$). VWF:Ag alone had the highest diagnostic efficacy (AUC=0.939), followed by VWF:GPIbR (AUC=0.708) in predicting cancer associated thrombosis (Figure 1).

Conclusions: Plasma levels of VWF antigen and activity in cancer patients were increased, and the growth of them was highly consistent. There was high value for VWF:Ag and VWF:GPIbR to predict thrombosis in cancer. Thus, elevated VWF:Ag and VWF:GPIbR levels could be novel markers in the occurrence of cancer associated thrombosis.

PB2109 | Incidence and Risk Factors for Venous Thromboembolism (VTE) among Lymphoma Patients in a Southeast Asian Population

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Background: The incidence of VTE in lymphoma patients ranges from 5% to 17% according to Western studies. However, data on the incidence and clinical presentations of VTE among Southeast Asian patients is sparse. VTEs complicate treatment, leading to morbidity and mortality.

Aims: We aim to determine the incidence and risk factors for VTE among Southeast Asian lymphoma patients and the impact of anticoagulation.

Methods: We identified all newly diagnosed lymphoma patients at the National University Hospital from 1 November 2012 to 31

TABLE 1 Venous thromboembolism among lymphoma patients in a Southeast Asian population

No.	Gender	Age	Lymphoma subtype	Location of VTE	Predisposing factors	Thrombotic event (+/- months from cancer diagnosis)	Ongoing chemotherapy (Yes/no cycle)	VTE treatment	Treatment duration (months)	Treatment complications	VTE recurrence
1	Female	47	Classical Hodgkin's Lymphoma	2 femoral popliteal and left brachial upper arm	Obesity, diagnosis: lymphoma	0	None	LMWH	Indefinite	None	No
2	Female	56	[illegible]	Right iliacal line augmented PE	Obesity, hospitalization, lymphoma	[illegible]	None	LMWH	4	None	No
3	Female	37	DLBCL	Right IJ above lines DVT	Obesity	7	None	LMWH	5	None	Yes Right LE DVT
4	Female	56	DLBCL (relapsed)	Left IJ above line DVT	no typical symptoms	1	None	LMWH	7	Line occlusion requiring removal	No
5	Female	28	Classical Hodgkin's Lymphoma	Right, IR PICC thrombosis	Venous catheter	2	72 ABVD	LMWH	3	None	No
6	Female	26	PMBCL	Bilateral venous with thrombosis	Obesity, hospitalization	30	74 CHEOP	LMWH	3	None	No
7	Male	42	NHL negative ALK	Right, IR PICC thrombosis	Hospitalization, venous catheter	2	72 CHEOP	LMWH	3	None	Yes Left LE PICC thrombosis
8	Male	63	type III BCLL	Left, the vein collateral veins, and IR to iliacal	Obesity, hospitalization, venous catheter	0	76 CHEOP	No (anemia EGFR)	NA	NA	No
9	Female	62	DLBCL	Left IJ DVT	Early diagnosis: lymphoma	0	None	LMWH	3	None	No
10	Male	27	PMBCL	Left IJ, IR DVT	Obesity, newly diagnosed lymphoma	0	None	LMWH	3	None	No
11	Male	26	PMBCL	Left IJ, IR DVT	Early diagnosis: lymphoma	0	None	LMWH	3	None	No
12	Female	50	NHL grade low BCLL	Left IJ thrombosis	Newly diagnosed lymphoma	0	None	LMWH	Indefinite	Vitreous bleeding – retinopathy of the	No
13	Female	67	DLBCL	Bilateral IJ, venous brace DVT	Obesity, NSCLC use, newly diagnosed lymphoma	7	None	LMWH	3	None	No
14	Male	52	DLBCL (relapsed)	PE	Hospitalization, venous catheter use, lymphoma relapse	0	None	LMWH	Ongoing	None	No
15	Female	30	DLBCL (relapse)	Right, IR, PICC thrombosis	Obesity, venous catheter, lymphoma relapse	0	#1 ABVD and 70 CHEO	LMWH	3 Stopped for survival in advanced	None	No
16	Male	36	Follicular lymphoma	Left IJ, above line DVT	Newly diagnosed lymphoma	0	None	LMWH	3	None	No
17	Female	32	Stage 3 Hodgkin lymphoma of CLBCL	Right, IR, thrombosis	Lymphoma (chemotherapy treatment)	0	#1 ABVD	No treatment (cancer advanced)	NA	NA	No
18	Male	32	PMBCL	Right, IR, thrombosis	Newly diagnosed lymphoma	0	#1 70 CHEOP	LMWH	3	None	No
19	Male	25	DLBCL	Right medial vein	Newly diagnosed lymphoma	0	None	Warfarin	Lifelong (Lupus anticoagulant, APS)	NA	No
20	Male	42	DLBCL	Massive PE	Obesity, hospitalization	7	#2 70 CHEOP	LMWH followed by LMWH	6	NA	Yes Right IR PICC thrombosis Yes
21	Female	27	Classical Hodgkin's lymphoma	Left, IJ, DVT	Newly diagnosed lymphoma	0	#1 ABVD	LMWH	12	NA	Left IR PICC thrombosis after six cycles
22	Female	30	DLBCL (relapse)	Left IJ above line DVT	Hospitalization, lymphoma relapse	0	#3 Mini-BEAM	DVT BMT	NA	NA	No
23	Male	30	Follicular lymphoma	Right, IR, PICC thrombosis	Newly diagnosed lymphoma	0	None	LMWH	6	NA	No

Definitions: Obesity: BMI, 32.5kg/m²; Recent Hospitalization: <3 month before VTE diagnosis

Legend:

Cases: CHEOP (cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone); ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine); Mini-BEAM (carmustine, etoposide, cytarabine, melphalan); DVT (deep venous thrombosis); PE (Pulmonary embolism); IJ (internal jugular); IR (iliacal line); BCLL (Bleeding Cerebrovascular Thrombosis);

PICC (Peripherally inserted central catheter); CVP (central venous pressure); SGAP (Salbutamol, Ipratropium, Methylprednisolone, Aminophylline); CHEOP (Cyclophosphamide, doxorubicin, vincristine, prednisolone);

BCBAM (Bleomycin, etoposide, cytarabine, carmustine); RTX (Rituximab); AB (Anti-Bacterial); rtPA (Recombinant Tissue Plasminogen Activator);

October 2017. We analysed patient demographics, time of lymphoma diagnosis, lymphoma treatment in relation to the VTE, duration of VTE treatment, reason for treatment cessation and complications from either the VTE or anticoagulation.

Results: Out of 600 lymphoma patients, after excluding patients in remission, 23 patients were diagnosed with VTE within one year of lymphoma diagnosis or relapse. 13 experienced a VTE prior to any chemotherapy. Four patients were diagnosed with VTE at lymphoma relapse. 10 developed VTE while on chemotherapy, with 40% of VTE occurring during the first chemotherapy cycle. There were five cases of catheter related thrombosis. Therapeutic anticoagulation was administered to 20 patients, with 15 completing at least three months of anticoagulation. No patient developed complications of VTE within 90 days, including those not anticoagulated. VTE recurred in four patients; three were line associated thromboses. Patients who were not anticoagulated or did not complete anticoagulation did not experience VTE recurrence.

Conclusions: VTE incidence in newly diagnosed or relapsed lymphoma was 3.8% in our population. Majority of VTEs were diagnosed at cancer diagnosis and the initial chemotherapy period. Catheter use accounted for a quarter of VTEs and is a major risk factor for VTE recurrence. Further studies are needed to determine the efficacy of reducing VTE with prophylactic anticoagulation during this at-risk period. Anticoagulation was safe with minimal bleeding complications.

PB2110 | Increased Coagulation Potential in Patients with Polycythemia Vera and Essential Thrombocythemia with Thrombotic History

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Background: Thrombosis represents one of the major life-threatening complications of polycythemia vera (PV) and essential thrombocythemia (ET). There is great evidence of coagulation activation in these pathologies whereas standard coagulation tests do not reflect prothrombotic tendency and show multidirectional changes. Hemostatic parameters studies in PV and ET give contradictory results especially in relation to thrombotic history of patients. Meanwhile, positive history of thrombosis is considered one of the most important risk factors of thrombosis development in PV and ET.

Aims: To evaluate influence of thrombotic history on coagulation potential in patients with PV and ET.

Methods: The study included 68 patients with PV and ET, 20 (29%) of them with history of thrombosis. Control group consisted of 30 healthy volunteers. Platelet-free plasma (PFP) was obtained using triple centrifugation. Coagulation potential was evaluated by thrombin generation test (TGT) using calibrated automated thrombogram method with triggers "PRP-Reagent" (tissue factor 1 pM) and

TABLE 1 Results of TGT with "PRP-" and "MP-reagent" in control group and patients with PV and ET depending on history of thrombosis (median, 50% CI)

	Control group (n=30)	Patients with PV and ET without thrombosis (n=48)	Patients with PV and ET with thrombosis (n=20)	p (between patients with and without thrombosis)
ETP (PRP-reagent), nM*min	471.84 (374.75-564.00)	511.65 (315.68-789.67)	689.69* (434.26-791.79)	0.162
Peak (PRP-reagent), nM	18.40 (11.89-20.40)	17.4 (10.79-27.59)	25.11* (16.47-34.15)	0.037
V (PRP-reagent), nM/min	1.59 (1.13-2.08)	1.24 (0.69-2.38)	2.18 (1.33-3.45)	0.023
ETP (MP-reagent+CTI), nM*min	566.50 (439.63-743.63)	707.60 (359.57-1116.25)	937.84* (579.82-1489.98)	0.087
Peak (MP-reagent+CTI), nM	46.25 (31.83-68.64)	54.86 (28.87-112.75)	83.81* (53.70-235.25)	0.056
V (MP-reagent+CTI), nM/min	7.34 (4.99-17.14)	10.38 (4.17-26.40)	17.14* (9.76-54.76)	0.072

* $p<0.05$ compared with controls.

"MP-reagent" (phospholipids 4 μM) + CTI 40 μg/mL. Endogenous thrombin potential (ETP), peak thrombin concentration (Peak) and thrombin generation velocity (V) were assessed. STATISTICA 6.0 was used, Mann-Whitney test applied, differences between groups considered significant with $p < 0.05$.

Results: There was no significant difference between patients without thrombosis and control group (Table1). Patients with positive history of thrombosis showed higher values of ETP and Peak with both reagents compared to controls, and differed significantly from patients without thrombosis by elevated Peak and V with "PRP-reagent" and a tendency to increase for other parameters.

Conclusions: PV and ET patients with history of thrombosis show increased thrombin generation quantity and velocity in PFP presumably associated with microparticles, enriched by negatively charged phospholipids and bearing tissue factor on their surface. The impact of procoagulant microparticles in enhanced coagulation potential apparently accounts for the elevated risk of thrombosis development in PV and ET patients with positive thrombotic history.

PB2111 | Non-Randomized Patient Outcomes. ADAM-VTE Registry Analysis

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Background: ADAM-VTE, a randomized clinical trial (RCT) for the treatment of cancer-associated acute venous thromboembolism (VTE) found superior efficacy with no major bleeding events in the apixaban arm compared to a dalteparin arm.

Aims: The ADAM-VTE Registry was established to assess the effect of anticoagulant choice on the safety and efficacy outcomes of the patients screened but who did not participate in the ADAM-VTE RCT at Mayo Clinic. The goal was to provide an internal validation of the clinical trial results.

Methods: Data of patients screened for trial participation from the Mayo Clinic Rochester MN campus who were not randomized in the ADAM VTE trial were reviewed to assess VTE recurrence, major bleeding, clinically- relevant non-major bleeding (CRNMB) and survival by treatment strategy.

TABLE 1 Demographics

Demographic variables, n (%)	ADAM-VTE Registry (n=131)
Any PE, n (%)	75 (57.3)
Any DVT, n (%)	84 (64.1)
Both PE and DVT, n (%)	28 (21.4)
Previous VTE, n (%)	14 (10.9)
Active chemotherapy, n (%)	71 (57.3)
Body weight kg, mean ± SD (range)	86.0 ± 25.6 (43.0-213.0)
Platelet count, mm3±SD (range)	243.3 ± 124.0 (18.0-642.0)
Creatinine Clearance, ml/min ± SD (range)	1.0 ± 0.4 (0.4-3.4)

Results: During trial recruitment (11/20/2015-10/2/2017), 288 patients at Mayo Clinic Rochester were screened for enrollment. Of these, 146 (50.7%) refused participation or did not meet inclusion criteria. One hundred thirty one patients (mean age 62.9±12.9 years, 45.9% females) (Table 1 demographics) were treated with either apixaban (n=53), enoxaparin (n=52), warfarin (n=12), rivaroxaban (n=6) or heparin (n=5). Three patients received more than one anticoagulant. The most common types of cancer were: gastrointestinal 23 (18.6%), genitourinary/ovarian 19 (15.3%), hematologic 19 (15.3%), pancreatic/hepatobiliary 16 (12.9%), lung 11 (8.9%), and breast 9 (7.3%). Major bleeding, CRNMB, and VTE recurrence were low and similar for apixaban and LMWH treatment groups (Table 2). Survival was significantly better for those patients treated with apixaban (p=0.03) potentially due to selection bias.

Conclusions: Although limited by small numbers, the ADAM VTE Registry found similar low rates of bleeding outcomes shown in the ADAM-VTE randomized clinical trial.

PB2112 | Are Patients with an Active Cancer and those with a History of Cancer Carrying the Same Risks of Recurrent VTE and Bleeding while on Anticoagulants?

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Background: Both direct oral anticoagulants (DOAC) and low molecular weight heparins (LMWH) are recommended as first-line option for the treatment of cancer-associated thrombosis (CAT) by recent

TABLE 2 Outcomes

Outcome at 6-months	All patients (n=131)	Apixaban (n=53)	Enoxaparin (n=52)	p-value
VTE recurrence	1 (0.8%)	0 (0%)	0 (0%)	NS
Major bleeding	9 (7.0%)	1 (1.9%)	3 (5.7%)	0.64
CRNMB	4 (3.1%)	1 (1.9%)	1 (1.9%)	1.0
Mortality	22 (17.2%)	3 (5.7%)	14 (26.9%)	0.03

guidelines, mainly based on the results of 2 dedicated noninferiority trials demonstrating that DOAC are equally or more effective than LMWH for the prevention of recurrent venous thromboembolism (VTE) in this specific population. The definition of "cancer patient" appears to differ substantially among studies and one out of these two trials included 22% of patients having their cancer "cured" at the time of the index VTE event.

Aims: To investigate whether patients with an active cancer and those with a history of cancer carry the same risks of recurrent VTE and bleeding.

Methods: We reviewed all the literature available data reporting the rates of recurrent VTE and bleeding in patients with an active cancer and those with a history of cancer.

Results: Scarce studies reported separately the efficacy and safety of anticoagulants in these 2 groups of cancer patients. Data are summarized in this review. While cancer patient subgroup analyses of the EINSTEIN-PE, EINSTEIN–DVT and HOKUSAI trials reported no difference between patients with an active cancer and those with a history of cancer, results from COMMAND-VTE, HOKUSAI VTE Cancer and AMPLIFY studies at least suggest that the bleeding risk might be potentially lower in patients with a history of cancer compared to those with an active cancer.

Conclusions: Whether or not these 2 groups of cancer patients carry the same risk of recurrent VTE and bleeding remains an unanswered question. A strict definition of active cancer accurately reflecting the high risks of recurrent VTE and bleeding associated with active cancer should be used in further trials assessing the efficacy and safety of anticoagulants for the prevention of recurrent VTE in patients with malignancy.

PB2113 | Effectiveness and Safety of Enoxaparin in Patients with Active Cancer and Venous Thromboembolism (VTE): A Systematic Literature Review

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Background: Low molecular weight heparins (LMWH) are indicated for thromboprophylaxis, and for extended secondary thromboprophylaxis beyond oral anticoagulants in patients with active cancer and venous thromboembolism (VTE).

Aims: To evaluate the effectiveness (recurrent VTE) and safety (bleeding rates) of extended (≥3 months) enoxaparin treatment in patients with active cancer and concurrent VTE.

Methods: A systematic literature review was performed by searching key biomedical databases (inception-September 2019) and conference proceedings (January 2016-September 2019) through combining search terms for 'cancers' and 'enoxaparin' (limited to English). Standard methodology from the Cochrane Handbook for Systematic Reviews of Interventions was followed. Quality assessment was completed using standardized tools according to study design. Results were reported as per PRISMA guidelines.

Results: Of the 3,577 total citations retrieved from the searches, 15 publications (three randomized controlled trials, two prospective, and nine retrospective cohort studies, and one case-control study) were included. Proportions of patients with recurrent VTE ranged from 3.1% to 21.7% in enoxaparin-treated patients, 0% to 12.5% in warfarin-treated patients, 2.7% to 3% in LMWH- (dalteparin) treated patients, and 0% to 10.53% in DOAC- (apixaban or rivaroxaban) treated patients. One observational study reported no significant difference in recurrent VTE rates for apixaban vs. enoxaparin (HR 1.14, 95% CI: 0.54-2.42), or rivaroxaban vs. enoxaparin (HR 0.85, 95% CI: 0.36-2.06). Major bleeding was the most commonly reported bleeding outcome, with proportions ranging from 1.2-15% for enoxaparin, 2.9-16% for enoxaparin + warfarin, 0-25% for apixaban, 0-11% for apixaban/rivaroxaban, 0-17% for rivaroxaban, and 0-20% for warfarin.

Conclusions: Affirmative conclusions cannot be drawn on the effectiveness and safety of extended treatment with enoxaparin in comparison to other therapies due to the limitations in the evidence and inability to conduct a meta-analysis with the available data. Further studies are needed to address the optimal treatment strategy for patients with cancer and VTE.

PB2114 | Long-Term Use of Tinzaparin for the Treatment of Cancer-Associated Thrombosis: A Subgroup Analysis of the Prospective Observational TROPIQUE Study

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Background: Real word data on the efficacy and safety of long-term use of tinzaparin for the treatment of cancer-associated thrombosis (CAT) are scarce.

Aims: To evaluate the benefit-risk ratio of long-term use of tinzaparin in patients with CAT and whether clinical outcomes differ according to the cancer sites.

Methods: We performed a subgroup analysis of all active cancer patients included in the previously published prospective multicenter observational TROPIQUE study who received long-term tinzaparin treatment. Main outcome was adherence to clinical practice guidelines (CPGs) based on recommended low-molecular-weight heparin treatment doses for at least 3 months in the absence of severe renal insufficiency.

Results: 301 patients were included in this subgroup analysis. At study entry, their mean age was 64.6±11.9 years and 143 (47.5%)

patients were men. The most frequent cancer type was gastrointestinal (24.3%), followed by breast (18.3%) and lung (15.3%) cancer. One hundred and seventy-four (57.8%) patients had metastatic disease and 245 (82%) were receiving chemotherapy at time of CAT diagnosis. The most frequent VTE were deep vein thrombosis (47.8%) and pulmonary embolism (32.2%). Mean tinzaparin treatment duration was 5.0±1.9 months. At 6 months of follow-up, the adherence rate to CPGs was 72.8%. Over 6 months of follow-up, the overall incidence of recurrent VTE was 6.0%, while the overall incidence of major bleeding was 6.6%. Recurrent VTE (9.3%) and major bleeding (9.3%) tend to be more frequent in lung cancer patients compared to other cancer patients, although not statistically significant. A high incidence of major bleeding was also observed in hematopoietic cancer patients (9.8%).

Conclusions: This subgroup analysis of TROPIQUE confirms the favorable benefit-risk ratio of tinzaparin for the long-term treatment of CAT. Clinical outcomes may differ according to the cancer site and individualized anticoagulation strategies are warranted in some cases.

PB2116 | Intracranial Hemorrhage with Direct Oral Anticoagulants in Patients with Brain Metastases

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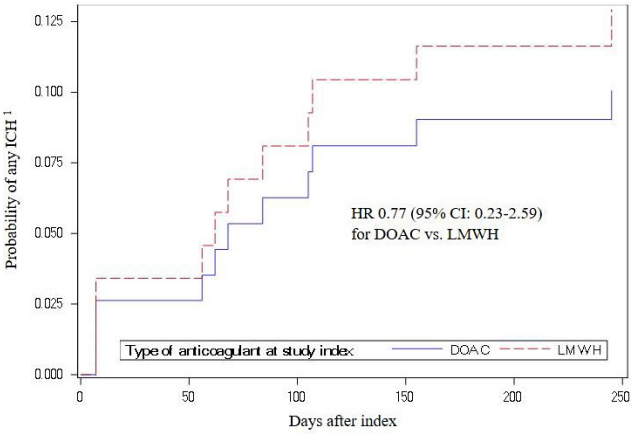
Background: Low-molecular-weight heparin (LMWH) has been the standard anticoagulant in cancer patients with venous thromboembolism (VTE). Based on recent studies, direct oral anticoagulants (DOACs) represent an alternative, but limited data exists regarding the safety of DOACs in patients with brain cancer. Brain metastases are prevalent in specific cancer types, some of which have a high risk of intracranial hemorrhage (ICH). One retrospective single-center study of patients with brain metastases and VTE demonstrated no difference in ICH rates with DOACs (n=21) compared to LMWH (n=84).

Aims: To determine the incidence of ICH with DOACs and LMWH in patients with brain metastases receiving anticoagulation for VTE or atrial fibrillation.

Methods: A retrospective cohort study in two academic centers in Israel and the Netherlands. Follow-up started on the first day of concomitant anticoagulation and brain tumor diagnosis. At least two brain imaging studies were mandated. The primary endpoint was the cumulative incidence of any spontaneous ICH at 12-months follow-up with death as a competing risk. Major ICH was defined as spontaneous, ≥ 10 mL in volume, symptomatic or requiring surgical intervention. ICH imaging studies were reviewed by a neuroradiologist blinded for anticoagulant type.



CI = confidence interval; DOAC = direct oral anticoagulant; HR = hazard ratio; ICH = intracranial hemorrhage; LMWH = low molecular weight heparin
[1] Death was considered a competing risk and type of anticoagulant was a fixed variable

FIGURE 1 12-month cumulative incidence of any spontaneous ICH

TABLE 1 Baseline patient characteristics and outcomes during 12-months follow-up. * The remainder had atrial fibrillation

Characteristics		DOAC, n (%) N=41	LMWH, n (%) N=55
Age (years), median [IQR]		**66 (58-74)**	**64 (57-71)**
Male sex		**28 (68)**	**28 (51)**
Cancer type	**Lung cancer**	**24 (59)**	**31 (56)**
	Esophageal cancer	7 (17)	4 (7)
	Breast cancer	1 (2)	8 (15)
	Other	9 (22)	12 (22)
Indication for anticoagulation	Venous thromboembolism *	22 (54)	49 (89)
Spontaneous ICH during follow-up	Any ICH [Major ICH]	4 (10) [2 (5)]	7 (13) [6 (11)]
Death [ICH-related]		25 (61) [0 (0)]	35 (64) [3 (6)]

Results: The cohort included 96 patients with brain metastases (41 DOAC, 55 LMWH). The 12-month cumulative incidence of any ICH was 10.1% in the DOAC-treated patients and 12.9% in those treated with LMWH (hazard ratio [HR] 0.77; 95% confidence interval [CI] 0.23-2.59, figure 1). When anticoagulation was analyzed as a time-varying covariate, neither the risk of any ICH nor the risk of major ICH differed between DOAC and LMWH treated patients (HR 0.98; 95% CI 0.28-3.40 and HR 0.57; 95% CI 0.12-2.87, respectively). Patient characteristics and outcomes are shown in Table 1.

Conclusions: This international multicenter study suggests comparable safety of LMWH and DOACs in patients with brain metastases, in line with a prior study.

PB2117 | Use of a Cancer Screening Tool as Part of a Conservative Approach to Screening for Occult Malignancy after Unprovoked DVT: The King's College Hospital Experience

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Background: The prevalence of occult cancer after unprovoked venous thromboembolism (VTE) appears to be lower than previously reported. As part of a conservative approach to cancer screening after unprovoked, a systems-review questionnaire, the 'cancer-screening tool' (CST), was introduced in the outpatient nurse-led clinic to promptly identify patients requiring further investigation. Inpatients continue to be assessed by the medical team. We sought to determine the incidence of cancer diagnosis following unprovoked deep vein thrombosis (DVT) and evaluate the use of the CST.

Aims: We sought to determine the incidence of cancer diagnosis following unprovoked deep vein thrombosis (DVT) and evaluate the use of the CST.

TABLE 1 Patients diagnosed with cancer following DVT Diagnosis

	Age	DVT Diagnosis	D Dimer (ng/mL)	Inpatient vs clinic	CST / outcomes	Events following DVT	Cancer diagnosis
1	49 F	Proximal DVT 29/05/18	>8000	DVT clinic	CST performed: Bloating	Early review. Ca125 high, CT performed and referred to Gynaecology MDM.	31/07/2018 Stage IIIc High Grade Serous Ovarian Cancer
2	72 M	Distal DVT 17/05/18	1300	DVT clinic	CST performed: LUTS only GGT 75	GP referral in progress to renal clinic at time of DVT diagnosis. Virology tests in renal clinic: Active HCV. Ultrasound: focal lesion	8/11/2018 Hepatocellular carcinoma
3	75 F	Distal 4cm 8/02/18 Distal 3.2cm 14/02/18	>8000	DVT clinic	No CST as 4cm distal DVT and no extension on repeat scan. Not anti coagulated	Treated for pneumonia at time of DVT diagnosis. Repeat CXR abnormal and referred to Lung Multidisciplinary meeting.	15/06/2018 Metastatic adenocarcinoma of likely lung primary
4	56 M	Proximal 04/05/18	>8000	Inpatient	Admitted with obstructive jaundice	Raised Ca199, CT confirmed pancreatic mass	10/05/2018 Metastatic adenocarcinoma of pancreatic primary
5	69 M	Distal 29/06/18	670	Inpatient	No symptoms on admission	CT AP performed on admission. Bladder diverticulum only. Haematuria 6/12 after DVT therefore referred to Urology.	04/03/2019 High grade carcinoma in situ of bladder

Methods: Clinical records of patients diagnosed with unprovoked DVT between 1st January and 31st December 2018 were retrospectively reviewed for 12 months following diagnosis. Provoked cases were excluded i.e. surgery, hospitalisation or lower-limb immobilisation (prior 12 weeks), pregnancy/postpartum, exogenous oestrogen use or cancer. Unprovoked cases included those associated with long-haul travel.

Results: 231 patients were diagnosed with DVT and 110 (44.5%) of these were classed as unprovoked. 74 (67.3%) were male. Median age was 56.5 years (range 19-97). Of unprovoked DVTs, 81 (73.6%) were diagnosed via the outpatient clinic. The remainder were inpatients. A total of 5 (4.5%) patients were diagnosed with cancer following DVT diagnosis and are summarised in Table 1. Of the 81 patients seen in DVT clinic, 57 (70.3%) had a CST completed. One had early consultant review due to the CST result and subsequent diagnosis of ovarian cancer.

Conclusions: Our findings confirm a continued low incidence of occult malignancy in unprovoked DVT. In the outpatient DVT clinic, the King's CST is a helpful adjunct for the early identification of patients requiring further investigation.

PB2118 | Increased Potential of Neutrophil Activation and Neutrophil Extracellular Trap Formation in Patients with Lung Cancer

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Background: Neutrophil heterogeneity and newly discovered functions are important foci of neutrophil research. Neutrophil extracellular traps (NETs) have been shown to be associated with thrombotic risk in patients with cancer. Neutrophil subpopulations, high and low density neutrophils (HDN/LDN) may have different roles with different abilities to undergo NET formation and thus promote thrombosis.

Aims: We aimed to investigate differences in neutrophil subpopulations, their potential of activation and NET formation between patients with cancer and healthy controls.

Methods: HDNs and LDNs from 20 patients with lung cancer (median age 67 years, range 46-82, 55% female) enrolled in the Vienna Cancer and Thrombosis Study (CATS) prior to anti-cancer treatment were isolated by density gradient centrifugation. 20 healthy individuals (62 years (50-72), 55% female) served as controls. Potential of activation (increase of CD11b surface expression) and NET formation (citrullination of histone H3 (H3Cit)) were investigated via flow cytometry. Cells were stimulated with ionomycin (IO) and phorbol 12-myristate 13-acetate (PMA). The percentage of CD11b^{high} cells (according to baseline set to 1%) and median fluorescent intensity (MFI) of H3Cit were analysed.

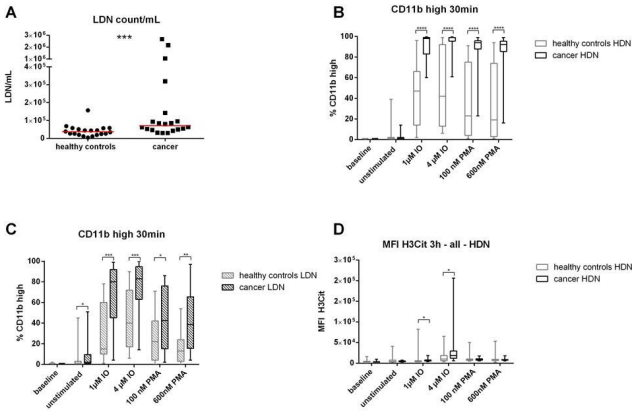

FIGURE 1 Cancer patients versus healthy controls: LDN count (A), increase of CD11b high in HDNs (B) and LDNs (C), increase of MFI H3Cit in HDNs (D)

Results: Comparison of neutrophil subpopulations isolated from patients and controls revealed 2-fold higher LDN numbers (patients median 71x10^3/mL blood (IQR 47x10^3-130x10^3), controls 37x10^3 (21x10^3-55x10^3), p< 0.001) (Fig. 1A). HDNs and LDNs from patients with cancer had a significantly higher potential of activation after 30min stimulation (Fig. 1B/C). H3Cit production was increased in HDNs from patients with cancer compared to HDNs from control individuals (Fig. 1D), whereas no differences in LDNs were observed between cohorts (data not shown).

Conclusions: Patients with cancer have more LDNs. HDNs and LDNs show a higher potential of activation and HDNs have a higher NET production, reflected by H3Cit. We conclude that neutrophil subpopulations, each in a specific way, may contribute to the increased risk of thrombosis in patients with cancer.

PB2119 | No Impact of Previous Venous Thromboembolism on the Survival of Patients Undergoing Autologous Hematopoietic Stem Cell Transplantation for Refractory Lymphoma

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Background: Venous thromboembolism (VTE) is an independent predictor of poorer survival in cancer patients, however, its impact on mortality of lymphoma patients undergoing autologous hematopoietic stem cell transplantation (ASCT) remains unclear.

Aims: To analyse the incidence of VTE and the impact on survival of patients undergoing ASCT for relapsed/refractory lymphoma.

Methods: 166 adult patients (average age 40 years, range 18-67, 56% men) undergoing ASCT due to relapsed (n=134) or refractory (n=32) non-Hodgkin's lymphoma (NHL, n=93) or Hodgkin's lymphoma (HL, n=73) in 2012-2018 were analysed.

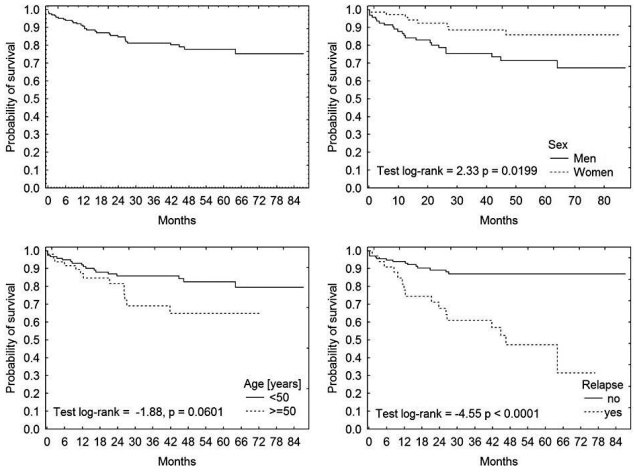

FIGURE 1 Kaplan-Meier estimates for cumulative survival probability of the patients with lymphoma undergoing ASCT

Results: 39 events of VTE (23%) were recorded during the follow-up period (median 38, range 2-87 months), including 6 cases during ASCT (3.6%). 32 patients (19.3%) died because of relapse, cardiovascular disease, infectious complications and other cancers. Although 7 of these patients had history of VTE, none of the deaths were VTE-related. More deaths were found among men (23/32, p=0.04). No differences in mortality were found between analysed lymphoma groups (HL vs. NHL, p=0.041). Of 32 patients who relapsed after ASCT, 16 died (p< 0.005). Kaplan-Meier analysis showed no difference in survival between patients with and without history of VTE. In the Cox proportional hazard model only relapse after ASCT (HR 5.19; 95% CI 2.44-11.05, p< 0.0005), age over 50 years (HR 3.04; 95% CI 1.37-6.76, p=0.0063) and male gender (HR 2.57; 95% CI 1.12-5.87, p=0.025) were significantly associated with mortality, Figure 1 shows Kaplan-Meier analyses. Other factors, including HL diagnosis (HR 1.16; 95% CI 0.49-2.76, p=0.74), relapse before ASCT (HR 1.31; 95% CI 0, 63-2.73, p=0.50), after >3 lines of treatment before ASCT (HR 1.29; 95% CI 0.58-2.90, p=0.53) and history of VTE (HR 0.67; 95% CI 0.28-1.62, p=0.38) had no impact on mortality.

Conclusions: Our analysis showed no negative impact of VTE both before and during ASCT on the survival of patients undergoing ASCT due to refractory lymphoma.

PB2120 | Risk Factors, Mortality and Costs of Cancer-Associated Thrombosis in Hospitalised Patients

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Background: Data on cancer-associated venous thromboembolism (VTE) in Southeast Asia are lacking.

Aims: We sought to determine cancer-associated VTE's prevalence and impact in Singapore.

Methods: This was a retrospective, single centre cohort, with nested case-control, study between 1 January 2015 and 31 October 2019. All patients admitted with a cancer diagnosis were identified from our hospital's ICD-10 database. VTE cases diagnosed within a year of cancer were identified; transplant patients were excluded. Outcomes were in-hospital mortality, bleeding complications and hospitalization costs over one year from the VTE/cancer diagnosis. For the purposes of outcomes analysis, the non-VTE and VTE groups were matched for age, ethnicity, cancer diagnosis, surgery within the last three months, and chemotherapy.

Results: 7967 cancer patients were included; 8.6% had VTE. Baseline characteristics are shown in Table 1.

VTE preceded cancer in 14.9% of patients, occurring a median of 77.5 (range 8 to 366) days prior. Of the 42.8% of patients who had a VTE after their initial diagnosis, median time to VTE was 82 days (range 1 to 352 days).

The absolute risk of VTE was 8.6%, and different by tumour type (figure 1). Patients on chemotherapy had a 10.6% risk of VTE, versus 7.0% without chemotherapy. VTE risk was 9.3% in patients with recent surgery (7.5% without).

TABLE 1 Characteristics of cancer patients without and with cancer-associated VTE

	No VTE Group - Before Matching	No VTE Group - After Matching	VTE Group
Number of Patients	7283	2052	684
Male Gender (%)	46.7	49.6	47.4
Age (mean ± SD)	62.1 ± 14.0	63.4 ± 13.1	62.1 ± 12.8
Atrial Fibrillation (%)	1.8*	1.9	3.1*
On Chemotherapy (%)	42.0*	53.2	53.2*
Surgery Within 3 Months (%)	61.3*	69.3	66.5*
Bleeding Complications	7.2*	8.0†	14.6*†
In-Hospital Mortality	20.7*	23.0†	28.2*†
Overall Mortality	27.8*	31.2†	39.8*†

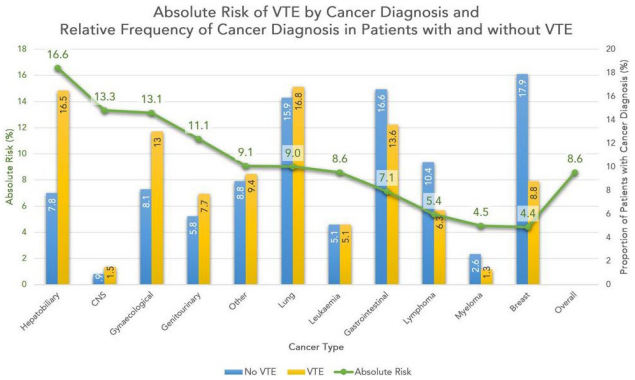

FIGURE 1 Characteristics of cancer patients with and without VTE

69.2% of the VTE group were anticoagulated. Of those not anticoagulated, diagnoses were splanchnic vein (62%), deep vein (15%) thrombosis and pulmonary embolism (15%).

Patients with VTE had inferior in-hospital mortality (28.2% versus 23.0%, *p*=0.007), more bleeding complications (14.6% versus 8.0%, p< 0.001) and hospitalization-related costs (178% that of the non-VTE group on average, p< 0.001). This was primarily driven by increased length-of-stay and medication charges.

Conclusions: VTE is prevalent in Asian patients and associated with increased mortality and healthcare costs. Prophylaxis of high-risk patients is warranted to reduce the incidence of cancer-associated thrombosis.

PB2121 | Assessment of the Ottawa Score in the Prediction of Recurrent Venous Thromboembolism in Patients Treated for Lymphoma

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Background: Data on the utility of the Ottawa score/modified Ottawa score for the prediction of recurrent venous thromboembolism (rVTE) in patients treated for lymphoma is scarce.

Aims: To evaluate the discriminatory performance of the Ottawa score/modified Ottawa score in stratifying or predicting recurrent VTE events in patients treated for diffuse large B-cell lymphoma (DLBCL) and Hodgkin lymphoma (HL).

Methods: To assess the occurrence of VTE and recurrent VTE, retrospective analyses were performed on 710 patients (median age 52). 419 were diagnosed with DLBCL and 291 had HL.

Results: During the 10-year observation of 144 (20%) patients who developed VTE, 25 had recurrent VTE (17%). According to the Ottawa Score, 64 (44.4%) patients were considered low risk and 80 (55.6%) patients were considered high-risk for rVTE. In comparison, when using the modified score, 17 (11.8%) patients were considered low risk, 62 (43.1%) patients intermediate risk and 65 (45.1%) patients high-risk for rVTE. Patients with HL and women had rVTE more frequently. Recurrent VTE occurred in 31% (n=25) of the high-risk patients according to the original Ottawa score and in 37% (n=24) high-risk patients and 1.6% (n=1) intermediate-risk patients according to the modified Ottawa score. In multivariate analysis, only a high modified Ottawa score (OR 94.3; 95% CI 11.5-769, p< 0.0001) and HL lymphoma (OR 4.30; 95% CI 2.41-8.57, p< 0.0001) remained significant for recurrent VTE. For the high modified Ottawa score, sensitivity was 96%, specificity was 65%, the ROC AUC was 0.811 (95% CI 0.74-0.88) and C statistic was 0.62. The high modified Ottawa score best discriminated HL patients with high risk of recurrent VTE (ROC AUC=0.87; 95% CI 0.77-0.97 and C statistic-0.73).

Conclusions: The modified Ottawa score could identify patients at a high risk of recurrent VTE, with high accuracy, particularly in patients with HL.

PB2122 | Massive Life-Threatening Intravascular Tumor Thrombus (ITT) as the Initial Manifestation of Renal Cell Carcinoma Complicated with Heparin-Induced Thrombocytopenia: A Case Study

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Background: Intravascular tumor thrombus (ITT) is a common complication in patients with renal cell carcinoma (RCC), with optimal management remaining uncertain.

Aims: To report the challenge and initial approach in a case of massive life-threatening ITT in the vena cava, right atrium and bilateral pulmonary embolism (PE) as the first manifestation of RCC.

Methods: We report a case study.

Results: A 33-year-old woman was admitted to hospital for acute shortness of breath. Physical examination showed arterial hypotension (70/50 mmHg), tachycardia (150 bpm) and tachypnea (35 bpm) requiring high concentration oxygen (FiO_2 40%). Electrocardography showed complete right blockade. Bedside ultrasound showed severe right ventricular dysfunction and dilation. Computed tomography

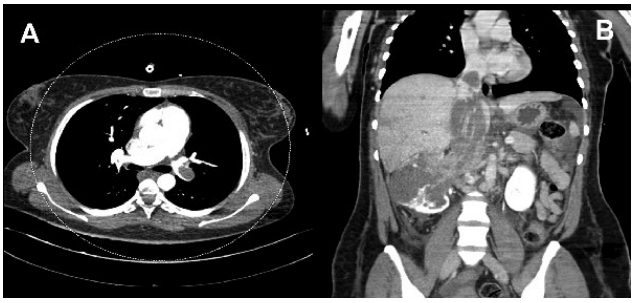

FIGURE 1 (A) Massive bilateral PE; (B) Expansive right renal mass associated with a ITT that extends into the IVC and right atrium

TABLE 1 Clinical and radiological findings over time

Date	Clinical findings	Radiological findings
November 9th 2019. Day +1	Acute dyspnea and tachypnea. Hypotension and tachycardia. Admission to ICU for vasoactive support, high flow oxygen and UFH.	Bedside ultrasound: right ventricular dysfunction and dilation; thrombus in the IVC and right atrium. Angio-CT: massive bilateral PE, right renal mass and ITT from the IVC to the right atrium.
Day +3	Vasoactive support withdrawn.	
Day +6	Transfer to the oncology ward. Switch to LMWH.	
Day +7	ECOG-PS 3. Tachypnea requiring high concentration oxygen (40%).	Angio-CT: thrombus persistence in bilateral pulmonary arteries with mild retraction.
Day +9	ECOG-PS 3. Grade 4 thrombocytopenia (26.000). Detection of anti-heparin platelet factor 4 antibodies. Switch to fondaparinux (7.5 mg/24h).	
Day +20	ECOG-PS 2. Oxygen withdrawal. Ambulation around the unit.	Thoracoabdominal CT: bilateral PE without changes; thrombus shrinkage in the hepatic IVC; thrombus progression in the infrarenal IVC with caudal extension to the left femoral vein. Echocardiography: thrombus persistence in the right atrium (18x14mm); no dilation nor dysfunction of the right ventricle; no signs of pulmonary hypertension.
Day +24	ECOG-PS 1. Renal biopsy performed. Hospital discharge.	
Day +40	ECOG-PS1. Outpatient treatment with nivolumab + ipilimumab initiated.	
Day +70	ECOG-PS0. Excellent tolerance. Third cycle of nivolumab + ipilimumab.	Echocardiography: thrombus persistence in the IVC but with shrinkage in the right atrium; no dilation nor dysfunction of the right ventricle; no signs of pulmonary hypertension.

angiography confirmed the presence of a massive bilateral PE (Figure 1A) and a right renal mass associated with a large ITT in the inferior vena cava (IVC) extending up to the right atrium (Figure 1B). Fibrinolytic therapy was performed with no hemodynamic improvement and vasoactive support and anticoagulation with unfractioned heparin (UFH) was continued. Endovascular or surgical thrombectomy was ruled out by a multidisciplinary team based on the large extension and tumoral thrombus nature. The main outcome features are shown in Table 1. Grade 4 thrombocytopenia due to heparin-induced thrombocytopenia (HIT) was diagnosed on day +9 and confirmed by detection of anti-heparin platelet factor 4 antibodies that normalized after switching low molecular weight heparin (LMWH) to fondaparinux. Renal biopsy confirmed the diagnosis of RCC and first-line anticancer therapy with nivolumab + ipilimumab was started as outpatient treatment on day +40.

Conclusions: This case illustrates the challenges associated with the management of a massive ITT and the subsequent development of HIT. Optimal management of ITT deserves further research.

PB2124 | Bleeding Risk Assessment Model for Older Multiple Myeloma Patients on Immunomodulatory Drugs (IMIDs)

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Background: Immunomodulatory drug (IMID) based multiple myeloma (MM) treatments are associated with high risk of venous thromboembolism (VTE). Several VTE risk models have been validated recently, including SAVED and IMPEDE VTE, to help stratify patients for thromboprophylaxis. Risk models for serious bleeding complications in this population are lacking.

Aims: We aim to derive a new model to predict major bleeding in IMID-treated older MM patients.

Methods: We selected patients 66 or older with newly diagnosed MM who concurrently received IMID and had complete fee-for-service coverage from the SEER-Medicare database over 7 years. Demographics and risk factors were ascertained from SEER and Medicare files. Major bleeding was defined as bleeding requiring hospitalization using inpatient ICD9 codes as previously validated (Arnason Thromb Res 2006). Patients were followed from IMID

TABLE 1 Bleeding risk assessment model for IMID-treated myeloma patients

	Hazard Ratio	P Value	95% CI	Points
Comorbidity last year				
__History of major bleeding (n=158)	2.88	0.01	1.26-6.57	2
__History of renal disease (n=624)	1.88	0.06	0.98-3.61	1
Age (baseline 66-75)				
__Age 76-85 (n=859)	1.38	0.35	0.70-2.71	1
__Age 86+ (n=192)	2.63	0.04	1.04-6.67	2
Model Discrimination				C Index
__Every 1 point increase	1.79	0.001	1.28-2.50	0.66
__2+ vs. 0-1 point	3.31	0.001	1.67-6.58	0.63

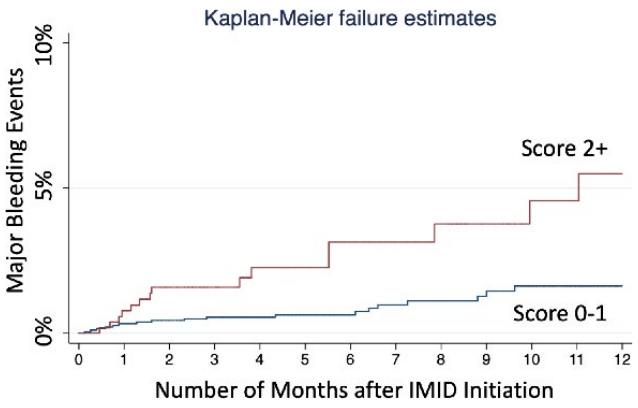

FIGURE 1 Bleeding risk stratification for IMID-treated myeloma patients

initiation to first major bleeding event; they were censored for death, disenrollment, discontinuation of IMID, or end of claims data. Cox regression model was used to determine significant risk factors for major bleeding. Integer points were assigned according to beta coefficients to create risk groups. The model's discrimination was assessed by Harrell's c index, hazard ratio (HR), and 95% confidence interval (CI).

Results: Among 2397 IMID-treated MM patients, inpatient major bleeding occurred in 40 during follow-up. Risk factors included history of major bleeding (HR 2.88), renal disease (HR 1.88), and older age (HR 1.38 to 2.63) (Table 1). The final model significantly predicted major bleeding for every point increase (HR 1.79, 1.28-2.50, c index 0.66). Patients with 2+ points (n=526) had more than 3-fold increase in major bleeding (HR 3.31, 1.67-6.58) compared to those with 0-1 point (n=1848) (Figure 1).

Conclusions: The incidence of major bleeding requiring hospitalization appears low in older MM patients. Nonetheless, a selective higher risk group may be identified. Our model, if externally validated, can help clinicians determine the best risk-benefit trade-off for thromboprophylaxis.

PB2125 | Empowering Cancer Patients for Non-Pharmacological Primary Prevention and Early Recognition of Cancer-Associated Thrombosis (CAT): The EMPATIC-CP Survey

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Background: In contrast to the relevance of cancer-associated thrombosis (CAT), recent data from qualitative studies and a European survey show a low level of awareness about the risk factors and suspicious symptoms of CAT.

Aims: The present study aimed to explore the current practices regarding education for non-pharmacological primary CAT prevention

TABLE 1

Table 1. Main results of the survey.

	Overall (48) N = (%)
Is health education carried out for early recognition of warning signs for **oncological emergencies**?	
o Always	27 (56.2)
o Never	1 (2.1)
o Not routinely, only in some situations	20 (41.7)
Is health education carried out for the **prevention and early recognition of CAT-related symptoms**?	
o Always	6 (12.5)
o Never	8 (16.7)
o Only in catheter carrier patients	2 (4.2)
o Only in catheter carrier patients or in any other specific situations	32 (66.7)
	Overall (37)* **N = (%)
What **professionals** perform health education for **prevention and early recognition of CAT-related symptoms**?	
o Specialist physician	30 (81.1)
o Day-care hospital nurse	21 (56.7)
o Resident physician	19 (51.3)
o Outpatient clinical nurse	9 (24.3)
o Hospital pharmacist	2 (5.4)
	Overall (37)* N = (%)
When you perform patient education for **prevention and early recognition of CAT** symptoms, what kind of **resources** do you use?	
o Verbal information	37 (100)
o Printed brochure	9 (24.3)
o Digital support	1 (2.7)
	Overall (48) N = (%)
Is health education carried out for prevention and early recognition of **recurrence of CAT**?	
o Always	33 (68.8)
o Never	15 (31.2)

*3 centres did not answer this question
**Numeric scale question (0 to 10). We considered that the Health Care Professional was actively involved in a specific centre when the participant voted ≥ 6

and early recognition of potential CAT-related symptoms in cancer patients.

Methods: We designed and conducted a specific electronic questionnaire from December 2018 until March 2019 to assess the current practices in a sample of 52 Spanish and 19 Portuguese Medical Oncology/Haematology Departments.

Results: Data from 48 out of 71 (68%) centres (35 in Spain and 13 in Portugal) were analysed. The main results are summarized in Table 1. Education specifically addressed to recognising warning symptoms for cancer-associated emergencies was routinely performed in 27 centres (56.2%). Specific patient education programmes for primary prevention and early recognition of CAT were reported as 'always' being carried out in only 6 centres (12.5%) and only in patients with indwelling central venous catheters or other specific situations in 32 (66.7%) centres.

Patient education (multiple-choice question) was reported to be mainly performed by specialist physicians (81.1%), day-care hospital nurses (56.7%) and resident physicians (51.9%). In all cases, the information was provided verbally and specific printed brochures for education in the prevention of CAT were rarely reported as being used. Patient education aimed at recognising signs of CAT recurrence and/or bleeding complications in cancer patients with previous CAT events was routinely performed in 33 (68.8%) of the participating centres.

Conclusions: Patient education for recognising potentially life-threatening symptoms related to cancer, anticancer therapies and cancer-associated complications such as CAT is poorly conducted in our setting.

The implementation of strategic educational programmes aimed at increasing patient awareness about cancer-associated emergencies and venous thromboembolism requires further research and development.

PB2126 | Venous Thromboembolism (VTE) Events with Cancer as a Risk Factor in a Teaching Hospital: 2015-2019

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Background: Cancer is a well-known risk factor for VTE. Data is presented on various metrics, for patients with VTE in the context of underlying Cancers.

Aims: Identify all VTE events diagnosed between 2015 and 2019 (5 years), associated with active cancer as a risk factor. Assess trends in management over the study period including anticoagulation use.

Methods: All VTE events identified from targeted radiological data, comprising CT pulmonary angiograms. SPECT-CT and Doppler ultrasounds of upper and lower limbs, were reviewed for evidence of active cancer as a risk factor. Cancer type and degree of metastasis was also assessed, together with anticoagulant used and duration

TABLE 1 Venous Thromboembolism events associated with cancer 2015 – 2019

Year	Total VTE Events	Number Cancer Risk Factor	%
2015	831	193	23.2
2016	861	188	21.8
2017	833	209	25
2018	822	179	21.8
2019	769	167	21.7
Total	4116	936	23

Results: A total of 4116 VTE events occurred over five years. 936 (23%) of these had active cancer as a risk factor. Of the 3210 community-acquired (CAT) events, 609 (19%) were cancer-associated, compared to 327 of the 906 hospital acquired (HAT) events (36%). Overall 174/936 (19%) demonstrated metastatic cancer. The most prevalent cancers were lung, prostate, breast and bowel, comprising 56% of all cancers associated with VTE. Low molecular weight heparin (LMWH) was the most widely prescribed treatment in 66% of cases, followed by Rivaroxaban at 23% and Apixaban at 6%. The use of direct oral anticoagulants generally, has increased significantly over the last two years.

Conclusions: Cancer is a major risk factor for VTE. Using the UK Department of Health, VTE risk assessment tool, only age (>60 years) demonstrated a larger risk within our VTE outcome data. The most common cancers identified are not those known to have the highest VTE risk, but are the more prevalent cancers, therefore explaining this outcome. Understandably, acknowledging current national guidance, LMWH is by far the most common anticoagulant used in the cancer risk cohort. This is likely to change soon.

PB2127 | Risk of Arterial Thromboembolism in Cancer Patients: A Population-Based Cohort Study

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Background: The relation between cancer and arterial thromboembolism (ATE) remains unclear.

Aims: To evaluate the association between cancer and ATE.

Methods: Danish registries were used to identify patients with a first-time cancer between 1997 and 2017, each matched to three cancer-free comparison individuals by age, sex, and year of the cancer case's diagnosis (index date). The primary outcome was a composite of ischemic/unspecified stroke, myocardial infarction, peripheral arterial occlusion, coronary artery bypass grafting, and

TABLE 1 Baseline characteristics of cancer cohort and matched comparison cohort, Denmark,1997-2017

	Cancer cohort N=458,462	Comparison cohort N=1,375,386
Female, n (%)	234,915 (51.2)	704,745 (51.2)
Age group, n (%)		
59 years or younger	109,572 (23.9)	328,716 (23.9)
60-74 years	204,819 (44.7)	614,457 (44.7)
75 years or older	144,071 (31.4)	432,213 (31.4)
Previous ATE, n (%)	54,516 (11.9)	149,294 (10.9)
Atrial fibrillation, n (%)	31,311 (6.8)	81,237 (5.9)
Antiplatelet therapy, n (%)	96,759 (21.1)	270,468 (19.7)
Anticoagulant therapy, n (%)	24,679 (5.4)	60,692 (4.4)

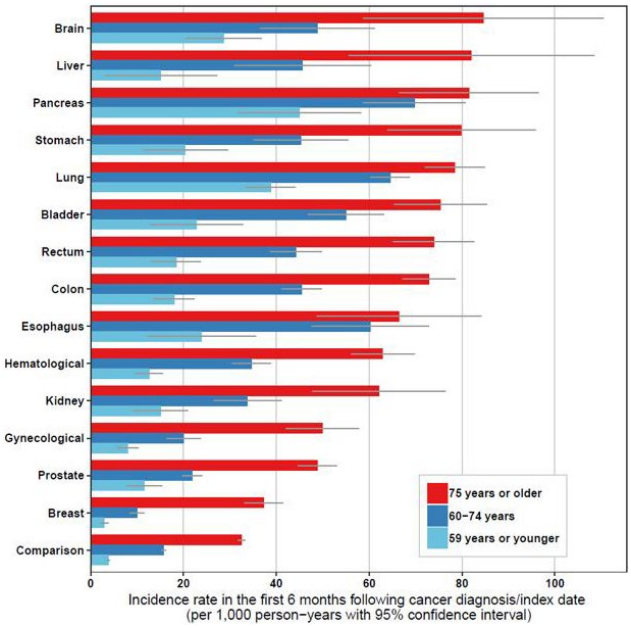

FIGURE 1 Incidence rate of ATE for each cancer type during the first 6 months after cancer diagnosis among patients ages 59 years or younger, 60-74 ys

percutaneous coronary intervention. A competing risk approach was used to compute cumulative incidences at 6 months after diagnosis. Incidence rates were calculated per 1,000 person-years. Groups were compared by calculating hazard ratios (HRs), using Cox regression models. Risk factors for ATE were assessed by computing subdistribution hazard ratios (SHRs).

Results: The study included 458,462 cancer patients and 1,375,386 matched comparison individuals (Table). In the 6-month period after the cancer diagnosis/index date, ATE incidence was 1.60% (95% CI, 1.57%-1.64%) in the cancer cohort and 0.89% (95% CI, 0.88%-0.91%) in the comparison cohort (HR 2.14; 95% CI, 2.07-2.21). ATE risk in cancer patients in the 6 months after cancer diagnosis varied across age groups as follows: 0.64% (95% CI 0.60%-0.69%) among patients < 60 years, 1.56% (95% CI, 1.51%-1.62%) among patients 60-74 years, and 2.39% (95% CI, 2.32%-2.47%) among patients >74 years. Risk factors for ATE were male sex (SHR, 1.64; 95% CI, 1.56-1.71), prior ATE (SHR, 4.37; 95% CI, 4.17-4.58), and diabetes (SHR 1.91; 95% CI, 1.78-2.04). The incidence rate in the first 6 months after cancer diagnosis varied substantially across cancer types and age, with the highest observed incidence in patients with brain, liver, and pancreatic cancer (Figure).

Conclusions: Cancer patients are at increased risk of ATE compared to the general population. Its risk depends on age, cancer type, history of ATE, sex, and diabetes.

PB2129 | Haemostatic State in Hodgkin Lymphoma Patients

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Background: Patients with Hodgkin Lymphoma(HL) have a high risk of VTE. However, the prophylaxis with LMWH do not prevent all TE thus routine laboratory monitoring is not recommended for LMWH. The identification of high-risk group of patients and the adequate laboratory control for preventing TE are still important and actual problems.

Aims: Examine the state of hemostatic system in primary patients and during the therapy.

Methods: 29 primary patients(median 31yr,18-60 yrs,13 men,16 women), staging by Ann Arbor II-12,III-7,IV-11, were enrolled in this study. The number of thrombotic RF by Khorana predictive model from 1 to 4. The patients were treated by BEACOPP-14. 24 patients receive LMWH 70-113IU/kg 2 times/day,6 patients have no anticoagulation therapy. Blood samples were collected after 4 hours after LMWH injection. ATIII, Hageman-factor-dependent fibrinolysis and D-dimer levels(DD), and the global assays Thrombodynamics (TD) and Thromboelastography(TEG) were performed to evaluate the hemostatic state of patients on diagnosis and during the 6 cycles of chemotherapy. Anti-Xa activity was used to monitor the anticoagulant activity.

Results: Hypercoagulation was detected in primary patients by TD(45%)and TEG(55%).DD were higher in 33% of patients.In 59% patients lysis time were extended, patients with high risk of TE had significant (p< 0.05)longer lysis time then patients with medium risk(41min&18min respectively).

Throughout the chemotherapy cycles in patients with ineffective LMWH prophylaxis(Anti-Xa< 0.5) was revealed the significant(p< 0.05)increase in TD and in DD compared to patients with effective prophylaxis(min 62 - max 5109ng/ml and min 53 - max 3571ng/ml respectively).

Despite LMWH prophylaxis the DD was significantly(p< 0.05)increased from 1st(min 71-max 5109) to 6th cycle (min 79-max 4376) of chemotherapy.

Conclusions: Primary patients with HL revealed hypercoagulation by TD,TEG and had extended lysis time before chemotherapy. The combination of TD, TEG, DD, lysis time and thrombotic RF is prospective in indicating high-risk group and monitoring and correction of LMWH prophylaxis in patients with HL.

PB2130 | Failure of the Ottawa Score to Predict the Risk of Recurrent Venous Thromboembolism in Patients with Cancer-Associated Thrombosis. A Pooled Analysis of two Prospective Cohort Studies

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Background: The risk of recurrent venous thromboembolism (VTE) despite therapeutic anticoagulation is 3-6 times higher in patients with cancer-associated thromboembolism (CAT) than in non-cancer patients. The ability to accurately predict this risk could have therapeutic implications. The only predictive risk-assessment sytem, the Otttawa score, was developed in a retrospective cohort and has not been externally validated in recent prospective cohorts of CAT patients.

Aims: To assess the predictive value of the Ottawa score and identify new potential predictive variables from data of two recent cohorts of CAT patients.

Methods: The prospective multicenter aXa (NCT02898051) and PREDICARE (NCT03099031) cohorts used identical inclusion criteria (symptomatic or asymptomatic lower limb proximal or distal deep venous thrombosis and/or pulmonary embolism), treatment (tinzaparin 175U/kg/d for a planned duration of 6 months) and primary efficacy endpoint (symptomatic or asymptomatic VTE recurrence within 6 months), to be pooled in a meta-analysis planned *a priori* (PROSPEROCRD42019119907). All clinical events were adjudicated by an independent committee. The competing risk of death was taken into account (Kalbfleisch and Prentice method).

TABLE 1 Potential additional prognosis variables of VTE recurrence adjusted for variables of the Ottawa score

Variables	Hazard ratios (95% CI)
Upper gastrointestinal primary	3.05 (1.00 ; 9.25)
Surgery/trauma <1 month	2.90 (1.23 ; 6.82)
Urologic cancer	2.65 (1.08 ; 6.47)
Proximal DVT	2.26 (1.23 ; 4.17)
Lung cancer*	2.19 (1.09 ; 4.44)
Female*	1.82 (0.95 ; 3.48)
Previous VTE*	1.44 (0.66 ; 3.13)
Stage I*	0.51 (0.07 ; 3.74)
Breast cancer*	0.17 (0.02 ; 1.27)

** not tested but used for adjustment*

Results: A total of 717 patients were included in 76 centers in France (aXa, n=308; PREDICARE, n=409). Mean age was 67.2 years, 66% of patients had metastatic disease, the main primary sites were lung (23%) colorectal (17%) and breast (13%) cancers. The 6-month cumulative incidences of VTE recurrence in patients with low (< 1; n=292) and high (≥1; n=399) Ottawa score were 4.2% (95%CI, 2.1-8.4) and 8.4% (95%CI, 5.9-12.0), respectively. The AUC for the Ottawa score was 0.63 (95%CI 0.59-0.66). Additional potential variables associated with the risk of VTE recurrence were identified In a multivariable analysis (table).

Conclusions: In this homogeneous prospective population of CAT patients treated with tinzaparin, the Ottawa score could not accurately predict VTE recurrence. Further research is needed to develop predictive tools that could help therapeutic decision making in this setting.

PB2131 | Biomarkers of Venous Thromboembolism Recurrence After Discontinuation of Anticoagulation for Cancer-Associated Thrombosis (HISPALIS- Study)

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Background: Optimal duration of anticoagulation for cancer-associated thrombosis (CAT) remains unclear. This study assessed D-dimer (DD), high-sensitivity C-reactive protein (hs-CRP) and

P-selectine (PS) after the withdrawal of anticoagulation treatment to predict the risk of venous thromboembolism (VTE) recurrence among patients with CAT.

Aims: To identify biomarkers of venous thromboembolism recurrence after discontinuation of anticoagulation for cancer-associated thrombosis.

Methods: Prospective multicenter study to evaluate CAT with more than 3 months of anticoagulation that was subsequently discontinued. Blood samples were taken when patients stopped the anticoagulation and 21 days later to determine the DD, PS and hs-CRP levels. All patients were followed up for 6 months to detect VTE recurrence.

Results: Between 2013 and 2015, 325 patients were evaluated and 166 patients were ultimately enrolled in the study. The mean age was 62 ± 13.5 years and nearly 40% had metastasis. Incidence of VTE recurrence at 6 months was 9.6%, 95% confidence interval [CI]: 6-15%). Male sex, ratio DD basal/DD 21 days >2, hs-CRP and PS levels after 21 days were associated with VTE recurrence. The subdistribution hazard ratios were 4.32 for male sex (95% CI 1.10-16.96), 7.53 for ratio DD >2 (95% CI 1.97-28.71), 5.15 for hs-CRP (95% CI: 1.37-19.34) and 5.60 for PS (95% CI: 1.48-21.08).

Conclusions: This study identified that ratio DD >2, hs-CRP and PS as potential biomarkers of VTE recurrence after discontinuation of anticoagulation in CAT. A risk-adapted strategy could identify the optimal time to stop the anticoagulation in CAT patients. Funding sources: Instituto de Salud Carlos III (PI15/01085) and SETH.

PB2132 | Use of Anti-Factor Xa Levels in Cancer Patients Taking Direct Oral Anticoagulants

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Background: Use of direct oral anticoagulants(DOACs) in patients with cancer is increasing but remains complex. Absorption of DOACs during chemotherapy and theoretical potential of drug-drug interactions are of concern.

Aims: To identify patients with active cancer who had anti-factor Xa(anti-Xa) levels measured on direct factor Xa inhibitors and rationale for this. To establish circumstances under which anti-Xa levels inform management.

Methods: Anti-Xa levels from March 2017-November 2019 in specialist coagulation laboratory at UCLH were reviewed. Retrospective case-note review established cancer type, DOAC indication, anti-Xa level indication and whether the level informed a DOAC dose adjustment. Anti-Xa levels were performed using assay-specific reagents, 2-4 hours post-dose.

Results: 65 patients with active cancer receiving a DOAC had 90 anti-Xa samples(Table 1). The indication for DOAC was treatment of cancer-associated VTE in 27/65(Figure 1). Suspected drug-drug interaction was the most common indication for checking levels(42/90), mostly PARP inhibitor(PARPi:niraparib/rucaparib/olaparib)(14/42). Other indications included absorption issues(18/90), renal impairment(12/90) and acute bleeding/thrombosis(6/90).

TABLE 1 Baseline characteristics

	Number of patients (n = 65)
Male sex (%)	18 (28)
Age, Median (range)	67 (12-89)
Creatinine clearance, ml/min, Median (range)	73 (11-154)
Cancer subtype (%) (n = 68*)	18 (26.5)
Ovarian	12 (17.6)
Haematological	8 (11.8)
Breast	6 (8.8)
Gastrointestinal	24 (35.3)
Other	
DOAC prescribed (%)	30 (46)
Apixaban	35 (54)
Rivaroxaban	

* 1 patient with 3 malignancies, 1 with 2.

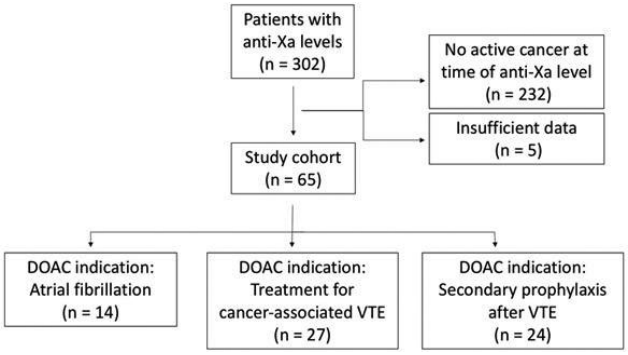

FIGURE 1 Data flow

Seven anti-Xa levels informed DOAC dose adjustments in six patients.All patients were taking rivaroxaban.5/6 patients' doses were reduced due to high levels secondary to drug-drug interaction or renal impairment.

One patient on therapeutic rivaroxaban and olaparib required DOAC dose reduction due to high anti-Xa levels. Anticoagulant dose was subsequently reduced empirically in the remaining 3/4 patients receiving PARPi and therapeutic DOAC. Median anti-Xa level in patients receiving PARPi and prophylactic rivaroxaban 10mg OD was 210 ng/ml(range 30-227),in keeping with levels expected from therapeutic anticoagulation. Rivaroxaban/apixaban anti-Xa levels taken during PARPi therapy were higher than predicted for dose in 9/14.

Conclusions: Drug-drug interaction concerns are a common reason for anti-Xa measurement in this population. PARPi appear to increase plasma levels of rivaroxaban and apixaban, probably via CYP3A4 and p-glycoprotein inhibition, but the clinical relevance of

this for bleeding risk is undefined. Doses of rivaroxaban/apixaban may need adjustment when co-prescribed with PARPi.

PB2133 | The Downregulation of Mucin1 Initiates Aberrant Calcium Signalling Pathways of Cancer Cells which Are Partly Responsible for the Procoagulant Phenotype

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Background: Intracellular calcium ions act as second messengers regulating numerous physiological functions. Alterations in calcium signalling have also been implicated in several pathological conditions including cancer.
Aims: To determine whether MUC1, a well-established tumor marker, plays a role in modulating calcium signalling and thereby alters the procoagulant properties of cancer cells.
Methods: MUC1 knockdown breast cancer MCF-7 cells were used to investigate whether the overexpression of MUC1 by cancer cells has an influence on calcium signalling pathways, as determined by analysis of the level of calcium signalling proteins, the degree of intracellular calcium mobilization, and calcium-induced cellular effects. The investigation was extended to normal human mammary epithelial, breast and pancreatic cancer cell lines, expressing different levels of MUC1.
Results: MUC1 downregulation in MCF-7 cancer cells was associated with aberrant expression of several calcium transporter proteins. In addition, the level of each of these calcium signalling pathway proteins was correlated with MUC1 levels in normal human mammary epithelial and human breast and pancreatic cancer cell lines. As a consequence, a number of proteins whose activities are calcium dependent, such as p-CaMKII, and procoagulant proteins Va, Xa and thrombin, were reduced in MUC1 downregulated MCF-7 cells. Moreover, PAR-1 and PAR-4 cleavage by thrombin as well as the resultant calcium mobilization were also reduced in MUC1 downregulated MCF-7 cells.
Conclusions: The downregulation of MUC1 is capable of modulating calcium cellular effects via altered expression of calcium signalling proteins as well as calcium mobilization in human cancer cells and associated procoagulant activity. Such properties contribute to a crucial role for MUC1 in numerous signalling networks within tumorigenesis. These findings suggest a potential role for MUC1 through modulation of Ca2+ signalling in cancer, which may help guide the development of therapeutic targeting of MUC1.

PB2135 | Current Status on Non-hematological Cancer Associated Thrombosis at Tertiary Care Hospital in India

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Background: Cancer associated thrombosis is a leading cause of morbidity and mortality in malignancy patients. It is multifactorial in origin, being related to type, site, stage as well as treatment of underlying malignancy. Prophylactic anticoagulation is often underutilized. Also, cost of LMWH and direct oral anticoagulants is a major barrier in developing countries.
Aims: To analyze the patient and disease related parameters in cancer associated thrombosis.
Methods: We did a retrospective analysis of all cancer associated thrombosis patients attending our thrombosis clinic between 2011-2015 at a tertiary level referral hospital based in North India. Patient demographics and disease related parameters were collected and analyzed.
Results: A total of 771 patients attended DVT clinic during study period, of which 64 were malignancy associated. Of these, 56% were female and 20% patients were bed ridden. Median age was 48.5 years with adenocarcinoma (48%) being the most common histological subtype, followed by squamous cell carcinoma (16%). Gynecological malignancies (20%) were the most common malignancies, followed by genitourinary (11%) and then CNS (9%) and gastrointestinal malignancies (7%). Most of the cases occurred during first year of diagnosis (51%), and only 14% occurred after 3 years. Most of the patients were on combination treatment, followed by surgical and chemotherapy based regimens. Lower limb thrombosis was most commonly seen (56%), abdominal and pulmonary thrombosis were seen in 5% each. Most of the patients were managed with LMWH and vitamin K antagonists (84.3%) and only 6.25% with LMWH alone.
Conclusions: Malignancy associated DVT is very common and presents an unmet need for prophylactic anticoagulation. Most of the cases occur early in disease course, and can be harbinger of relapse. Most cases involve lower limb veins, but pulmonary and abdominal veins may also be thrombosed. Early suspicion and prompt treatment can improve quality of life in such patients.

PB2136 | A Newly Developed Kit for High-Sensitivity Measurement of TF-Dependent Procoagulant Activity on Microvesicles Purified by High-Speed Centrifugation

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Background: Standardized and ultra-sensitive measurement of MV-associated TF-dependent activity is mandatory to evaluate thrombotic risk. Although several versions of FXa generation assays have been described, those may not be sensitive and specific enough. Our highly sensitive new method (Vallier, Thromb. Res. 2019) has been adapted into a kit, switching detection technology from fluorescence to absorbance for wider applicability.

Aims: The aim of this study was to compare chromogenic versus fluorogenic assay versions and illustrate performance of this new MV-TF Activity kit.

Methods: MVs were purified from platelet-free plasma by high-speed centrifugation (HS-C - 24,000 g - 1h). Robustness was ensured by easy pipeting volumes, a lyophilized mix of FVII + FX and a wide recTF calibration range over 2 decades in log scales. The FXa substrate was switched from fluorogenic to chromogenic wo losing sensitivity. Precision measurements were realized using the kit controls and kit's performance tested using 1) TF liposomes, 2) MV-free plasma spiked with tumoral TF+ vesicles and 3) normal and pathological plasma samples.

Results: Noteworthy, for samples with detectable but low values (< 30 fM) in optimal pre-analytical conditions, increased TF activities were observed when one of paired blood tubes was purposely agitated during transportation. The FXa generation assay itself is robust with CV< 5% for repeatability and < 15% for inter-operator reproducibility. However, the pre-test HS-C step still induces significant inter-operator variability (CV ~25%). An alternative approach for MV extraction using magnetic beads is considered.

Conclusions: This new kit allowing robust and sensitive measurement on purified MVs may help clarifying the biomarker value of MV-TF activity in future studies. It advocates for the presence of active MV-TF in normal plasma and for taking care of transportation conditions.

PB2137 | Thromboprophylaxis with Rivaroxaban in Patients with Malignancy and Central Venous Lines (TRIM-line): a Multicentre Open-label Pilot Randomized Controlled Trial

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Background: Upper extremity deep vein thrombosis affects 2-13% of cancer patients with indwelling central venous catheters (CVC) which are commonly used to administer chemotherapy and supportive treatments. While the AVERT and CASSINI trials showed that low-dose direct oral anticoagulants reduce venous thromboembolism (VTE) in high-risk cancer patients, patients with CVCs were under-represented in these studies. The role of thromboprophylaxis following CVC insertion is uncertain.

TABLE 1 Baseline characteristics

	Rivaroxaban 10mg N=25	Standard of Care N=25
Age(mean), mean (SD)	59 (12)	61 (14)
Female, n (%)	17 (68)	15, (60)
Weight(kg), mean (SD)	86 (16)	81 (20)
BMI (kg/m²)	31 (6)	29 (8)
Concurrent ASA/NSAID, n (%)	5 (20)	4, (16)
ECOG Status, n (%)		
ECOG 0	19 (76)	16 (64)
ECOG 1	6 (24)	6 (24)
ECOG 2	0	1 (4)
Cancer Type, n (%)		
Abdominal NOS	0	1 (4)
Brain	1 (4)	0
Breast	9 (36)	5 (20)
Colorectal	7 (28)	8 (32)
Genitourinary	1 (4)	0
Gynecological	2 (8)	4 (16)
Lung	0	2 (8)
Lymphoma	1 (4)	1 (4)
Pancreas	1 (4)	3 (12)
Stomach	3 (12)	1 (4)
CVC Type, n (%)		
PICC	22 (88)	22 (88)
Port-a-cath	3 (12)	3 (12)

ASA – Acetylsalicylic Acid, NSAID- Nonsteroidal Anti-Inflammatory Drug, ECOG – Eastern Cooperative Oncology Group Performance Status, NOS – Not Otherwise Specified, PICC – Peripherally Inserted Central Catheter, CVC- Central Venous Catheter

TABLE 2 Clinical outcomes at 90 days

	Rivaroxaban 10mg N=25	Standard of Care N=25
Adherence to therapy (>80% indicated study drug taken), %	96	N/A
VTE at 90 days, n (%)		
Symptomatic PE	0	1 (4)
Upper extremity DVT	0	1 (4)
Other DVT*	1 (4)	0
Bleeding at 90 days, n (%)		
Major bleeding	0	0
CRNMB	4 (16)	0
Minor bleeding	1 (4)	0
Catheter Complication, n (%)		
Line migration	2 (8)	2 (8)
Infection	1 (4)	1 (4)
Pain	1 (4)	0
Rash	1 (4)	1 (4)
Line blockage	0	1 (4)

ASA – Acetylsalicylic Acid, NSAID- Nonsteroidal Anti-Inflammatory Drug
ECOG – Eastern Cooperative Oncology Group Performance Status, NOS – Not Otherwise Specified
PICC – Peripherally Inserted Central Catheter

Aims: To assess the feasibility of conducting a multicentre randomized controlled trial (RCT) to assess the safety and efficacy of low-dose rivaroxaban to prevent VTE in patients with active cancer and indwelling CVC.

Methods: Multicentre open-label, pilot RCT conducted in 2 Canadian centres in patients with active cancer and insertion of a CVC within 72 hours. Patients were randomized 1:1 to rivaroxaban 10mg by mouth daily or standard of care (observation) for 90 days. The primary feasibility outcome was the number of participants recruited per centre per month. A sample size of 50 patients over approximately 6 months was estimated with an enrollment target of 4-6/month at the coordinating centre and 2/month at additional

sites. Secondary outcomes included consent rates, loss to follow up and adherence to therapy defined as over 80% of study drug taken (NCT03506815).

Results: Target enrolment of 50 patients was achieved in 6 months and 12 days. The enrolment rate was 7.5/month at the coordinating Ottawa Hospital site and 2.0/month for Juravinski Hospital site. Baseline characteristics are shown in Table 1. Clinical outcomes at 90 days are shown in Table 2. No patients were lost to follow up and the overall consent rate after screening was 33%.

Conclusions: The pilot trial achieved its enrollment targets and supports that a large multicentre RCT is feasible in this area.

PB2138 | Elevated Plasma Factor VIII Levels Are Associated with Hypercoagulability and Shorter Survival in Patients with Non-Hodgkin Lymphoma

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Background: Mechanisms underlying venous thromboembolism (VTE) vary among cancer types. Pathogenesis of VTE in non-Hodgkin lymphoma (NHL) remains undefined.

Aims: To investigate the hypercoagulability and its clinical impact on NHL patients.

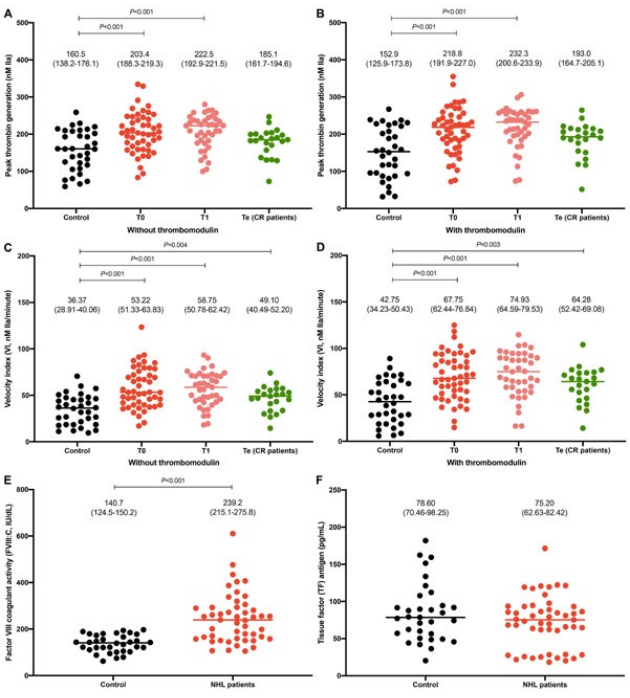

FIGURE 1 Peak thrombin (A-B), velocity index (C-D), FVIII:C (E) and TF antigen (F) of NHL patients compared with controls

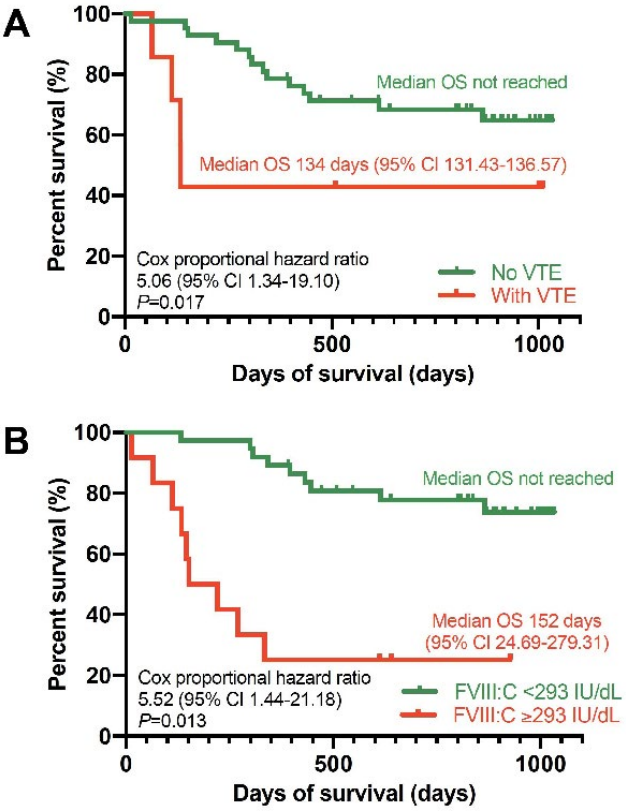

FIGURE 2 Kaplan-Meier estimations of overall survival considering VTE (A) and elevated FVIII:C (B)

Methods: During 2017-2019, platelet-poor plasma from NHL patients was collected at the diagnosis (T0), after 1-2 courses of chemotherapy (T1), and 4-8 weeks following the last treatment (Te). Normal controls were matched healthy volunteers (N=34). Global hemostasis was evaluated by thrombin generation assays (TGA) using 1.25-pM tissue factor (TF) and 1-μM phospholipids with/without 1-nM thrombomodulin (TM). D-dimer, fibrinogen, factor VIII coagulant activity (FVIII:C), von Willebrand factor antigen (VWF:Ag), C-reactive protein (CRP) and TF antigen were measured as the secondary outcomes.

Results: Of 49 NHL patients, diffuse large B-cell lymphoma was the most common diagnosis (51%). The median age was 59.2 yr (range 19.3-80.3) and 53.1% were male. TGA of NHL group at T0-T1 revealed significantly elevated peak thrombin, velocity index (VI, Figure 1, A-D) and endogenous thrombin potential (ETP) with TM. While ETP without TM, lag time and time to peak were not different between groups. Unlike peak thrombin, VI at disease remission (Te) remained high. D-dimer, fibrinogen, FVIII:C, VWF:Ag, CRP, but not TF antigen, levels (Figure 1, F), were elevated in NHL group (P< 0.001). However, only fibrinogen (Spearman r=0.274, P=0.001) and FVIII:C (r=0.302, P< 0.001) exhibited significant correlations with peak thrombin. Bulky disease (odds ratio [OR] 57.3, P=0.03) and poor performance status (OR 20.8, P=0.037), but not Khorana and ThroLy scores or hemostatic parameters, were associated with symptomatic VTE occurring in 14.3%. Notably, NHL patients with

symptomatic VTE or FVIII:C ≥293 IU/dL had significantly diminished overall survival (Figure 2).

Conclusions: Without increase in circulating TF, NHL patients showed hypercoagulability by enhanced thrombin generation which was correlated with elevated fibrinogen and FVIII:C levels. High FVIII:C and VTE were associated with poor survival outcomes.

PB2139 | Plasma Levels of Procoagulant and Anticoagulant Factors in Patients with Acute Cancer-associated Thrombosis

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Background: The role of coagulation factors to the development of cancer-associated thrombosis (CAT) has not been completely clarified.

Aims: To evaluate levels of coagulation factors in CAT compared to VTE not cancer-related.

Methods: Consecutive patients with a first VTE between 2010-2019 were enrolled. Procoagulant (II, VIII, IX, X, XI and fibrinogen), anticoagulant (antithrombin, protein C and protein S), and fibrinolysis factors (plasminogen and plasminogen activator inhibitor-1) were analysed at admission. Outcomes were the difference in coagulation profile between CAT and VTE patients, and the association between coagulation profile at admission and VTE recurrence or bleeding complications at follow-up.

Results: 659 patients with a first VTE were enrolled, 104 (16%) CAT and 555 (84%) VTE without cancer. We showed a significant increase in the mean levels of procoagulant factors in patients with CAT. Factor VIII was increased in CAT (200 ± 65%) compared to VTE (180 ± 60%, p = 0.002), as well as factor IX (142 ± 37% vs. 130 ± 43%, p = 0.009) and factor XI (127 ± 33% vs. 119 ± 32%, p = 0.042). No difference was found for anticoagulant and fibrinolysis factors. During follow-up, 38 (48.1%) CAT underwent recurrence vs. 137 (30.9%) non cancer-VTE (p = 0.003). Twelve (19.4%) CAT patients experienced bleeding complications vs. 36 (9.8%) VTE, p = 0.028. Cancer patients with factor VIII >192% at admission showed a significantly increased risk of VTE recurrence (OR 8.3 [95% CI 1.01-68.8] while FIX levels < 150% was protective (OR 0.62 [95% CI 0.4-0.92]). Factor levels were not associated with the occurrence of bleeding complications

Conclusions: Patients with CAT showed a hypercoagulable state mainly due to higher levels of procoagulant factors. Factors VIII and IX measured at CAT diagnosis may be an easy potential tool to early reveal patients at increased risk of VTE recurrence.

PB2140 | Apixaban Levels In Cancer-Associated Thrombosis In Low-Weight Individuals

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Background: Apixaban, a direct factor Xa inhibitor, has been shown to be at least as safe and probably more effective than dalteparin for the treatment of Cancer Associated venous Thrombosis (CAT), as reported in the ADAM-VTE trial. In the latter study however, only 10.7% of patients weighed less than 60Kg while hospitals in other countries may treat a significantly higher percentage of low-weight individuals. At our CAT Clinic 40% of individuals weigh under 60Kg and 25.4% under 55Kg. Lower weight-based doses are supported by cancer-specific studies such as for edoxaban (Hokusai-VTE Cancer) as well as rivaroxaban in childhood VTE (EINSTEIN Jr).

Aims: We routinely treat patients with CAT weighing between 60-120Kg with full-dose apixaban and those bellow 60Kg receive full-dose anticoagulation for the first month and decrease to 2.5 mg BID thereafter. We sought to determine whether low-weight individuals are being similarly anticoagulated as determined by apixaban plasma levels.

Methods: Apixaban plasma through levels were determined after a minimum of 7 days on steady dose treatment.

Results: Full-dose anticoagulation in low weight patients (40-60Kg) doubled the median through apixaban plasma levels (median: 229, min/max: 70-578 p:0.67) while those receiving 2.5 mg BID attained similar plasma levels as compared to those patients over 60Kg given full dose anticoagulation (median: 82 min/max:12-359 versus median:106 min/max:13-381, respectively, p:0.723). We found an inverse relation between apixaban levels and albumin (p:0.001).

Conclusions: Patients with CAT and low-weight may be underrepresented in published studies and may be over-anticoagulated with regular therapeutic-dose apixaban. Prospective studies assessing thrombotic and bleeding risk in these individuals are warranted.

PB2141 | Venous Thromboembolism in Patients with Cancer in Palliative Care

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Background: The prevalence of venous thromboembolism (VTE) in cancer patients is particularly high at disease progression or relapse, causing additional morbidity. Patients cared for in specialized palliative care units (SPCU) are rarely included in VTE studies, thus the actual impact of VTE and need for prophylaxis in this setting are not well known.

Aims: We studied the prevalence, clinical characteristics, and survival of individuals with VTE.

Methods: We retrospectively included 2301 consecutive individuals with active cancer managed at our SPCU. Data were summarized using descriptive statistics. Overall survival was estimated by Kaplan-Meier and comparisons by log-rank test. Thrombotic events were confirmed by imaging.

Results: We studied 1646 (71.5%) women and 655(28.5%) men. The prevalence of VTE was higher in women (9.7% vs 2.5%), particularly women with gynecological tumors (cervical: 19.2%, breast: 14.7%, ovarian: 13.5%). The overall prevalence of VTE was 12.1%, with only 1.7% occurring after the first consultation in the SPCU. Median overall survival was longer for patients without VTE at 3, 6, and 12 months (48.2%, 34.2%, 23.4%) compared to patients with thrombosis (42.6, 25.1%, 15%).

Conclusions: The prevalence of VTE varied by tumor origin and impacted overall survival. Though median survival was short, some patients were followed over months suggesting that therapeutic anticoagulation to decrease the risk and morbidity of rethrombosis should be considered. On the other hand, few patients developed symptomatic VTE during SPCU care, making generalized primary prophylaxis probably unwarranted. Customizing treatment to the risk of hemorrhage, physical performance, and prognosis is important.

PB2143 | Catheter-Associated Upper Extremities Deep Vein Thrombosis in Cancer Patients: Does Anticoagulation Time Matter?

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Background: Catheter-related upper extremities deep vein thrombosis (UEDVT) represents a major issue for cancer patients. Consensus opinion for the treatment of catheter-related UEDVT in cancer patients is still not completely defined.

Aims: To describe a single-center clinical casistic of cancer patients with catheter-related UEDVT and to determine rate of VTE recurrences in patients undergoing a short-course (3 months) versus a longer course of anticoagulant therapy.

Methods: Retrospective study. Enrollment of consecutive patients with active cancer and catheter-related UEDVT from Cardiovascular Medicine database from August 2016 to August 2018. Diagnosis of catheter-related UEDVT obtained by compression ultrasonography. Minimum follow up of 6 months up, maximum of 24 months. Individualized duration of therapy was established by the vascular medicine specialist.

Results: A total of 84 patients with cancer and catheter-related UEDVT were enrolled. The most common device were PICC (75%) and Port-a-cath (16.3%). Types of cancer varied from solid tumors (breast 19%, colorectal 10.7%, lung 7.1%) to hematologic malignancies (lymphomas 23.8%). 29 patients (34.5%) died within 2 years of follow-up. Cancer represented the first cause of death (72.4%) followed by sepsis (11%), hemorrhagic events (7.4%) and fatal pulmonary embolism (3.4%). Total rate of VTE recurrences was 22.2% in patients undergoing ≤ 3 months anticoagulation and 20.8% in longer therapies; deaths were 36% versus 33% ($p>0.05$). On the opposite, total hemorrhagic events were more frequent in the first group (16.7% versus 6.4%), despite the short term of therapy, in relation to the more frequent critical thrombocytopenia

Conclusions: Present data are in line with previous studies. Recurrent thrombotic risk wasn't negligible independently from anticoagulant duration. Hemorrhagic rates in the second group were related to high individual hemorrhagic risk rather than duration of anticoagulation. Our results suggest that a defined duration of anticoagulant therapy can't be generally established, therefore personalized approaches must be considered.

PB2144 | Deep Venous Thrombosis and Intraductal Papillary and Mucinous Tumor of the Pancreas: about 3 Cases

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Background: Venous thromboembolism (VTE) is a common problem in cancer patients and the incidence is increasing. Common features of these patients, like advanced stage, male gender and old age are risk factors of VTE. Intra-papillary and mucinous nonneoplastic cyst of the pancreas (IPMNP) are benign neoplasm due to the proliferation of the ductal epithelium. It is usualy diagnosed fortuitously.

Aims: To determine the link between IPMNP, a precancerous condition and DVT.

Methods: We report 3 cases of deep venous thrombosis (DVT) associated with IPMNP.

Results: Tow men and 1 woman with a mean age of 78 years (70, 83 and 81 years) were admitted for management of DVT of the lower limb treated with heparin then oral anti-coagulant drug during 12 months. The biological analysis were within the normal range. An abdominal CT scan revealed a pancreatic mass in all patients. A pancreatic magnetic resonance imaging showed a cystic lesions in cephalic body of the pancreas in 2 cases with other cystic lesions of caudal pancreas in the other case. Then, our patients were diagnosed with DVP associated with IPMNP. Only 1 patient developed pancreatic insufficiency. As the concern for malignant transformation to pancreatic cancer is great, a magnetic resonance imaging was underwent every 1 year in the 3 cases with no malignant transformation.

Conclusions: DVT can reveal a malignant and asymptomatic neoplasm. We describe 3 cases of thromboembolic event associated with a benign pancreatic tumor. Because the potential of IPMNPs for malignancy is high, which suggest a magnetic resonance imaging control every 12 months espacially when associated with DVT.

PB2145 | Aspects of Cancer-Related Venous Thrombosis in a cohort of 207 Tunisian Patients

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Background: Venous Thromboembolism (VTE) and neoplasms are closely related. Indeed, VTE can precede their discoveries as it can occur during their care.
Aims: The aim of our work was to study the relationship between these two diseases in a cohort of Tunisian patients.
Methods: We conducted a descriptive retrospective study of 19 years (from 2000 to 2018) in the internal medicine department of La Rabta Hospital. All patients who were hospitalized for VTE and whose etiological research revealed a neoplasm were included.
Results: Our study collected 207 patients with a sex ratio of 121/86. The average age was 63.2 years [19-90 years]. An age under 40 years was found on 6.8% of patients, while 50.7% of patients were over the age of 65 years. VTE was unusual in 86.5% of patients. Solid neoplasm was found in 78.3% of cases. VTE was indicative of neoplasm in 51.7% of cases. On these patients, the average diagnostic time for cancer was 1.45 months [0-15]. Among the patients already being followed for neoplasm, 19.2% were undergoing chemotherapy.
A recurrence of MVTE on antivitamins K was observed on 11 patients.
Conclusions: The occurrence of VTE in an elderly subject requires the search for a neoplasm especially when it is of unusual siege. This investigation must be redone at the slightest sign since the VTE may precede the neoplasm of a certain period of time. Cancer chemotherapy is an added risk factor.

PB2146 | Clinical Outcome of Splanchnic and Usual Site Venous Thrombosis in Patients with Solid Cancer

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Background: Solid cancer represents a leading risk factor for splanchnic vein thrombosis (SVT). Limited information is available on the outcomes of patients with cancer-associated-SVT.
Aims: To evaluate differences in clinical characteristics, treatment, and outcomes between two cohorts of cancer patients with SVT and usual site venous thromboembolism (VTE).
Methods: SVT patients enrolled in a multicenter, international, prospective cohort study from May 2008 to January 2012 were eligible if they had known solid cancer. The comparison cohort included patients with usual site VTE (deep vein thrombosis and/or pulmonary embolism) and solid cancer from a multicenter cohort of patients enrolled between 2012 and 2018. All patients had at least one-year follow-up. Recurrent VTE, major bleeding, and mortality were evaluated.
Results: A total of 264 patients (132 patients for each cohort) were enrolled. Patients with SVT were younger than patients with usual site VTE (median age 61 vs 70 years; $p< 0.001$). SVT patients were less likely to receive chemotherapy at the time of diagnosis than patients with usual site VTE (17.4% vs 59.2%; $p< 0.001$). Liver and lung cancer were the most frequent cancer types in SVT and usual site VTE patients, respectively. Fewer patients in SVT than in the comparison cohort received anticoagulation (68.9% vs 99.2%; p<

0.001), mainly parenteral and the median duration of anticoagulation was shorter (6.0 vs 11.0 months; p=0.001). The rates of major bleeding (3.2 vs 6.3 per 100 patient-year; p=0.33) events were non-significantly lower in patients with SVT than usual site VTE, whereas those of recurrent VTE (6.4 vs 7.3 per 100 patient-year; p=0.81) and mortality were comparable (56.5 vs 51.4 per 100 patient-year; p=0.62).

Conclusions: Despite less intensive anticoagulant treatment, incidence of recurrent VTE tends to be similar between patients with cancer-associated SVT and with cancer-associated usual site VTE. Mortality rates are also similar and are high in both groups.

PB2147 | Decreased Thrombin Generation Potential in Lymphoma Patients Is Associated with Increased D-dimer, CRP, vWF and TNF-α. Interrelationship between Thrombogenesis and Inflammation

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Background: Inflammation and other vascular factors contribute to the pathogenesis of thrombotic complications in lymphoma patients. Biomarkers of hemostatic activation, vascular dysfunction and inflammation are upregulated in lymphoma.

Aims: This study was designed to compare the thrombin generation potential and its relevance to the generation of various biomarkers of hemostatic activation process and inflammatory responses.

Methods: Citrated blood samples from 90 patients with confirmed diagnosis of lymphoma were collected from University of Belgrade, Serbia. 50 samples of normal human plasma (NHP) were included as control. Thrombin generation studies were carried out using a commercially available kinetic system. Thrombin generation parameters such as peak thrombin (PT), lag time (LT) and area under the curve (AUC) were compiled. Plasma levels of D-Dimer, CRP, vWF and TNF-α were measured by using commercially available Sandwich ELISA assays.

Results: On a cumulative basis, lymphoma patients showed increase in LT (2.91.17) compared to NHP (2.7). The PT levels (119.046.2) and AUC was decreased (670.2290.0) in the lymphoma patients compared to NHP (PT: 159.731.1 and AUC: 700.9). When the lymphoma patients were sub-grouped, PT levels were similar in HL, NHL and CLL and were decreased compared to NHP. However, the AUC was increased in HL, decreased in NHL and was comparable for CLL compared to NHP. The lymphoma patients also exhibited elevated levels of D-Dimer CRP, vWF and TNF-α compared to NHP. In HL group D-Dimer and vWF correlated well with PT, in this group CRP, vWF and TNF-α also showed a good correlation with AUC. In the NHL group, CRP correlated well with LT.

Conclusions: The observed decrease in thrombin generation potential in these patients may be due to the consumption of prothrombin and other coagulation factors. Wide variations in circulating levels of various biomarkers and thrombin generation parameters between various lymphoma groups are noted.

PB2148 | Differential Augmentation of Thrombin Generation by Andexanet Alfa in Lymphoma Patients

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Background: The prevalence of thrombosis in lymphoma patients is reportedly high and ranges from 3 - 10%. Vascular malfunction and inflammatory processes further contribute to the thrombotic activation processes in these patients. Andexanet alfa (AA) is an antidote for factor Xa inhibitors and its usage has been reported with thrombotic complications.

Aims: This study was designed to compare effect of AA on the thrombin generation potential.

Methods: Citrated blood samples from 78 patients with confirmed diagnosis of non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL) and Chronic lymphocytic leukemia (CLL) were collected from the University of Belgrade, Serbia. 50 samples of normal human plasma (NHP) was used for referencing purposes. Individual samples were supplemented with AA at 100 ug/ml. Thrombin generation studies were carried out using a commercially available a fluorogenic substrate method. Thrombin generation parameters such as peak

TABLE 1 Correlation coefficient demonstrating the relevance of various biomarkers to thrombin generation parameters

Samples	D-Dimer (ng/ml) AUC	D-Dimer (ng/ml) PT	CRP (ug/ml) AUC	CRP (ug/ml) PT	vWF (%) AUC	vWF (%) PT	TNF-α (pg/ml) AUC	TNF-α (pg/ml) PT
HL (n=7)	0.7275	0.4664	0.5266	0.4988	0.3290	0.4813	0.2794	0.1825
NHL (n=61)	0.0149	0.0137	0.0104	0.0004	0.0083	0.0004	0.0509	0.0294
CLL (n=22)	0.0228	0.0027	0.0792	0.0064	0.0068	0.0267	0.1373	0.0889
Lymphomas	0.0362	0.0311	0.0401	0.0070	0.0123	0.0125	0.0440	0.0220

TABLE 1 Inhibition of thrombin generation potential in lymphoma cohort by andexanet alfa

Groups	Peak Thrombin Pre Andexanet	Peak Thrombin Post Andexanet	AUC Pre Andexanet	AUC Post Andexanet	Lag Time Pre Andexanet	Lag Time Post Andexanet
Control	159.20 ± 4.8	188.15 ± 7.4	756.46 ± 34.5	829.14 ± 50.2	2.06 ± 0.2	1.94 ± 0.1
HL	128.16 ± 68.2	180.75 ± 35.4	781.91 ± 343.8	938.31 ± 220.2	3.67 ± 1.3	3.38 ± 1.0
NHL	113.99 ± 45.4	154.16 ± 36.8	627.81 ± 221.0	744.52 ± 222.6	3.07 ± 1.2	2.50 ± 0.6
CLL	129.77 ± 31.9	142.46 ± 33.8	738.18 ± 402.5	725.26 ± 224.2	2.23 ± 0.6	2.32 ± 0.5
Lymphoma	120.34 ± 44.3	153.70 ± 37.2	668.85 ± 284.3	758.32 ± 226.4	2.87 ± 1.2	2.52 ± 0.7

thrombin (PT), lag time (LT) and area under the curve (AUC) were compiled.

Results: On a cumulative basis, lymphoma patients showed an increase in LT in comparison to NHP which decreases with AA. The PT and AUC levels were decreased in comparison to NHP and increases with AA. When the lymphoma patients were sub-grouped, PT levels were similar in all groups and decreased compared to NHP and increases with AA. The AUC was increased in the HL and decreased in NHL and CLL compared to NHP. However, with AA the AUC values increases for HL and NHL and decrease for CLL in comparison to control. Variations in lag time were noted in the three groups.

Conclusions: Lymphoma patients represent a heterogenous group of patients in which both the hypercoagulable state and inflammatory responses simultaneously occur. Differential increase in thrombin generation in post andexxa supplemented samples suggest that the use of this agent may potentially be associated with thrombotic complications.

PB2149 | Pulmonary Embolism as a Presenting Manifestation of Cerebral Glial Tumor

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Background: Cerebral gliomatosis (CG) are a group of rare infiltrative tumor entity of the brain whose prognosis is reserved.

Aims: Thromboembolic event (TE) as a presenting manifestation of this entity is exceptional excluding the post-operative context.

Methods: We present a new case of cerebral glial tumor revealed by pulmonary embolism.

Results: A 53-year-old man presented with respiratory distress secondary to a pulmonary embolism (PE). Chest CT scan showed several micronodules without any malignant feature, Abdominopelvic ultrasound showed hypertrophy of the prostate and cervical ultrasound was normal. Tumor markers (AFP, ACE,CA 19-9 antigen, PSA) were normal. The patient was treated with heparin and anti-vitamin K drugs. Three weeks after the diagnosis of PE, he was admitted to the emergency room for generalized convulsions without trauma or fever. Laboratory tests were normal and brain imaging showed cerebral gliomatosis. He was treated with corticosteroids 20 mg/day and valproic acid. A stereotaxic biopsy to determine the phenotype of the CG could not be achieved. The patient died 18 months after the onset of the diagnosis in a context of intracranial hypertension.

Conclusions: The association between venous thromboembolic diseases and neoplasia is widely established however the discovery of a cerebral gliomatosis as neoplastic etiology of the VTE is rare. Indeed, the thromboembolism events are common in the postoperative period in cerebral neoplasia and specifically in glial tumors. TE accidents associated with these pathologies are generally attributed to the postoperative context, corticosteroid treatment or the chemotherapy. This observation is particular by the occurrence of the VTE as a first manifestation of the disease.

PB2150 | Uncovering Clinical Gaps in Treatment of Cancer-Associated Thrombosis: A Clinical Practice Assessment

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Background: Guideline-recommended anticoagulant therapy is widely underused in the setting of cancer-associated thrombosis (CAT), resulting in poor patient outcomes.

Aims: This study's objective was to assess clinical gaps, attitudes and barriers related to current treatment practices in the setting of CAT.

Methods: A 24-question, online, continuing medical education (CME) survey was developed, including knowledge, case-based (treatment), confidence and attitude questions. Questions were mapped to clinical themes and analyzed to uncover physician gaps by specialty.

Results: A total of 512 physicians, 46% of whom were cardiologists and 20% of whom were hematologists/oncologists completed the survey. 65% of participants were hospital-based. On average 79% of participants manage 1-5 patients with CAT per week, 14% managed 6-10 patients/week and 8% see more than 10 patients/week. Analysis showed that physicians had poor knowledge of LWMH and NOAC clinical trial data in CAT, the risk of bleeding and the current guideline recommendations for managing CAT. The majority also had difficulty selecting appropriate anticoagulant treatment for patients with CAT (Figure). The most important barriers to implementing an effective anticoagulant therapy for patients with CAT were perceived risk of bleeding (56%), cost and poor medication coverage (25%), knowledge of guidelines (23%), and patient adherence (18%).

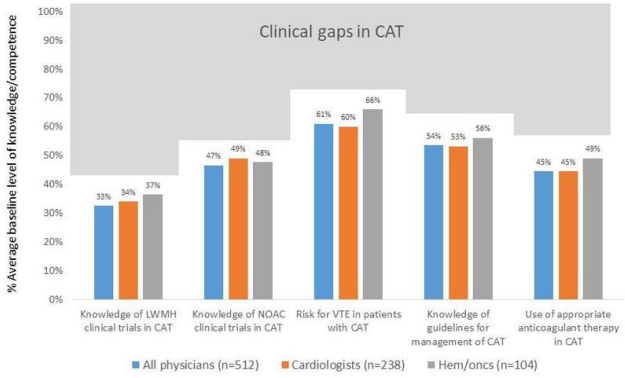

FIGURE 1 Clinical gaps in CAT

On average only 21% of participants felt confident in selecting the appropriate anticoagulant strategy for patients with CAT.

Conclusions: This educational research study uncovered significant knowledge and treatment gaps among physicians who treat patients with CAT. These findings support the need to continue to educate cardiologists, hem/oncs and other physicians in order to align physicians' knowledge and performance to improve outcomes and reduce the risk for thrombotic events in patients with CAT.

PB2151 | Influence of Tumor Site on Clinical Outcomes in Cancer Patients with Venous Thromboembolism. Insights from the Prospective PREDICARE Cohort Study

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Background: Predicting the risk of venous thromboembolism (VTE) recurrence in patients with cancer-associated thrombosis (CAT) could help choose the best treatment option in such patients. The PREDICARE study, a prospective cohort of CAT patients designed to validate the Ottawa VTE recurrence risk scoring system (NCT03099031), failed to confirm the predictive value of this score. There is also no established consensus on a predictive model for bleeding in this population.

Aims: To reconsider the influence of the primary site of cancer on VTE recurrence and bleeding rates during therapeutic anticoagulation in CAT patients.

Methods: PREDICARE is a prospective multicenter observational cohort of cancer patients with VTE treated with tinzaparin for an expected duration of 6 months (NCT03099031). The primary endpoint was the recurrence of symptomatic or asymptomatic VTE within 6 months of enrollment. Other endpoints included major bleeding, and all-cause mortality. All clinical events were adjudicated by an independent committee. Cumulative incidences of VTE recurrence and major bleeding according to the site of cancer were estimated taking into account the competitive risk of death (Kalbfleisch and Prentice method).

TABLE 1 Incidence of clinical outcomes according to the type of cancer during the 6-month treatment period

Primary site	Number of patients	VTE recurrence n % (95% CI)*	Major bleeding n % (95% CI)*	Death n % (95% CI)**
Lung	128	13 **10.2%** (6.5-15.9)	3 **2.4%** (0.8-7.2)	64 **52.9%** (43.0-62.7)
Colorectal	57	2 **6.6%** (1.5-29.8)	2 **3.5%** (1.0-11.7)	10 **17.8%** (7.8-27.8)
Breast	57	1 **1.7%** (0.2-12.5)	2 **3.5%** (0.9-13.2)	12 **23.2%** (11.3-35.1)
Gynaecologic	35	2 **5.6%** (1.6-20.5)	0 -	8 **23.5%** (9.2-37.8)
Urologic	26	3 **11.2%** (3.6-34.8)	3 **11.8%** (4.5-31.3)	8 **31.0%** (13.1-48.8)
Pancreas	24	1 **4.1%** (0.5-32.6)	2 **8.2%** (1.8-38.0)	11 **45.8%** (25.9-65.8)
Hematologic	23	2 **8.5%** (1.8-41.0)	1 **4.3%** (0.8-22.9)	5 **21.7%** (4.9-38.6)
Upper gastrointestinal	18	2 **10.8%** (3.0-38.8)	0 -	10 **55.6%** (32.6-78.5)
Other	51	3 **5.8%** (1.8-18.7)	2 **3.9%** (0.9-16.1)	19 **39.2%** (24.9-53.6)

Other: prostate (n=18); ear, throat, nose (n=10); liver (n=9); central nervous system (n=7); soft tissue sarcomas (n=2); peritoneum (n=1); skin (n=3); unknown (n=1)
n: number of patients with at least one event
* Cumulative incidence estimated by Kalbfleisch and Prentice method
** Cumulative incidence estimated by Kaplan-Meier method

Results: In the 409 patients of the cohort, mean age was 67.6 years and 67% of patients had metastatic disease. At 6 months, 144 patients had died (cumulative incidence 36.2%; 95%CI 31.3-41.0), mainly of cancer (133 patients).The overall 6-month cumulative incidence of recurrent VTE was 7.3% (95%CI 4.9-11.1). VTE recurrences were frequent mainly in urologic, upper gastrointestinal and lung cancers (Figure). The overall 6-month cumulative incidence of major bleeding was 3.7% (95%CI 2.3-6.0). Major bleeding rates were comparatively lower than VTE recurrence rates across most tumor sites.

Conclusions: This prospective study confirms that CAT patients with lung, upper gastrointestinal, and urologic cancers appear at high risk of VTE recurrence despite anticoagulation. Cancer-specific risk assessment research should be considered.

PB2154 | Anticoagulation Therapy as Prevention of Cancer Relapce

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Background: The bidirectional relationship of cancer and hemostasis are well-known: they interact in a "vicious cycle", where cancers overexpress thrombin, which in turn promote both prothrombotic potential and tumor growth, invasion, and spread. Indeed, venous thromboembolism occurs frequently as a paraneoplastic event. Thus, anticoagulants could be accepted not only as a classic treatment for

prevention thrombotic complications, but also as an anti-cancer therapy.

Aims: To estimate the rate of cancer recurrence in the group of patients receiving anticoagulant therapy and among patients not receiving anticoagulants.

Methods: In our case-control study 168 patients with advanced ovarian cancer were examined. In concordance with the identified risk of thrombosis (Khorana and Vienna CATS scale), as well as the presence of genetic thrombophilia (FV Leiden, Prothrombin mutation, MTHFRG mutation), the first group of patients (n=69) with a high risk of thrombosis received anticoagulant therapy (LMWH and NOAK), the second group (n=88) did not. Patients were under our supervision for 60-72 months.

Results: Our study showed that the group taking anticoagulants performed a significantly lower recurrence rate than the group of patients who did not receive regular anticoagulant therapy: 30.4% and 67% respectively. However, thrombotic complications, even with anticoagulant therapy, were more common in the first group - 15.9% versus 7.95%. in the second group. It is necessary to emphasize that in the first group were patients with a higher risk of thrombosis than in the second.

Conclusions: Available data confirm the role of the hemostatic system in the growth and metastasis of cancer, but we cannot confidently assert that anticoagulation therapy should be included in the secondary cancer prevention. The duration of study was sufficient, but the number of patients involved in the study needs to be expanded to draw fulfilled conclusions.

PB2155 | Dynamics of ADAMTS13 and vWF Activity Change in Ovarian Cancer Patients

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Background: The imbalance between vWF and ADAMTS13 activity are associated with thrombotic complications in cancer patients, while low ADAMTS13 activity demonstrates prognostic potential.

Aims: To determine the dynamics of vWF and ADAMTS13 activity changes depending on the stage of ovarian cancer spread.

Methods: 127 patients with ovarian cancer of various stages in the perioperative period were examined: 42 patients with stage I ovarian cancer, 41 patients - stage II ovarian cancer and 44 patients with advanced ovarian cancer.

Results: In patients with I stage ovarian cancer, the median activity of ADAMTS13 and vWF was within normal ranges, while the ADAMTS13/vWF ratio was within 75 percentile. Patients with II stage of ovarian cancer had ADAMTS13 and vWF activity also within normal ranges, but their ratio was within 45 percentile. In patients with advanced ovarian cancer, the median of vWF activity was close to the upper limit of normal and ADAMTS13 to the lower limit of normal, their ratio was within 30 percentile, but the level of ADMATS13 inhibitor was also within the upper limit of normal.

Conclusions: We have shown a clear trend towards increasing vWF activity and decreasing ADAMTS13 activity depending on the degree of ovarian cancer spread. In addition, the ADAMTS13/vWF ratio tended to decrease down to 30 percentiles. An increase in the level of ADAMTS13 inhibitor was also demonstrated. In malignant neoplasms, a change in the proportional ratio of ADAMTS13/vWF may be the result of excess metalloproteinase consumption due to excess ULvWF expression by activated endothelium cancer cells.

PB2156 | Impact of Obesity in Pulmonary Embolism among Patients with Solid Malignancies

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Background: Obesity has been associated with decreased mortality among the general population who develop pulmonary embolism (PE). Obesity effect it is not described in patients with both: PE and malignancies.

Aims: We sought to portray the effect of obesity on mortality among patients with solid malignancies and PE.

Methods: We queried the National Inpatient Sample (NIS) database from 2010 to 2014 for adults with active solid malignancies who had PE as the primary diagnosis of admission. We stratified patients by the top 4 more deadly cancer types (lung, prostate, breast and colon) and by the Khorana Risk Score for Venous Thromboembolism in Cancer Patients. Categorical variables were compared using the

TABLE 1 ADAMTS13 and vWF activity in patients with ovarian cancer

	Normal range	Ovarian cancer I stage	Ovarian cancer II stage	Advanced ovarian cancer
vWF activity	70-130%	96±14.8	112±19.7	128±22.1
ADAMTS13 activity	40-130%	72±9.2	51±13.7	39±17.8
ADAMTS13 inhibitor level	<12 IU/ml	2.1±0.4	6.5±1.2	11.5±3.7
ADAMTS13/vWF ratio		75%	45.5%	30.5%

chi-square test. Logistic regression evaluated the effect of obesity on patients with active solid cancer and PE.

Results: From 2010 to 2014, we found a weighted population of 192471 patients defined as in-hospital adults with active solid malignancies who were admitted due to a primary diagnosis of PE. Obesity was related to a decreased in-hospital mortality of patients with active solid malignancies in general aOR 0.70 (0.64 - 0.76). After stratification, obesity decreased in-hospital mortality in breast cancer patients aOR 0.54 (0.44 - 0.68). No significant effect was found on colon aOR 0.75 (0.55 - 1.03), lung aOR 0.87 (0.72 - 1.06) and prostate aOR 0.91 (0.38 - 2.17) cancers. Obesity decreased the risk of in-hospital mortality in patients having Khorana Risk Score: 1 while increased mortality in score: 2 aOR 0.74 (0.65 - 0.85) and aOR 1.48 (1.05 - 2.08), respectively.

Conclusions: Obesity has not a homogenous effect among patients with an active diagnosis of cancer. A beneficial effect was found only in one of the top 4 more deadly cancers in US (i.e. breast cancer). Obesity increased the risk of in-hospital mortality in patients with malignancies with the highest risk of developing PE.

PB2157 | Fondaparinux versus Enoxaparin in the Treatment of Venous Thromboembolism in Cancer Patients: Retrospective Cohort Study - "ONCOTEV"

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Background: Patients with cancer-associated venous thromboembolism (VTE) experience high rate of anticoagulation failure and bleeding events and VTE is the second leading cause of death.

Aims: To evaluate the net clinical benefit of fondaparinux (F) compared with enoxaparin (E) through the composite outcome of: recurrent VTE, major and clinically relevant non-major bleeding, all-cause mortality.

Methods: Retrospective, monocentric cohort study. Consecutive patients with VTE and active cancer (i.e. metastatic cancer or on treatment or last treatment within the previous 6 months at the time of VTE diagnosis) treated with F or E. The data were collected by "ONCOTEV" Register of the Cardiovascular Medicine Unit of Reggio Emilia Hospital. This study was approved by the local Ethic Committee. Written informed consent was collected according to local practice.

Results: A total of 368 patients were enrolled, 81 treated with F (group A) and 287 treated with E (group B). Stage III-IV cancer was 73% in group A and 70% in group B. The most frequent index events are upper extremities deep vein thrombosis, lower extremities deep vein thrombosis and pulmonary embolism (PE): 27.2%, 21.0%, 18.5% respectively in group A and 32.4%, 18.8%, 15.6% in group B. The first cause of death was cancer progression (69.2% in A and 75% in B). The survival curve free from primary composite outcome shows a positive trend in E group, however it isn't statistically significant. VTE recurrences and their timing are shown respectively in Table 1 and Table 2.1

Conclusions: Although these data refer to a retrospective analysis, they show comparable efficacy, safety and compliance of F versus E. Further randomized clinical trials are advisable to explore the difference between F and E in avoiding PE and fatal PE recurrences. It's essential to carefully personalize anticoagulant therapy in term of doses and optimal duration.

TABLE 2 Timing of VTE recurrences

	Fondaparinux (F) n (%)	Enoxaparin (E) n (%)	p
Full anticoagulant therapy	3 (10.3)	4 (6.45)	ns
Personalized anticoagulant therapy (50-75% of the anticoagulant dose, 2nd-6th month)	5 (17.2)	20 (35.3)	< 0.05
Prophylactic dose	5 (17.2)	10 (16.1)	ns
No therapy	16 (55.2)	28 (45.2)	ns

TABLE 1 VTE recurrences

	Fondaparinux (F) n (%)	Enoxaparin (E) n (%)	p
Total	29 (35.8)	62 (22.0)	ns
Lower extremities deep vein thrombosis (LEDVT)	7 (24.1)	15 (24.5)	ns
Upper extremities deep vein thrombosis (UEDVT)	6 (20.7)	13 (21.0)	ns
Isolated distal deep vein thrombosis (IDDVT)	4 (13.8)	11 (17.7)	ns
Lower extremities superficial vein thrombosis (LESVT)	8 (27.6)	7 (11.0)	< 0.05
Upper extremities superficial vein thrombosis (UESVT)	1 (3.4)	3 (4.8)	ns
Pulmonary embolism (with or without DVT) - Non Fatal - Fatal	2 (6.9) - 2 - 0	11 (17.7) - 8 - 3	< 0.05
Splanchnic vein thrombosis (SVT)	1 (3.4)	2 (3.2)	ns

PB2158 | Histopathological Features of Cancer-Associated Venous Thromboembolism in Human

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Background: Cancer-associated thrombosis (CAT) is a major cause of mortality in cancer patients. It is presumed that primary organs and/or histological types of cancer affect the underling mechanisms of CAT.
Aims: We investigate histopathological features of cancer-associated venous thromboembolism (VTE) in human autopsy cases.
Methods: We examined 119 cases of cancer-associated VTE, and investigated cancer primary organs and histopathological types. We also histologically examined presence or absence of cancer cells in venous thrombi and/or pulmonary emboli, and expression of prothrombotic factors in cancer cells using antibodies for cytokeratins (epithelial marker), tissue factor, and podoplanin.
Results: The primary organs of the cancers were lung (n=29, 24%), stomach (n=16, 13%), hematopoietic and lymphoid system (n=12, 10%), pancreas (n=11, 9%), biliary system (n=7, 6%), esophagus (n=6, 5%), colorectal system (n=5, 4%), and the others (n=26, 22%). Seven cases (6%) had multiple primary cancers. The histological types of cancers were adenocarcinoma (n=51, 43%), lymphoma (n=11, 9%), neuroendocrine carcinoma (n=9, 8%), squamous cell carcinoma (n=8, 7%), and the others (n=40, 34%). Histologically, venous thrombi occasionally developed at the site of direct intravenous invasion of cancer cells. Immuhistochemically, cancer cells as small clusters in thrombi were found in about one-half cases. Cancer cells of adenocarcinoma and neuroendocrine carcinoma expressed tissue factor, and those of peritoneal cancer expressed podoplanin.
Conclusions: Direct cancer invasion or circulating cancer cells can contribute to thrombogenesis in cancer-associated VTE. Tissue factor and podoplanin expressed by cancer cells may induce coagulation reaction and platelet aggregation.

PB2159 | Anticoagulation Therapy Management in Oncological Patients: Experience in a Center

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Background: Anticoagulation therapy in oncological patients is relatively frequent, being low molecular weight heparin (LMWH) the most commonly anticoagulant prescribed. In this context, the anticoagulation is associated to a higher rate of mayor and minor hemorrhages clinically relevant. Direct oral anticoagulants (DOACs) could be an excellent therapeutic option in such a complex group of patients.
Aims: To evaluate the efficacy and safety of DOACs in oncological patients.
Methods: Descriptive and retrospective observational study, accomplished in Clinical Hospital "Lozano Blesa", in Zaragoza, from January 2018 to February, 2020. There's been an analysis on medical records of anticoagulated patients by atrial fibrillation (AF) and/or venous thromboembolism (VTE), all of them diagnosed with an active oncological disease.
Results: 43 patients, 67'4% (29/43) male and 32'6% (14/43) female, average age of 72'9 years.
Solid tumors 81'4% (35/43), lung and gastrointestinal tract tumors are the most prevalent. Metastasic disease 51'1% (18/35). The most frequent reason of anticoagulation has been FA, 79% (34/43), previous AF 58'1% (25/43) and 72% (18/25) of them were previously anticoagulated with acenocumarol. Regarding VTE, every case was diagnosed during the context of the oncological disease. LMWH has been, for these patients, the therapy of choice during the acute phase and subsequent change to DOACs by indication of long term anticoagulation.
Edoxaban has been the most used anticoagulant with 65'1% (28/43) along with Apixaban 18'6% (8/43). From these, 32'6% (14/43) needed dossage reduction. Complications rate related to the anticoagulant treatment has been 11'6% (5/43). The most common complication was hemorrhage, being severe in 2 patients and leading to the suspension of anticoagulation on those. Both patients were treated with Edoxaban. There were no tromboembolic complications reported.
Conclusions: DOACs are a very interesting terapeutical option in patients with AF and/or VTE and an active neoplasia.

PB2160 | The Activated Coagulation Factor X (FXa) Directly Stimulates Colorectal Cancer Cell Migration and Proliferation - Characterizing the Underlying Molecular Mechanisms

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Background: Colorectal cancer (CRC) is associated with a high risk of thrombosis. Besides its key function in hemostasis, the activated coagulation factor-X (FXa) directly stimulates cellular effects via activation of the G-protein-coupled protease-activated receptors PAR1 and PAR2.
Aims: The aim of the current study was to characterize direct effects and underlying signaling mechanisms of FXa in CRC cell migration and proliferation.
Methods: Human (HCT116, CaCo2) and murine (MC38) CRC cell lines were stimulated with a range of physiological FXa concentrations. Selective agonist peptides for PAR1 and PAR2 were used to characterize their functions. Cell migration was determined in wound scratch and Boyden chamber migration assays; proliferation by BrdU

(bromdesoxyuridin) incorporation and cell counting. Signaling pathways were analyzed by Western blotting.

Results: Stimulation of murine MC38 cells with FXa (0.1-30 nM) significantly increased directed and undirected cell migration within 24 hours up to 2-fold. In comparison, proliferation of MC38 cells was at a high basal level already and was not further stimulated by FXa. Human CaCo2 cells exhibited an attenuated migration response, while proliferation was significantly increased after stimulation with FXa. In HCT116 cells, FXa significantly enhanced both cell migration and proliferation. These effects were mimicked by peptide-stimulated activation of PAR2 but not PAR1. In addition, incubation with FXa significantly increased phosphorylation of p44/42, p38 and AKT in MC38 and HCT116 cells in a time-dependent manner. The EGF (epidermal growth factor) receptor inhibitor Erlotinib markedly attenuated FX-induced cell migration in CRC cells, indicating transactivation of the EGF receptors as an underlying mechanism.

Conclusions: FXa directly stimulates proliferation and migration of various CRC cell lines *in vitro*. This involves p44/42, p38 and AKT signaling pathways and may involve transactivation of the EGF receptor. Use of suitably FXa inhibitors to reduce the thromboembolic risk in CRC patients may simultaneously attenuate tumor progression.

PB2161 | Direct Oral Anticoagulants in Patients with Hematological Malignancies

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Background: The recommended anticoagulant treatment for patients with hematological malignancies is low molecular weight heparin (LMWH), considered the safest in this particular patients setting. Although direct oral anticoagulants (DOACs) have proven their efficacy and safety in patients with cancer, their use can be challenging in patients with hematological malignancies due to the peculiarity of these neoplasms: possible rapid onset of thrombocytopenia, coagulopathy and drug-drug interactions with concomitant anti-cancer therapies.

Aims: Purpose of our study was to evaluate the efficacy and safety of DOACs for venous thromboembolism (VTE) or atrial fibrillation (AF) in patients with hematological malignancies and any pharmacokinetic interferences with the concomitant anti-cancer therapy.

Methods: We retrospectively evaluated patients with hematological malignancies who received DOACs for VTE or AF until a platelet count ≥50x10^9/l. In case of bleeding or thrombotic complications and during concomitant anti-cancer treatment, we performed DOACs plasma levels (trough and peak).

TABLE 1 1. Hematological malignancies

Diagnosis	Patients (n=134)
Ph negative Myeloprolipherative Neoplasms	49
Non-Hodgkin Lymphoma	26
Hodgkin-Lymphoma	3
Chronic Lymphocytic Leukemia	13
Myelodisplastic Syndrome	13
Acute Leukemia	11
Multiple Myeloma	10
Chronic Myeloid Leukemia	9

TABLE 2 2. Concomitant anti-cancer therapies

Concomitant therapy	Patients (n=134)
Chemotherapy and/or immunotherapy	28
Tyrosine kinase inhibitors	23
Immunomodulatory agents	7
BCL-2 inhibitor	3
Hypomethylating agents	3
Supportive care/ cytoreductive therapy	70

Results: The patients evaluated were 134. The hematological malignancies are summarized in table 1 and the concomitant therapies are reported in table 2. The median follow-up was 12 months. We did not observe nor thrombotic neither major hemorrhagic adverse events. Minor bleedings occurred in 12 patients: DOACs dose was reduced in 5/12 patients due to concomitant high DOACs peak plasma levels. All these 5 patients were performing tirosine kinase inhibitor therapy: DOACs dose reduction in these patients resulted safe without any further hemorrhagic or thrombotic complications. Six patients discontinued DOACs during disease progression for a platelet count < 50x10^9/l after a median time of 8.4 months.

Conclusions: DOACs resulted effective and safe in patients with hematological malignancies. Anticoagulants plasma level can be helpful in suggesting an early dose adjustment to prevent hemorrhagic adverse events. Larger prospective studies are warranted to confirm the safety and efficacy of DOACs in hematological patients.

PB2162 | Incidence, Risk Factors and Clinical Outcome of Venous and Arterial Thromboembolic Events in Patients Treated with Immune-checkpoint Inhibitors: A Single-center Cohort Study

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Background: Neither venous nor arterial thromboembolic events (VTE/ATE) have been reported in phase III trials evaluating immune-checkpoint-inhibitors (ICI) in patients with cancer. Little is known about the risk of VTE and ATE in patients with cancer treated with ICI.

Aims: To quantify risk of VTE and ATE in a cohort of patients treated with ICI, explore its impact on mortality and identify potential risk factors.

Methods: Patients treated with an ICI approved by the European-Medical-Association at the Medical University of Vienna between 2015 and 2018 have been identified using the in-house pharmacy records (n=582; most frequent entities: melanoma: 35.6%, lung-cancer: 29.3%; stage-IV-disease: 89%). A retrospective chart-review was performed to screen for occurrence of VTE and/or ATE. Competing-risk analysis was performed to estimate cumulative incidences of VTE and ATE. Differences between subgroups were investigated by proportional sub-hazards regression model. The impact of VTE and ATE on mortality was studied within a multi-state model.

Results: Over a median follow-up of 13.1 months, 43 VTE 17 ATE and 259 deaths (median-survival: 23.4 months) occurred. The cumulative incidences of VTE and ATE were 10.8% [95%CI: 7.1-15.4)] and 3.5% [2.1-5.4], respectively. Occurrence of VTE was associated with increased mortality (transition hazard-ratio (THR): 3.05 [95%CI: 2.00-4.66]. ATE occurrence was not associated with mortality (1.38 [0.68-2.81]). Disease-stage (all VTE occurred in stage 4) and history of VTE (SHR: 3.23 [1.68-6.20]) were strong clinical predictors of VTE. No association with VTE risk was observed for ECOG performance-status, Charlson-Comorbidity-Index or tumour type. In 93 patients with available data on PD-L1 expression, higher levels were associated with VTE risk (HR per 10% increase: 1.20 [1.01-1.32]).

Conclusions: Patients with cancer undergoing ICI-therapy frequently developed VTE or ATE. Occurrence of VTE was associated with a prior history of VTE and PD-L1 expression and was associated with higher risk of early mortality.

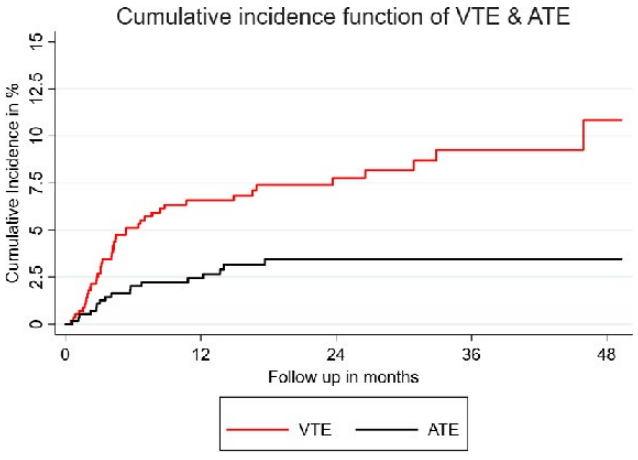

FIGURE 1 Cumulative incidence function of venous thromboembolic events (VTE) and arterial thromboembolism (ATE)

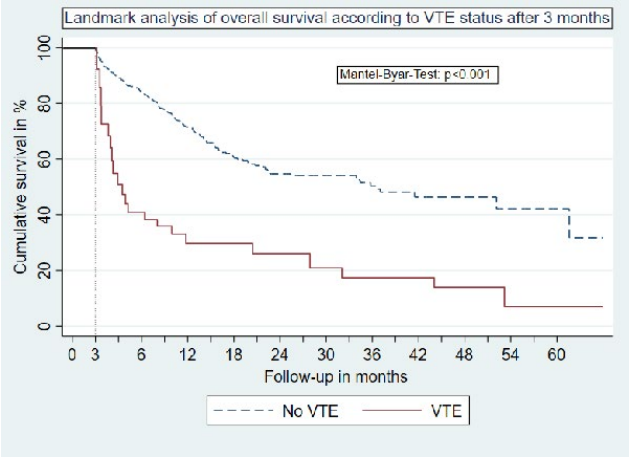

FIGURE 2 Landmark analysis of overall survival stratified by occurence of VTE within the first 3 months of observation

PB2163 | Cerebral Sinus Venous Thrombosis in Children with Leukemia: Single-institution Report

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Background: Cerebral sinus venous thrombosis (CSVT) is considered one of the significant complications of childhood leukemias. According to published literature the incidence of CSVT varies from 1.5 to 9% depending on the chemotherapy regimen. The reported

mortality which can be directly or indirectly attributed to CSVT ranges from 10 to 40%.

Aims: The aim of this study was to explore the incidence of CSVT in pediatric patients with acute leukemias treated at our Center.

Methods: A retrospective review of the medical records of children with acute leukemias diagnosed and treated at the Hematology Center after Prof.R.H.Yeolyan between 01/2017 and 12/2019 was conducted.

Results: 94 children (0-18y) with leukemia, from which 12 acute myeloid leukemia (AML) and 82 acute lymphoblastic leukemia (ALL), were diagnosed at our Center. 2 ALL patients without receiving any treatment went abroad, 1 patient with ALL abandoned treatment at the beginning and 1 patient with ALL died at the ICU at diagnosis.

From the remaining 90 patients, the mean age was 7.4y (median - 7y). Male/female ratio was 1.05. Patients with ALL received treatment according to ALL-BFM 2009 and AML patients with AML-BFM 2004 protocols. 10(11%) patients died during the observed 3 years, from which 6 patients had AML.

Out of 90 patients only 2(2.2%) cases of CSVT were reported, which were found by diagnostic MRI. Patients had not clinical signs and were diagnosed at the beginning of the initial workup. Both patients received treatment with low molecular weight heparin and both are alive and doing well.

Conclusions: The incidence of CSVT at our Center was comparable with the reported literature data. For the majority of patients with fatal outcome we didn't have information on the cause of mortality, therefore we believe that the actual incidence of CSVT is higher, and further studies need to explore this.

PB2164 | Patients Experience of Living with Cancer Associated Thrombosis in Singapore (PELICAN)

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Background: Previous research has identified several areas of unmet clinical and support need for cancer patients diagnosed with venous thromboembolism. Data have suggested differences may reflect the diversity of cultures and difference in healthcare services. It is not known whether such experiences can be extrapolated to Eastern and Asian countries where cultural approaches to healthcare have been previously identified.

Aims: To explore patients' experience of cancer-associated thrombosis (CAT) within Singapore.

Methods: Purposive sampling of twenty- one patients with CAT were recruited from attending a regional thrombosis clinic in Singapore. Semi-structured were analysed using an applied framework matrix with an inductive approach to identify any new themes. This was in order to explicate differences not apparent in the previous datasets.

Results: Twenty-one patients aged 20-80 participated. The main commonality observed with Western populations was the lack of information regarding CAT. Participants adopted a passive unquestioning role whereby the doctor was always right.

Three major themes were identified:

1. The lack of desire to understand their CAT diagnosis.They were largely indifferent to their diagnosis and the potential complications of this condition.
2. Paternalisic Doctor-Patient relationships which further propagates the educational gap, as many patients lack the initiative/willingness to ask questions to clarify their understanding.
3. A mindset of (unfounded) optimism towards their health: discouraging thinking 'in a bad way', with an emphasis on a 'positive outlook'.

Conclusions: Singapore patients with CAT adopt a passive role with respect to information requirements, as illustrated by the paternalistic treatment of doctors. This societal hierarchy creates a 'one-way' communication style and establishes a minimal desire to understand CAT and an unfounded acceptance/optimism.

PB2165 | Cooperation about the Treatment of the Venous Thromboembolism in Cancer Institute Hospitals and Neighboring Cooperation Acute Hospitals

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Background: When performing various treatment of cancer, prevention of thrombosis and treatment of thrombosis management are very important. Cancer associate thrombosis (CAT) is well known for the report of Prof. Trousseau. It also needs to be warned that CAT forms thrombosis in both of artery and vein. In the cancer hospital and research institute, prevention of venous thromboembolism has widely used.

Aims: We aimed at standardizing the treatment of CAT by cooperation of the cancer hospitals and research institutes and the acute hospitals.

Methods: Two cancer hospitals and research institutes and two acute term hospitals of cooperated, and we managed the curative program.

Results: Defining adaptation of the treatment of VTE case which can be performed in a cancer hospital and research institute, the clinical case with the high mortality risk plan to transfer hospital treatment by acute hospital, and we created the flow chart of VTE treatment with cancer.

Conclusions: A cancer care hospital becomes there are very few doctors of a circulatory speciality, therefore management for CAT is insufficient compared with many specialized physicians of cancer care exist. And so we cannot carry out advanced circulation management in our organization for 24 hours.I report and consider our institution cooperation.

PB2166 | Effectiveness and Safety of Direct Oral Anticoagulants in Patients with Ph-negative Myeloproliferative Neoplasms and Venous Thromboembolism

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Background: Data on the use of direct oral anticoagulants (DOACs) in patients with Ph-negative myeloproliferative neoplasms (MPN) are scarce.

Aims: To assess effectiveness and safety of DOACs in MPN patients with venous thromboembolism (VTE).

Methods: We retrospectively selected from our monocenter cohort of 1,100 MPN patients those having received DOACs for atrial fibrillation (AF) or after VTE. We estimated the incidence rate (IR) % pt-yrs of recurrent thromboses and bleeding events.

Results: We selected 38 patients on DOACs as primary prophylaxis in AF (n=15) or secondary prophylaxis after VTE (n=23) (Table). Circulating levels of DOACs were checked at least once and were therapeutic in all patients. Among the VTE patients on DOACs, 18 were first-users and 5 were shifted from vitamin K-antagonists (VKA); 17 received cytoreduction (hydroxyurea=16, ruxolitinib=1). Four recurrent thromboses (Table) were recorded in the first-users after a median duration of 19.5 months on DOACs (range 1-52); a fifth patient with previous CVT was shifted to DOACs after 18 years on VKA after an intracranial hemorrhage but after 27 months a splanchnic vein thrombosis occurred. All patients except one were on hydroxyurea. The IR of recurrence on DOACs was 12.6% pt-yrs, with a 2 yrs-cumulative probability of 21.5% (95%CI 0-44.4). This compares unfavorably with the 11.5% (95%CI 5.0-18.0) 2 yrs-cumulative probability of thrombosis on VKA recorded in MPN patients with VTE (Leukemia 2016;30:2032). The IR of bleeding events % pt-yrs was 5.1 in VTE patients and 6.8 in the whole cohort.

Conclusions: Obvious limitations of this study are the small number of patients, the short duration of exposure to DOACs, and the retrospective design. However our data induce caution in prescribing DOACs after VTE in MPN. Those findings urgently call for a multicenter study aimed to assess effectiveness and safety of DOACs in MPN on a statistically powered patient sample size.

TABLE 1

	DOACs after VTE (N=23)	DOACs in AF (n=15)
Sex (M/F)	12 / 11	9 / 6
Age at diagnosis, median years (range)	66 (33-82)	64 (35-83)
Diagnosis: Polycythemia vera, N / Essential thrombocythemia, N / Myelofibrosis, N	12 / 7 / 4	6 / 9 / 0
First thrombotic event: DVT and/or pulmonary embolism, N / Splanchnic vein thrombosis, N / Cerebral vein thrombosis, N	14 / 4 / 5	Not applicable
Type of DOAC: Apixaban, N / Dabigatran, N / Rivaroxaban, N	3 / 0 / 20	9 / 2 / 4
Months on DOACs, median (range) - Total observation time, years	18 (1-57) mo. - 39.5 yrs	26 (3-63) mo. - 33.6 yrs
Thrombotic events on DOACs, N - IR per 100 pt-yrs (95%CI)	5 [a] - 12.6 (4.1-29.5)	2 [b] -5.9 (0.7-21.5)
Bleeding events on DOACs: Major events, N / Clinically relevant non-major events, N - IR per 100 pt-yrs (95%CI)	0 / 2 [c] - 5.0 (0.6-18.2)	1 [d] / 2 [e] - 8.9 (1.8-26)
Legend [a] Pulmonary embolism=1, cerebral vein thrombosis=1, splanchnic vein thrombosis=1, intraventricular thrombosis1, TIA=1 [b] Ischemic stroke=1, acute myocardial infarction=1	Legend [c] Epistaxis=1, metrorrhagia=1	Legend [d] Intracranial hemorrhage=1 [e] Gastrointestinal bleeding=1, hematoma after insertion of central venous line=1

PB2167 | Differential Expression of Neutrophil Activation Markers in Venous Thromboembolism Patients with and without Association to Cancer

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Background: Venous thromboembolism (VTE), including pulmonary embolism (PE), require new diagnostic strategies and therapeutic approaches. Upon activation, neutrophils release their content by degranulation or NETosis. NETs are highly prothrombotic networks made of DNA, myeloperoxidase (MPO), calprotectin and nucleosomes, among other neutrophil molecules. Furthermore, new biomarkers are needed to early identify cancer patients with high thrombotic risk.

Aims: To study the markers of neutrophil activation in PE and cancer-associated VTE patients.

Methods: 18 (8 PE patients and 10 cancer-associated VTE patients) and 12 healthy subjects were prospectively recruited. Plasma markers of neutrophil activation (cell-free DNA, MPO, calprotectin and nucleosomes) were measured in the totality of the samples. Statistical analysis of differentially expressed neutrophil activation markers among all groups was performed using GraphPad Prism (v8.0.1).

Results: We compared each plasma markers of neutrophil activation by an ANOVA analysis. Compared to controls, PE patients had higher concentration of plasma calprotectin (P=0.028), cfDNA (P=0.0067) and MPO (P=0.028). Similarly cancer-associated VTE patients had increased levels of plasma calprotectin (P< 0.0001) and DNA (P< 0.0001) than controls. A significant correlation was observed between calprotectin and cfDNA levels (Spearman r=0.812, P< 0.001), between calprotectin and nucleosomes levels (r=0.538, P=0.003), calprotectin and MPO levels (r=0.557, P< 0.002) and between MPO and cfDNA levels (r=0.508, P=0.005).This significant correlations indicate that they all had to some extent the same origin. As expected, no differences were observed between PE and cancer-associated VTE patients since both are highly prothrombotic states.

Conclusions: An increase in plasma markers of neutrophil activation may reflect a highly prothrombotic state and, when confirmed, may be valuable for the identification of high-risk patients including those with a neoplastic disease. Funding sources: Instituto de Salud Carlos III (PI15/01085, PI17/00495) and SETH.

PB2168 | Antimetastatic Profile of Bovine Intestinal Heparin and its Low Anticoagulant Activity Derivative

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Background: Heparin is frequently used to treat thromboembolism in cancer patients. Interestingly, heparin therapy is benefic, prolonging the overall survival due to a mechanism unrelated to its anticoagulant activity. Porcine intestinal mucosa is the main source of commercial heparin (HPI). However, bovine mucosa (HBI) is also used in some countries. In 2017, the Brazilian Pharmacopeia was the first to publish separate monographs for HPI and HBI, based of contrasting anticoagulant potencies and chemical differences. The distinct chemical features allowed us to isolate a low anticoagulant fraction from HBI (LABH).

Aims: The aim of the present study was to determine the anticoagulant profile of LABH and investigate the antimetastatic potential of both, LABH and pharmaceutical-grade HBI.

Methods: The *in vitro* anticoagulant potencies of the heparins were estimated by anti-FIIa and anti-FXa activities. The hemorrhagic effect was evaluated by the tail-bleeding assay in mice. The effect of the heparin preparations on metastasis was investigated by experimental models in mice, using melanoma (B16F10) and colon carcinoma (MC38) cells.

Results: HBI is about two-times less anticoagulant than HPI. LABH is significantly less potent with a specific activity of 22 UI/mg. LABH, HBI and HPI attenuates metastasis at the same extent, reducing 10% of the lung metastatic foci in both melanoma and colon carcinoma model at the dose of 4mg/kg. At the antimetastatic dose, HPI and HBI increased blood loss approximately ten times, whereas LABH did not increase blood loss comparing with control.

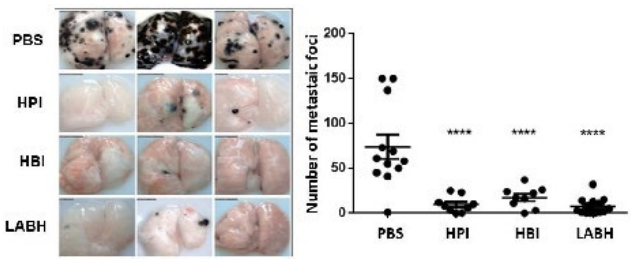

FIGURE 1 Experimental lung metastasis of B16F10 melanoma cells is attenuated by HBI and LABH treatment

Conclusions: HBI and LABH present significant antimetastatic effects. The low anticoagulant profile of LABH indicates its use in further cancer studies. The introduction of heparin from bovine mucosa has a worldwide interest since the current heparin production is based on porcine products. Any information about in vivo properties of HBI will contribute to its clinical use.

PB2169 | Developing an *ex vivo* Method to Study Thrombosis in Cancer Patients: Cancer-associated Thrombosis-on-a-Chip

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Background: Cancer patients have a 4- to 7- fold increased risk of venous thromboembolism (VTE). Cancer-associated thrombosis is a major cause of morbidity and one of the main causes of death in these patients. Currently, mouse models are used to study the mechanisms involved in this pathology and to allow preclinical testing. However, mouse models cannot be used to properly mimic the human pathology, since mice do not develop thrombosis spontaneously. The biological mechanisms involved in cancer-associated thrombosis are still unknown.
Aims: Develop a humanized organ-on-a-chip model to study cancer-associated thrombosis.
Methods: The OrganoPlate Graft (Mimetas BV) was used as a platform to mimic a cancer-associated thrombosis environment. Human umbilical vein endothelial cells (HUVECs) were isolated, expanded and seeded in the perfusion channels from the OrganoPlate Graft. In parallel, spheroids of HT-29 human colorectal cancer cell line were generated using 10,000 cells/well which were seeded in non-adherent round bottom 96-wells plates. After 3-4 days of HUVEC and spheroid seeding, pro-angiogenic growth factors were added to the perfusion channel, and/or colorectal cancer spheroids were added to the graft chamber. After another 3 days of co-culture, the model was assessed for cell morphology, barrier permeability, and thrombin formation.
Results: Preliminary data showed that the addition of pro-angiogenic growth factors or colorectal cancer spheroids increased the permeability of the endothelial barrier. Pre-incubation of endothelial cells with TNF-α induced thrombin formation after the addition of citrated human plasma and calcium. Moreover, there was more thrombin formation when pro-angiogenic growth factors were added in comparison with conditions that included colorectal cancer spheroid and control.
Conclusions: Our data showed that the cancer-associated thrombosis model is a promising model to study this pathology *in vitro* incorporating a 3D environment in a microfluidic system. This work was funded by Health~Holland.

PB2170 | ThroLy and Khorana Scores Comparation in a Group of Lymphoma Patients

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Background: The incidence of thromboembolic complications in patients with lymphoma ranges from 1.5% to 59.5%. Studies showed that lymphoma patients had a10-fold higher risk for the development of venous thrombosis, than patients with other cancers.Despite this datathromboprophylaxis in these patients is underused.
Aims:
- Compare Throply Score, model based on individual clinical and laboratory patient characteristics designate for lymphoma patients, with Khorana score.
- Study the effect of cardiovascular risk factors on thrombotic risk.

Methods: This is a non-interventional study, which included all patients diagnosed with non-hodgkin lymphoma or Hodgkin lymphoma in our center between January 1, 2017 and December 31, 2019. A total of 187 patients, with a median age of 60 years (18-89 years), 63% were man.
Data for specific demographic, clinical, and laboratory variables were collected at diagnosis (VTE/AMI/stroke, reduced mobility (ECOG 2-4), obesity (BMI>30 kg/m^2), extranodal localization, mediastinal involvement, neutrophils< 1 3 10^9/L, hemoglobin level< 100 g/L) for 100 patients with lymphoma diagnosis. Additionally, cardiovascular classical risk factors were obtained from each patient. Khorana and Throly scores were applied to all patients.
Results: In the global analysis there were 163 patients and 7 thrombosis (in 4.3% of the patients) in the low and intermediate Throly vs 19 patients and 6 thrombosis (in 31.6% of the patients) in the high Throly. In the LBDCGB analysis there were 65 patients and 4 thrombosis (in 6.2% of the patients) in the low and intermediate Throly vs 11 patients and 5 thrombosis (in 45.5% of the patients) in the high Throly. Korana score showed a specificity an VPN inferior.
In patients who developed thrombosis, dyslipidemia, arterial hypertension and advanced age, were risk factor more prevalent compared with no thrombosis patients.
Conclusions: Throly score is more specific to predict thrombosis complicartion in lympohoma patientsThe incluison of risk factor could increase de specificity os lymphoma scores.

PB2171 | Effectiveness and Safety of Rivaroxaban in Patients with Cancer-associated Venous Thromboembolism

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Background: Guidelines recommend oral factor Xa inhibitors for the treatment of cancer-associated thrombosis (CAT) in patients considered low-risk of bleeding and without a potential for drug-drug interaction with systemic therapy.

Aims: To evaluate the effectiveness and safety of rivaroxaban versus low molecular-weight heparin (LMWH) for the treatment of CAT.

Methods: Using US Surveillance, Epidemiology and End Results (SEER)-Medicare-linked data from 2013-2016, we identified adults with primary lung, breast, pancreatic, prostate, ovarian or bladder cancer, experiencing a hospitalization or emergency department admission with a primary diagnosis code for venous thromboembolism (VTE) within 2-years of cancer diagnosis or while actively treated for cancer, ≥12-months of continuous benefits prior to the index CAT and who received rivaroxaban or a LMWH as their first outpatient anticoagulant. Patients with oral, esophageal, stomach, colorectal or brain cancer were excluded. Rivaroxaban and LMWH cohorts were 1:1 propensity-scored matched (standard differences< 0.10 achieved for all covariates). Outcomes included the composite of recurrent VTE or major bleeding at 12-months and recurrent VTE, major bleeding and all-cause mortality individually. Cohorts were compared using Fine-Gray (competing risk) Cox regression.

Results: We included 684 rivaroxaban and 684 LMWH-managed patients. Thirty-three percent of patients were ≥75-years-of-age, 43.8% had a pulmonary embolism as their index event and most were diagnosed with stage 2 (45.5%) or 3 (52.0%) disease. Nearly 72% of CAT occurred within 2-years of patients' initial cancer diagnosis, with 67.9% of patients undergoing active cancer treatment at time of thrombosis. At 12-months, there was no difference in the composite endpoint of recurrent VTE or major bleeding, either component alone or all-cause mortality between the rivaroxaban and LMWH cohorts (Figure).

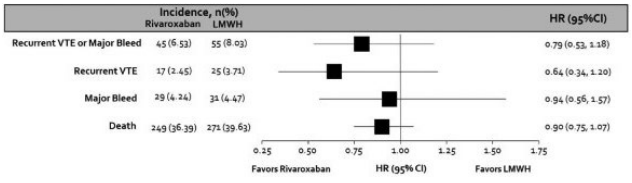

FIGURE 1 Incidence rates and hazard ratios for recurrent VTE, major bleed, and death

Conclusions: In this real-world study of rivaroxaban- and LMWH-managed CAT patients, no difference in patients' risk of experiencing the composite outcome, recurrent thrombosis, major bleeding or all-cause mortality were observed between anticoagulant strategies.

PB2173 | Cancer Patients Has Lower Plasmin Generation by Simultaneous Thrombin and Plasmin Assay Evaluation

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Background: Cancer has the ability to alter all mechanisms linked to the hemostatic system, and therefore, venous thromboembolism (VTE) is a frequent complication in these patients. Until the present, no study has evaluated the fibrinolytic potential by the method of simultaneous thrombin and plasmin generation (STP) on this setting.

Aims: Evaluate the fibrinolytic activity of patients with cancer, in the absence and presence of VTE, using the STP method.

Methods: A multicenter prospective study to assess the development of VTE in all cancer patients is underway on Unicamp, SP, Brazil. The global fibrinolytic capacity was assessed using the simultaneous thrombin and plasmin generation assay by fluorometric methodology.

Results: One hundred thirteen patients with newly diagnosed cancer without chemotherapy and 31 healthy individuals were included. During the six-months follow-up eleven patients developed VTE (CAT). The median age with and without DVT was 58 (19 - 90) and 53 (31 - 78) years, respectively. The male was predominant among CAT patients (54.54%). The Lymphoma/Myeloma were more prevalent in our study population (57.84%) and among CAT patients were Brain, Stomach and Pancreas cancer subtypes. The results of four parameters from the curve of plasmin and thrombin generation are in table1. Cancer patients showed a shorter lag time and a higher thrombin production speed, but the concentration of thrombin generated was similar between the groups (Figure 1). The plasmin concentration generated was lower in cancer patients, accompanied by an early lag time. In addition, plasmin production speed was shown to be more accelerated in patients who developed thrombosis when compared to those who did not.

Conclusions: Cancer patients showed a state of hypercoagulability, characterized by a fast thrombin generation without increased concentration. The lower production of plasmin and hypofibrinolysis in cancer patients can contribute to VTE risk.

TABLE 1 Comparison of parameters between cancer patients (n=102), CAT patients (n=11) and healthy controls (n=31)

Parameters	Healthy controls	Cancer patients	CAT patients	P-Value
MA - Thrombin	863.48 (441.15 - 1021.15)	866.43 (98.85 - 1440.35)	877.51 (119.60 - 1064.35)	0.4358
Time to MA (min) - Thrombin	39.24 (15.75 - 216.75)	23.48 (6.75 - 235.50)	16.77 (6.75 - 31.50)	<0.0001
Vmax (AU/min) - Thrombin	266.81 (131.80 - 461.8)	390.30 (22.13 - 789.53)	292.16 (19.17 - 560.53)	<0.0001
Lag time - Thrombin	8.71 (4.50 - 12.75)	13.57 (0 - 239.25)	45.95 (0 - 240.0)	<0.0001
MA - Plasmin	772.99 (630.50 - 977.75)	666.21 (38.10-1155.80)	632.45 (40.20 - 977.65)	0.0016
Time to MA (min) - Plasmin	149.36 (86.25 - 240.0)	141.21 (78.75 - 240.00)	164.25 (0.75 - 237.75)	0.0691
Vmax (AU/min) - Plasmin	30.34 (1.53 - 56.67)	24.27 (2.09 - 51.07)	49.65 (5.80 - 288.14)	0.0175
Lag time - Plasmin	23.06 (14.24 - 33.75)	17.85 (1.50 - 60.0)	48.68 (9.75 - 239.25)	<0.0001

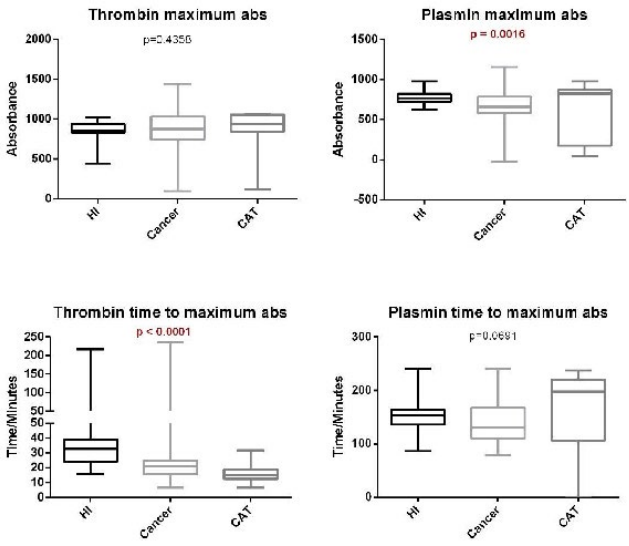

FIGURE 1 Comparison of thrombin and plasmin generation between cancer patients (n=102), CAT Patients (n=11) and healthy controls (n=31)

PB2174 | Venous Thromboembolic Risk in Ambulatory Cancer Patients: Awareness Needed!

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Background: Venous thromboembolism (VTE) is an important cause of morbidity and mortality among cancer patients. Comprehensive management of VTE in these patients includes not only effective treatment, but also identification of patients who will benefit from thromboprophylaxis. Khorana score (KS) is the main validated tool for VTE risk-stratification.

Aims: This study aims to determine VTE risk, using KS, in ambulatory cancer patients, and to evaluate thromboprophylaxis prescription in high-risk patients.

Methods: This is an ongoing prospective observational study (FIIT: Factor II - Thrombin) from a Portuguese central hospital, designed to develop a predictive model of VTE. Court study population includes patients with cancer diagnosis, proposed for anti-tumor treatment (chemotherapy, immunotherapy or targeted therapies). Patients with major bleeding in the last 3 months, major surgery in the last 28 days, on anticoagulation/antithrombotic therapy or previously submitted to bone marrow transplantation were excluded. Patients, disease characteristics and blood count values were collected, and KS calculated. The study was approved by local ethics committee. Informed consent was obtained prior to study inclusion.

Results: From April-November 2019, 182 patients were included with a mean age of 57±12 years, 51% were female. The majority had breast (22%), colorectal (20%) and gastric (12%) malignancies, 45% stage IV disease. According to KS, 46% were stratified as low-risk, 44% intermediate-risk and 10% high-risk. In high-risk patients 6-month VTE incidence can reach 17.7%. Results for VTE incidence in our population are expected during 2020. Although not routinely recommended, thromboprophylaxis might be offered to outpatients with KS ≥2. In the study 26.9% (n=49) patients had KS≥2, none initiated thromboprophylaxis before systemic treatment.

Conclusions: A noteworthy number of patients starting anti-tumor treatments have high-risk of VTE. Evidence for thromboprophylaxis criteria in these patients should be clarified. This prospective-study intends to develop a comprehensive risk-model adapted to current realities considering the diversity of available anti-tumor treatments.

PB2175 | No Agreement among Risk Prediction Scores for Venous Thromboembolism in Cancer Patients: A Prospective Cohort Study

M. Evelyn Alves de Almeida; G. dailva Souza Gois; T. Ramires Rodrigues Alencar; S. Aparecida de Lima Montalvão; B. Moraes Martinelli; I. Macedo Toni; J. Barreto Carvalheira; M. Etchebehere; N. Adami Andreollo; J. Cesar Teixeira; J. Maria Annichino-Bizzacchi; J. Maria Annichino-Bizzacchi; ADVENTH Cancer Investigators
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Background: Scores has been proposed to predicting incidence of cancer-associated venous thromboembolism (CAT) among patients starting chemotherapy.
Aims: Evaluate the performance of the Khorana, PROTECHT, and Vienna scores to predict venous thrombosis in a cohort of cancer patients.
Methods: In a prospective multicenter cohort study on Unicamp, CAISM and Hospital de Clínicas, SP, Brazil, patients newly-diagnosed with moderated to advanced cancer (stage II or III solid cancer) were eligible in the absence of chemotherapy or had started chemotherapy in the previous three months.
Results: A total of 429 patients were enrolled (Table 1), of whom 83 (20%) had not yet received chemotherapy. Fourteen patients (3.3%) developed CAT (64.3% DVT and 35.7% PE) with all cases occurring in the 6-months of analysis. The scores classified 12.9% *vs.* 23.2% of patients as high-risk, 58.6% *vs.* 57.2% as intermediate-risk, and 28.5% *vs.* 19.6% as low-risk for Khorana and PROTECHT, respectively. Of the patients with VTE in the first six months, only 13% and 23% had been classified as high risk according to the Khorana and PROTECHT scores. In a preliminary result 80/429 (19%) patients were also evaluated according to VIENNA CATs score. Among three scores VIENNA CATs appears to discriminate better between low- and high-risk patients (Figure 1). The agreement evaluation among three scores was -0.048 (no agreement) according to Kappa coefficient.

TABLE 1 Demographic data and risk prediction scores for venous thromboembolism in cancer patients

Variable	CAT Patient (N=14)	Cancer Patient (N=415)
Age	52 (31-78)	56 (18-92)
Male (%)	43%	42%
Cancer Stage (%)	Stage IV (55%)	Stage II (40%)
Cancer site - Higher frequency (%)	Stomach (30%)	Mama (30%)
Gemcitabine Chemotherapy (%)	0	07
Platina Chemotherapy (%)	05	90
P-selectina (ng/L)	59 (14-100)	36 (0-100)
D-dimer (ug/L)	2 (0-25)	0 (0-9)

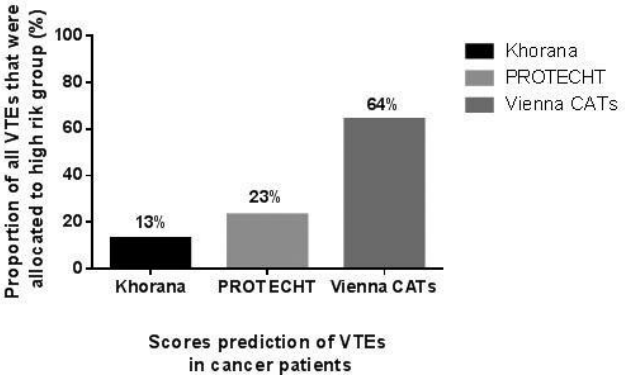

FIGURE 1 Proportion of all VTEs that were allocated to high-risk group according to Score prediction

Conclusions: Khorana and PROTECHT scores performed poorly in predicting VTE in cancer patients in this population and present no agreement. However, the biomarkers D-Dimer and P-selectine used to calculate VIENNA CATs appear to be important to classify at a risk, since this score presented a high prediction value. Further inclusion of patients to validate this result are needed considering before they can be considered for introduction into clinical practice.

PB2176 | Clinical Presentation and Outcomes of Cancer-associated Distal Deep Vein Thrombosis: Data from the RIETE Registry

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Background: Distal deep vein thrombosis (D-DVT, infra-popliteal DVT without pulmonary embolism (PE)) is usually considered as a minor form of venous thromboembolism (VTE). it is associated with a much lower rate of adverse outcomes (death, VTE recurrence or bleeding) than proximal DVT (P-DVT). Cancer-associated thrombosis (CAT) is associated with poorer outcomes than thrombosis developing in the absence of cancer. Few data are available on the clinical presentation and prognosis of CAT D-DVT.
Aims: To assess clinical presentation and prognosis of CAT D-DVT.

TABLE 1 Clinical characteristics of patients with CAT D-DVT (n=755), non-CAT Distal DVT (N=5,218) and CAT P-DVT (n=4,798); ‡ indicates p < 0.001

	CAT Distal DVT	CAT Proximal DVT	Non-CAT Distal DVT
Age (years), mean ±SD	67±12	68±13	60±18
Male sex, n (%)	391 (52%)	2,529 (53%)	2,553 (49%)
Weight, mean (±SD)	72±15	71±14	77±16
Inpatient status, % (n)	240 (33%)	1,280 (27%)	1,185 (23%)‡
Surgery, n (%)	162 (22%)	588 (12%)‡	770 (15%)‡
Immobilization, n (%)	114 (15%)	959 (20%)	1,280 (25%)‡
History of VTE, n (%)	81 (11%)	616 (13%)	782 (15%)
Severe renal impairment, n (%)	37 (4.9%)	393 (8.2%)	168 (3.2%)
Recent major bleeding, n (%)	17 (2.3%)	157 (3.3%)	101 (1.9%)

TABLE 2 Clinical outcomes on anticoagulant therapy of patients with CAT D-DVT (n=755), non-CAT Distal DVT (N=5,218) and CAT P-DVT (n=4,798)

	CAT Distal DVT N, %PY (95%CI)	CAT Proximal DVT N, %PY (95%CI)	Non-CAT Distal DVT N, %PY (95%CI)
Median duration of follow-up: days (IQR)	199±327	221±336	247±409†
Death	163, 40.0 (34.1-46.6)	1361, 47.1 (44.6 - 49.7)*	64, 1.8 (1.4-2.3)‡
Fatal PE	0, -	23, 0.8(0.5 - 1.2)	3, 0.1 (0.02 - 0.3)
Fatal bleeding	2, 0.5 (0.1 - 1.8)	44, 1.5 (1.1 - 2.1)	3, 0.1 (0.02 - 0.3)
Recurrent DVT	34, 8.3 (5.8 - 11.6)	204, 7.1(5.8 - 11.6)	117, 3.3 (2.8 - 4.0)‡
Recurrent PE	14, 3.4 (1.9 - 5.8)	119, 4.1(3.4 - 4.9)	36, 1.0 (0.7 - 1.4)†
Major Bleeding	20, 4.9 (3.00 - 7.58)	229, 7.9 (6.9 - 9.0)*	51, 1.5 (1.1 - 1.9)‡
Comparisons between patients with Distal DVT and cancer (ref) vs. proximal DVT and cancer and vs. distal DVT without cancer:	*p <0.05; †p <0.01; ‡p < 0.001.		

Methods: Multicentre, international, observational RIETE registry of patients with objectively confirmed VTE. We compare clinical presentation and rates of adverse outcomes during anticoagulant treatment of patients with CAT D-DVT vs. CAT P-DVT or vs. non-CAT D-DVT.

Results: 755 patients with CAT D-DVT, 4,798 patients with CAT P-DVT and 5,218 patients with non-CAT D-DVT were available for analysis. Their median duration of follow-up was 148 days (95% confidence interval (93-294)), 156 days (90-339) and 193 days (110-392) respectively. Their clinical characteristics are depicted in Table 1. As compared with patients with CAT P-DVT, those with CAT D-DVT had less frequently metastatic diseases (56% vs. 47%, p< 0.001). However there was no marked differences in terms of tumor site (all p values>0.001 except for bladder cancer, twice more frequent in case of CAT P-DVT), time elapsed between cancer diagnosis and DVT (>3 months in 66% and 58% of cases, p>0.05) and proportion of patients on chemotherapy at time of DVT diagnosis (50% vs. 51%, p>0.05). Rates of adverse outcomes of patients with CAT D-DVT were higher than for patients with non-CAT D-DVT and similar to patients with CAT P-DVT (Table 2).

Conclusions: Whereas non-CAT D-DVT appears to have a relatively favorable prognosis, CAT D-DVT shares the same poor prognosis as CAT P-DVT and presents overall similar clinical presentation.

PB2177 | Cancer Site and Distant Metastasis at Diagnosis Predict the Risk of VTE in Cancer

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Background: Patients with active cancer have a high risk of venous thromboembolism (VTE) during the course of their disease. However, prophylactic treatment is challenging due to a concomitant high risk of bleeding, and development of prediction models to

enable assessment of a beneficial balance between thrombosis and bleeding risk are important.

Aims: To test whether a simple risk assessment model for cancer-related VTE based on cancer site, metastasis and genetic information could be used to identify patients at high risk of VTE.

Methods: We used a case-cohort derived from two Norwegian population-based cohorts (Tromsø study and Nord-Trøndelag Health Study). All patients with VTE within one year after cancer diagnosis (n=131) and a randomly sampled subcohort of cancer patients (n=1290) were included. We created a risk score model for one-year prediction of cancer-related VTE based on cancer site (high-, moderate- and low risk= 0/1/2 points) and metastasis (distant=1 point), and a second model adding prothrombotic genotypes (5-SNPs). Cox regression models were used to estimate hazard ratios for VTE with 95% confidence intervals, and Harrel's C was calculated to determine the discriminatory power.

Results: The simple model containing cancer site and metastasis ranged from 0-3 points, with a HR of VTE per point increase of 2.04 (95% CI: 1.68-2.47). The Harrel's C was 0.67 (95% CI: 0.62-0.71), and the one-year cumulative incidence of VTE for those with 0, 1, 2 and 3 points were 1.1%, 1.5%, 2.9% and 4.9%, respectively. Adding the 5-SNP genetic risk score did not change the discriminatory power of the model (Harrel's C: 0.67, 95% CI: 0.63-0.72).

Conclusions: A simple risk score based on cancer site and metastasis could be used to assess risk of VTE in cancer patients. Addition of prothrombotic genotypes to the model did not improve the discriminatory power.

PB2178 | Risk of Venous Thromboembolism in the Clinical Course of Osteosarcoma

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Background: Patients with cancer are at high risk of venous thromboembolism (VTE), a risk dependent among others on type and stage of the tumor. Osteosarcoma cells express abundant levels of tissue factor (TF), which may indicate that patients with osteosarcoma are at high risk of cancer-associated venous thromboembolism (CAT). Few studies on the incidence of CAT in osteosarcoma are available in daily clinical practice.

Aims: To assess the cumulative incidence of (symptomatic and incidental) VTE in the clinical course of osteosarcoma and to study its prognostic impact on overall survival.

Methods: We performed a post-hoc chart review of patients diagnosed with osteosarcoma and treated in the Leiden University Medical Center (LUMC) between 1984 and 2018. The institutional review board in Leiden waived the need for informed consent from study participants. Patient charts were scrutinized for index tumor characteristics, clinical patient characteristics and the VTE events. All endpoints were adjudicated by an independent expert.

Results: The baseline characteristics of the 530 included patients are shown in Table 1.The cumulative 24-month incidence of VTE in our cohort of osteosarcoma patients was 9.0% (95%CI 6.5-12). None of the VTE events was fatal. Seventy-four percent of VTE events was symptomatic and the most frequent presentation was upper

TABLE 1 Baseline characteristics of 530 osteosarcoma patients

Baseline characteristics	N=530
Age at diagnosis – years	
Mean (+/- SD)	32 (20)
Range	3.5 - 84
Gender – no (%)	
Male	295 (56)
Female	235 (44)
Presence of distant metastases at time of diagnosis – no (%)	110 (21)
Tumour grade – no (%)	
High grade sarcoma	459 (87)
Low grade sarcoma	40 (7.5)
Intermediate grade	10 (1.9)
Undefined	21 (4.0)
Surgical anti-tumor treatment – no (%)	461 (87)
Presence of CVC – no (%)	
Yes	399 (75)
No	94 (18)
Unknown	37 (7.0)

Note: N: number of patients, SD: standard deviation, CVC: central venous catheter

TABLE 2 VTE characteristics in osteosarcoma patients (time period 24 months from osteosarcoma diagnosis)

Characteristics	N= 46
Type and location of VTE – no (%)	
DVT upper limb	18 (39)
DVT lower limb	12 (26)
Pulmonary embolism	11 (24)
Other	5 (11)
Symptomatic – no (%)	34 (74)
Mean time after diagnosis osteosarcoma in months – (SD)	4.1 (4.7)
CVC related – no (%)	16 (89)
Fatal – no (%)	0 (0)

Note: VTE: venous thromboembolism, DVT: deep venous thrombosis, CVC: central venous catheter, N: number of patients, SD: standard deviation

extremity thrombosis (n=18, 39%); 16/18 of the latter were related to a central venous catheter (Table 2). The overall cumulative 24-month mortality rate was 27% (95%CI 23-30). A diagnosis of VTE was not associated with higher mortality for a crude hazard ratio of 1.4 (95% CI 0.86-2.4).

Conclusions: Osteosarcoma is associated with a considerable incidence of VTE, although mostly related to indwelling catheters. Future studies should be performed to reduce this high incidence of catheter related thrombosis in this young age patient group.

PB2179 | Cancer Associated Thrombosis: Are DOACs a Treatment Option? Systematic Review and Meta-analysis of Randomised Controlled Trials

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Background: Venous thromboembolism (VTE) in patients with cancer is an important cause of morbidity and mortality and associated with a significant risk of VTE recurrence as well as bleeding associated with anticoagulation.

Aims: To compare the efficacy and safety of direct oral anticoagulants (DOACs) to standard of care in the treatment of cancer associated thrombosis (CAT).

Methods: Sytematic literature review of prospective RCTs including patients with cancer and VTE receiving DOAC: recurrent VTE as an efficacy outcome and major bleeding (MB) and clinically relevant non-major bleeding (CRNMB) as safety outcomes. Pooled incident rates and relative risks with 95% CIs calculated with The Cochrane Collaboration Review Manger 5.3 using Mantel-Haenszel random effects model.

Results: Nine RCTs identified, 3434 patients, 6 compared DOAC v LMWH/ VKA and 3 compared DOAC v LMWH. Pooled incidence rate for recurrent VTE with DOAC was 83/1734 (4.8%) versus 139/1700 (8.2%) with LMWH/VKA or LMWH a relative risk reduction of 0.30 (0.42-0.79) I2 0% p=0.03. Sub-analysis of 3 DOAC v LMWH studies 50/870 (5.7%) v 86/869 (9.9%) VTE recurrence, RR 0.50 (0.26-0.98) I2 48% p=0.04. Pooled incidence rate of MB and CRNMB with DOAC was 14.7% versus 13.7% with LMWH/VKA & LMWH alone a non-significant RR of 1.07 and similarly DOAC v LMWH 16.3% v 10.9% RR 1.58.

Conclusions: DOACs are associated with a reduced risk of recurrent VTE in patients with cancer associated thrombosis when compared to both LMWH/VKA or LMWH alone. There appears to be a non-statistically significant trend towards more bleeding with DOACs which may be related to cancer site or treatment related drug interactions. Further investigation is needed to identify patients with cancer with a low risk of bleeding who will benefit most form treatment with a DOAC.

GENETIC RISK FACTORS OF THROMBOSIS

PB2180 | Thrombophilia Gene Screening in Pediatrics Solid Tumors with Thrombosis

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Background: Malignancy leading to a hypercoagulable state is a well established concept There is limited data on the influence of inherited thrombophilia on the occurrence of thrombosis in the pediatric solid tumors.

Aims: to correlate of the type of thrombophilia genetic mutations to the prevalence of thrombosis in pediatric solid tumors.

Methods: This study was carried out Hematology &Oncology unit of Pediatric Department of Zagazig University Hospital during the period from 2012 t0 2018., on fifteen pediatrics with solid tumors with thrombosis. All patients were subjected to full history and complete examination. The following CBC, PT, PTT and D Dimer were done. Radiology as Sonar, MRI as well as Doppler was done. Thrombophilia screening gene mutation was done for all patients, the Procedure includes single multiplex PCR for the amplification of a relevant sequence in the respective genes followed by reverse hybridization of biotinylated amplification products to oligonucleotides probes on the test strip. The assay covers the following 12 mutations: FV G1691A (Leiden), FV H1299 R(R2), Prothrombin G20210A, Factor XIIIV34L,B-Fibrinogen- 455G>A,PAI-1 4G/5G,GPIII L33P (HPA-1),MTHFR C677T, MTHFRA1298C,ACEI/D, Apo B R3500Q, and Apo E22/E3/E4.

Results: The frequency of occurrence Wilms′ tumor in 6 cases (40%), neuroblastoma in 3 cases (20%), on Hodgkin′s lymphoma in 2 cases (13.3%) and 4 cases (26.7%) with other solid tumors. Out of fifteen cases, Deep Venous thrombosis were detected in 9 cases (60%), cerebral venous thrombosis [sinus] was in 3 cases (20%), While portal vein thrombosis in 2 cases a (13.3%) and superior vena cava in 1 patients. Ten cases out of Fifteen cases of thrombosis revealed abnormal gene mutations in the heterozygous or homozygous form either in single gene or multiplex. Most frequent gene mutation was MTHFRA1298C in 6 cases.

Conclusions: Thrombophilia gene mutations may share in the pathogenesis of thrombosis in pediatric solid tumors.

PB2181 | Does blood Group Predicts Thrombosis Risk? A Pilot Study Assessing Thrombin Generation

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Background: ABO blood group has been described to play a role in thrombosis risk, being higher in those no-O. Classical coagulation tests do not reflect the thrombophilic profile of patients. The thrombin generation test (TGT) is a promising tool to assess hemostatic disorders.

Aims: Evaluate ABO blood group prothrombotic risk by using the thrombin generation test.

Methods: This was a descriptive study including 37 healthy adult patients (48.65% males, 23 year-old median age (19-64 years old)). Patients were classified according to blood group: group A (n=13), group B (n=12), group O (n=12). Plasma samples were obtained to determine the prothrombin time (PT), activated partial thromboplastin time (aPTT) coagulation factor VIII (STA STAGO, France) and von Willebrand factor activity (vWF) (BCS, Siemens, Germany). Thrombin generation test (TGT) was performed by means of GENESIA device (STAGO, France) with Thromboscreen kit. The ethic committee of the institution approved the study. All included patients signed the informed consent.

Results: We found differences in time to peak (p=0.008) and in maximum peak (p=0.007) without thrombomodulin depending on patients' blood group; differences were stablished between group B and O (4.1 vs 5.4 and 111.5 vs 79.4, respectively). Moreover, a correlation between peak time without thrombomodulin and factor VIII (r=0.37, p=0.025) was found. No differences were seen in any parameter after the addition of thrombomodulin. No correlation of TGT parameters with vWF was obtained.

No differences with aPTT and PT were found between groups. Those patients with group O had lower levels of vWF and factor VIII, having a good correlation between them (r=0.8, p< 0.001).

aPTT correlated with FVIII (r=-0.53, p< 0.001) and with vWF (r=-0.38, p=0.019).

Conclusions: Group B patients show a procoagulant profile according to TGT results. These findings suggest that TGT can identify a prothrombotic states related to ABO blood groups.

PB2182 | Genetic Study for Idiopathic Venous Thromboembolism in Japanese Using Short-Read and Long Read Sequencers: A Pilot Study of Japanese Group of Idiopathic Thromboembolism

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Background: Idiopathic venous thromboembolism (VTE) is characterized with family history of thrombosis, thrombosis at a young age (≤ 40 years), recurrent thrombosis, or thrombosis in unusual vascular territories. Short-read DNA sequencer facilitates the genetic analysis in patients with VTE. However, long-read sequencer has never been utilized for patients with idiopathic VTE.

Aims: We collected patients with idiopathic VTE consecutively. Using short-read and long-read sequencers, we identified small genetic variations and large structural variations in patients with VTE.

Methods: We selected 10 VTE candidate genes, *SERPIC1*, *PROC*, *PROS1*, *F2*, *F5*, *FGA*, *FGB*, *FGG*, *F9*, and *THBD*, and established a long-read sequencing using PacBio RS II (Pacific Bio) and short-read sequencing using Miseq (Illumina) for the candidate gene approach.

Results: Targeted sequencing using short-read as well as long-read sequencers identified 8 loss of function (LOF) variations in anticoagulant proteins in 27 VTE patients, including 2 LOF variations in *SERPINC1* in 2 families, 2 LOF variations in *PROC* in 3 families, and 4 LOF variations in *PROS1* in 7 families. In addition, we identified a thrombophilic variation, p.Arg596Gln, in the *F2* gene in three patients with two families that conveys antithrombin resistance. We also identified copy number variation in *F2* intron 4 and translocation in *FV* intron 6, which functions remained to be analyzed.

Conclusions: Our study provided fundamental resources for idiopathic VTE in Japan. Targeted sequencing using both short- and long-read sequencings would open a new paradigm for diagnosis and treatment for idiopathic VTE.

PB2184 | The Sex-Related Risk of Venous Thromboembolism Attributed to Recognized Prothrombotic Genotypes

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Background: Family- and twin studies have indicated that the genetic risk of venous thromboembolism (VTE) may be different in men and women. The proportion of VTE-risk attributable to known prothrombotic genotypes has been scarcely investigated in men and women.

Aims: To estimate the population attributable fraction (PAF) of VTE for recognized prothrombotic genotypes in men and women using a population-based case-cohort.

Methods: Cases with incident VTE (n=1,493) and a randomly sampled subcohort (n=13,069) were derived from the Tromsø Study (1994-2012) and the Nord-Trøndelag Health Study (1995-2008). DNA-samples were genotyped for 17 single nucleotide polymorphism (SNPs) previously associated with VTE. Hetero- and homozygous carries were defined as risk allele carriers. PAFs with 95% bias-corrected confidence intervals (CI) (based on 10,000 bootstrap-samples) were estimated for SNPs significantly associated with VTE, and 5-SNP cumulative models were constructed for both sexes by adding SNPs one-by-one in order of the individual PAF values.

Results: In women, the five highest PAF-estimates were observed for F11 rs2036914 (PAF 17.6, 95% CI: 0.4-34.1), ABO (PAF 16.9, 95% CI: 5.8-28.0), F11 rs2289252 (PAF 15.1, 95% CI: 3.4-26.7), TC2N (PAF 11.6, 95% CI: -1.3-24.4) and FVL (PAF 8.7, 95% CI: 5.4-12.3). In men, the five highest PAF-estimates were observed for ABO (PAF 21.3, 95% CI: 9.6-32.5), F11 rs2036914 (PAF 12.2, 95% CI: -5.5-29.2), F11 rs2289252 (PAF 10.4, 95% CI: -2.0-22.4), FGG (PAF 7.8, 95% CI: -0.5-16.0) and FVL (PAF 7.5, 95% CI: 4.0-11.2). The cumulative PAF for the 5-SNP model was 52.4% for men, and 52.7% for women.

Conclusions: Our findings suggest that 52% of the VTEs can be attributed to known prothrombotic genotypes in both men and women, and four of the five SNPs in the cumulative PAF-model were similar between the sexes. Additionally, FGG contributed to the PAF model in men, while TC2N contributed to the PAF model in women.

PB2185 | Role of *ADAMTS13*, *VWF* and *FVIII* in Deep Vein Thrombosis

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Background: We previously reported the association between rare *ADAMTS13* single nucleotide variants (SNV) and deep vein thrombosis (DVT). Furthermore, we showed that DVT patients carriers of at least one rare *ADAMTS13* SNVs had a lower ADAMTS13 activity than non-carriers.

Aims: To confirm the association of *ADAMTS13* SNV with DVT and plasma ADAMTS13 activity levels in a large DVT population. To investigate the role of *VWF*, *FVIII* variants in DVT.

Methods: *ADAMTS13*, *VWF* and *FVIII* were sequenced using NGS (Illumina) in 594 Italian DVT patients and 571 controls. Genetic association testing of low-frequency/common (minor allele frequency, MAF≥1%) and rare (MAF< 1%) variants was performed using logistic regression and gene-based burden tests, respectively. ADAMTS13 activity was measured in 365 cases (median 9, IQR 3-32 months after the event) and 292 controls using FRETS-VWF73 assay. Association between rare *ADAMTS13* SNVs and plasmatic activity was determined using a linear regression model. All models were age-, sex- adjusted.

Results: We identified 324 variants, 48 low-frequency/common and 276 rare. Of low-frequency/common SNVs, 9 were associated with DVT (FDR≤0.24), 7 in *ADAMTS13* (all protective) and 2 in *VWF* (1 risk, 1 protective) (Table 1). Two groups of *ADAMTS13* SNVs were in high linkage disequilibrium (rs28641026, rs28503257 [r^2=1]; rs3124768, rs3118667, rs739469 [r^2>0.74]). Rare *ADAMTS13* variants were associated with DVT using the burden, variable threshold and UNIQ tests (P< 0.05), but not with C-ALPHA and SKAT tests. Rare *VWF* or *FVIII* variants were not associated with DVT.

TABLE 1

Table 1

rsID	Chr	Position	Class	A1/A2	Amino Acid Position	MAF (Cases/ Controls)	OR (95%CI)*	P	FDR
rs28641026	9	136314952	exonic	T/C	p.V970V	0.02/0.04	0.53 (0.31-0.89)	0.02	0.15
rs28503257	9	136319589	exonic	A/G	p.A1033T	0.02/0.04	0.53 (0.31-0.89)	0.02	0.15
rs695523	9	136310908	exonic	T/C	p.A900V	0.07/0.10	0.74 (0.55-0.99)	0.05	0.24
rs3124768	9	136304497	exonic	A/G	p.T572T	0.43/0.50	0.78 (0.67-0.92)	0.003	0.06
rs3118667	9	136291063	exonic	C/T	p.A140A	0.36/0.43	0.78 (0.66-0.92)	0.003	0.06
rs739469	9	136298729	intronic	G/C	-	0.43/0.49	0.78 (0.67-0.92)	0.004	0.06
rs3124767	9	136308542	exonic	C/T	p.G760G	0.45/0.50	0.82 (0.69-0.97)	0.02	0.15
rs1900302	12	6126380	exonic	T/C	p.R1399H	0.01/0.001	3.26 (1.18-8.98)	0.02	0.15
rs7962217	12	6061559	exonic	T/C	p.G2705R	0.05/0.07	0.67 (0.47-0.95)	0.03	0.16

A1-minor allele; A2 major allele; MAF-Minor allele frequency; OR – odds ratio; 95% CI – confidence interval; FDR – false discovery rate; * age-, sex- adjusted.

Carriers of rare ADAMTS13 variants had lower ADAMTS13 activity than non-carriers (ß -6.2%, 95%CI -10.9, -1.5). This association was stronger in DVT cases than controls (ß -7.5%, 95%CI -13.5, -1.5 vs ß -2.9 95%CI -10.4, 4.5).

Conclusions: We identified common/low-frequency variants associated with DVT in *ADAMTS13* and *VWF*, but not in *FVIII*. Carriers of *ADAMTS13* rare variants showed slightly reduced ADAMTS13 activity and higher risk for DVT.

PB2186 | Clinical and Laboratory Characteristics of Patients with Hereditary Thrombophilias in the North-Western Region of Russia

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Background: The role of hereditary trombophilias in the genesis of thrombosis is one of the most important. The clinical peculiarities of different thrombophilia forms are still discussed.

Aims: To study clinical and laboratory characteristics of patients with different forms of hereditary thrombophilia.

Methods: 68 case-records of patients, who underwent treatment in Russian Scientific Research Institute of Hematology and Transfusiology in 2017-2019, were studied. Inclusion criteria: verified arterial or venous thrombosis and verified hereditary thrombophilia. Exclusion criteria: JAK2, CALR, MPL mutations. The following groups of patients were formed on the base of the molecular genetics and coagulation tests: I - isolated FV Leiden mutation (n=12), II - isolated mutation in Prothrombine gene G20210A (n=5), III - isolated FVIII elevation (n=8), IV - isolated hyperhomocysteinemia (MTHFR, MTRR mutations, confirmed phenotypically) (n=4), V - isolated primary antiphospholipid syndrome (n=4), VI - three thrombophilia markers combination (n=2), VII - two strong thrombophilia markers combination(n=2), VIII - strong and moderate thrombophilia markers combination (n=32). Clinical and laboratory data of those groups were analyzed.

Results:

1. Isolated thrombophilias were found in 47.06% of cases, combined - in 52.94%. The FV Leiden mutation was the most common isolated thrombophilia (37.5%). Combination of FV Leiden mutation with hyperhomocysteinemia was the most common combined thrombophilia (38.9%).
2. The median age of first thrombosis was 39.8±1.8 years (from 1 year to 84 years).
3. Women/men ratio was 61.76%/38.24%.
4. Venous thrombosis was found in 73.53%, arterial - in 11.77%, combined - in 14.7% of patients.
5. Provocative factor was established in 54.41%.
6. Non-inflammatory hyperfibrinogenemia was found in 27.94% of cases.
7. In analyzed patients incidence of B (III) blood group was 3.03 times higher than in average population.

Conclusions: Thrombophilia screening is necessary for patients with venous/arterial thrombosis. Blood group B (III) can be considered as an additional thrombophilia risk factor.

PB2187 | Characterisation of a Novel F5 Gene Mutation (Ala2114Asp, Factor V Besançon) Associated with Factor V Deficiency and Recurrent Venous Thrombosis

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Background: Coagulation factor V (FV), present in plasma and platelets, has multiple pro- and anticoagulant functions. FV deficiency is usually associated with a bleeding diathesis.

Aims: We investigated a 37-year-old male with FV deficiency (FV:C 3%, FV:Ag 4%) and recurrent venous thrombosis (11 events).

Methods: The patient provided informed consent to blood sampling for the purpose of this study. Thrombin generation in frozen-thawed platelet-rich (PRP) and platelet-poor plasma (PPP) was measured by Calibrated Automated Thrombography. The cofactor activity of FV for activated protein C (APC) in factor VIIIa inactivation was determined with the Immunochrom assay. The mutant FV C2 domain structure was analysed by molecular dynamics simulation. The functional properties of $FV(a)_{Besançon}$ were studied by kinetic assays using highly diluted patient's PPP as a source of $FV_{Besançon}$.

Results: The patient carried no thrombophilic defects, except for the *F2* 20210G>A mutation. Thrombin generation showed a hypocoagulable state in the patient's PPP, but a hypercoagulable state in the patient's PRP. The APC-response was impaired in both PPP and PRP, partly due to the absence of APC-cofactor activity of FV (Immunochrom APCsr 1.44 *vs.*2.04 in normal plasma) and to the low tissue factor pathway inhibitor level (24%). The patient's FV deficiency was attributable to a novel missense mutation (Ala2114Asp, $FV_{Besançon}$) that favours a "closed conformation" of the C2 domain and interferes with FV(a) binding to phospholipids. Compared to normal FVa, $FVa_{Besançon}$ had 2-fold lower affinity for factor Xa, slightly (< 1.5-fold) unfavourable kinetic parameters (K_m, V_{max}) of prothrombinase, but a considerably lower rate of APC-catalysed inactivation in the presence of protein S.

Conclusions: $FV_{Besançon}$ induces a hypercoagulable state *via* quantitative (markedly decreased FV level) and qualitative

(phospholipid-binding defect) effects that impact the anticoagulant reactions (anticoagulant activities of FV, FVa inactivation) more strongly than the procoagulant reactions (prothrombinase). A possible specific role of platelet FV is still under investigation.

PB2188 | Assessment of Fibrinolysis Markers in Patients with Lower Limb Deep Venous Thrombosis: A Prospective Case-control Study

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Background: Hypofibrinolysis has been linked to the development of thrombosis, both venous and arterial. Previous studies have suggested a variable association of the fibrinolysis markers with thrombosis in different settings. There is a paucity of literature from India regarding the role of various fibrinolytic parameters in deep venous thrombosis (DVT).

Aims: To assess the fibrinolytic parameters in patients with DVT.

Methods: A total of 52 patients and 52 controls were included in the study. The quantitative determination of plasminogen activator inhibitor-1 (PAI-1), plasminogen, thrombin activable fibrinolysis inhibitor (TAFI), α2-antiplasmin and tissue plasminogen activator (t-PA) was performed by enzyme-linked immunosorbent assay. Thrombophilic parameters including protein C, protein S, anti-thrombin III, antiphospholipid antibodies and Factor V Leiden mutation (FVL) mutation were assessed. The association of continuous variables between cases and controls was compared using student t-test or Mann-Whitney U test. The categorical variables were compared using Chi-square test.

Results: The patient group comprised of 27 males and 25 females and the control group had 35 males and 17 females. DVT was unprovoked in 61.5% cases and idiopathic in 40.38% cases. TAFI, plasminogen and t-PA levels were significantly higher in cases than controls. PAI-1 was significantly lower in cases than controls. α2-AP levels were statistically insignificant (Table1). TAFI, plasminogen and t- PA levels were significantly higher in unprovoked and idiopathic DVT cases than controls. The most common inherited thrombophilic risk factor was FVL mutation followed by protein C and anti-thrombin III deficiency.

Conclusions: Plasma TAFI, plasminogen and t-PA levels were significantly higher in cases than controls suggesting a positive association with DVT. A similar trend was also observed in unprovoked and idiopathic DVT cases.

PB2189 | Afibrinogenemia and Leiden FV Within the Same Family

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Background: Afibrinogenemia is a rare dyscrasia characterized by a constitutional deficit in fibrinogen. About 150 families have been reported around the world since the first German observation published by Rabe in 1920. Hemorrhagic manifestations vary, ranging from a minimal or moderate bleeding to cataclysmic hemorrhage. Afibrinogenemia may be paradoxically associated with venous and arterial thrombotic complications. These complications could occur spontaneously or after a massive substitution in fibrinogen concentrate or in very rare cases associated with a biological risk factor for thrombosis.

Aims: Interest to complete the hemostasis assessment to identify the associated deficits.

Methods: We report here the case of two brothers known for severe fibrinogen deficiency since childhood. In adulthood, one of the two brothers had severe deep vein thrombosis complicated by pulmonary embolism. A complete thrombosis checkup was undertaken in the patient and other members of his family in search of a biological risk factor for thrombosis.

Results: In addition to severe fibrinogen deficiency with a fibrinogen level < 0.1 g / l in activity and in antigen, the thrombosis assessment revealed resistance to activated protein C found in the afibrinogenemic brothers and the other members of family. This resistance to

TABLE 1 Comparison of fibrinolytic parameters between cases and controls

Fibrinolytic marker	Cases (median)	Controls (median)	Cases (range)	Controls (range)	Cases (SD)	Controls (SD)	p-value
PAI-1 (ng/ml)	0.8548	1.1638	0.3047-4.2088	0.4167-3.8215	0.7670	0.7706	0.032
TAFI (μg/ml)	3.9948	3.157	0.087-33.593	1.2159-4.8325	7.5559	0.6548	0.002
t-PA (ng/ml)	11.21	8.625	4.13-28.99	2.34-15.789	4.9626	3.2262	0.003
α-1 anti-plasmin (μg/ml)	78.92	70.347	6.76-108.95	42-140.95	25.4237	19.4941	0.678
Plasminogen (mg/dl)	2.807	2.207	1.6992-6.1600	0.312-4.1907	0.9734	0.7316	0.678 <0.001

activated protein C corresponds to a mutation of FV Leiden in the heterozygous state.

Conclusions: Congenital afibrinogenemia is a rare, autosomal recessive disorder that results in multiple life-threatening haemorrhagic manifestations. The occurrence of thrombosis remains exceptional. The achievement of a complete hemostasis assessment is required for the diagnosis of rare associated deficits of hemostasis responsible for hemorrhagic and thrombotic syndrome.

PB2191 | "Gene-gene Interaction" between FXIII-A VAL34LEU and PAI-1 - 675 4G/5G Polymorphisms Can Modify the Risk of Pulmonary Embolism in Young VTE Patients

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Background: Risk of pulmonary embolism (PE) development in patients with venous thromboembolism (VTE) could depend on fibrin clot structure and the rate of thrombus lysis. Factor XIII A-subunit (FXIII-A) Val34Leu polymorphism modifies kinetics of fibrin monomer crosslinking and affects stabilization of fibrin clot. Allele -675 4G of plasminogen activator inhibitor type 1 (PAI-1) gene is associated with decrease of fibrinolytic capacity.

Aims: To assess effect of the FXIII-A (Val34Leu) and PAI-1 (-675 4G/5G) polymorphisms on the risk of PE development in young VTE patients.

Methods: We included 250 young patients with VTE (123 men and 127 women, mean age 37.4±10.9 years) in the study. Isolated deep-vein thrombosis was diagnosed in 158 (63.2%) patients ("DVT only" group), whereas the signs of PE (DVT complicated by PE or isolated PE) were detected in 92 (36.8%) persons. Genotyping for the FXIII-A Val34Leu and PAI-1 -675 4G/5G polymorphisms was performed by PCR-RFLP. Odds ratios (OR) and p-values were determined by Fisher's exact test to characterize intergroup differences in distributions of genotypes and their combinations.

Results: Genotypes distributions of the FXIII-A and PAI-1 polymorphisms were similar in patients with isolated DVT and those with PE. Simultaneous presence of the PAI-1 4G/4G and FXIII-A 34 Val/Val variants was more frequently seen in PE patients (60.0% vs. 34.5% in "DVT only" group, OR=2.8; p=0.018). At the same time, presence of the FXIII-A 34Leu variant in patients with PAI-1 4G/4G genotype was associated with decrease of PE risk. The frequency of this combination was higher in "DVT only" group when compared to patients with PE (58.2% vs. 34.2%, respectively, OR=2.7; p=0.028).

Conclusions: Simultaneous presence of PAI-1 4G/4G and FXIII-A 34 Val/Val genotypes is associated with thromboembolic complications in young VTE patients. In turn, presence of FXIII-A 34Leu variant could have protective effect on PE risk in patients with PAI-1 4G/4G genotype.

PB2192 | Homozygous State of Leiden Mutation in Factor V Associated with Other Genetic and Biochemical Risk Factors and Late Onset of Thrombotic Episodes in Five Clinical Cases

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Background: Leiden mutation in Factor V (FVL) is the most common genetic risk factor related to venous thromboembolism (VTE). This genetic variant results in dysfunctional enzyme resistant to the activated protein C prolonging the enzyme activity time. However, clinical significance of this risk factor is variable. The most heterozygous individuals never develop thrombosis, and some have reccurent VTE before the age of 30. Patients with FVL in homozygous state are at greater risk for developing the first VTE at a younger age.

Aims: In our Clinic we have registered 5 cases with homozygous FVL associated with other genetic risk factors but without any clinical evidence of VTE or with a late onset of clinical thrombotic episodes and late detection of genetic risk factors. The aim of this study is to elucidate significance of other genetic risk factors, coagulation status, biochemical parameters and lifestyle to appearance of thrombosis and level of their predictive value.

Methods: Patients were tested for FVL, G20210A variant in the prothrombin gene (FII), C677T variant in MTHFR gene and 4G/5G variant in the PAI-1 gene. Also, we have measured coagulation parameters, protein C, protein S, antithrombin III and LA antibodies.

Results: In three cases homozygous FVL was associated with homozygous 4G/4G in PAI-1 gene, and one of them was heterozygous for G20210A variant in FII, one case was combined with heterozygous MTHFR and 4G/5G in PAI-1 gene, and the latter was homozygous FVL solely.

Conclusions: On the basis of data from the literature, 40% homozygous individuals have had VTE by the age of 33, however in our Clinic 2/5 (40%) of our homozygous FVL patients have experienced thrombosis episodes in their older age, after 50, and 3/5 (60%) homozygous FVL individuals haven't had any episodes of VTE even in their late life stage.

PB2193 | "Gene-gene Interaction" between PAI-1 - 675 4G/5G and TNF-A -308 G/A Polymorphisms Can Modulate the Risk of Pulmonary Embolism in Young Patients with Venous Thromboembolism

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Background: The most common clinical manifestations of venous thromboembolism (VTE) are deep-vein thrombosis (DVT) and pulmonary embolism (PE). Little is known about the factors associated with PE development in VTE patients. Individual genotype-dependent features of hemostatic system could influence the risk of VTE and peculiarities of its clinical presentation.
Aims: To assess effect of the plasminogen activator inhibitor type 1 (PAI-1) -675 4G/5G and tumor necrosis factor alpha (TNF-A) -308G/A gene polymorphisms on the risk of PE development in young VTE patients.
Methods: We included 400 young VTE patients (184 men and 216 women, mean age 33.1±8.3 years) originated from the North-Western region of Russia in the study. Isolated DVT was diagnosed in 245 (61.3%) patients. DVT complicated by PE (DVT+PE) was present in 94 (23.5%), whereas isolated PE - in 61 (15.2%) patients. Genotyping for PAI-1 -675 4G/5G and TNF-A -308 G/A polymorphisms was performed by PCR-RFLP. Odds ratios (OR) and p-values were determined by Fisher's exact test to characterize intergroup differences in distributions of genotypes and their combination.
Results: Genotype frequencies for PAI-1 and TNF-A polymorphisms were not significantly different between the groups of patients with isolated DVT and those with PE. When compared to "isolated DVT" group, simultaneous presence of the PAI-1 4G/4G genotype and TNF-A -308A allele was detected more frequently in patients with DVT+PE (14.9% vs. 4.9%, respectively, OR=3.4, p=0.005) and isolated PE (13.1% vs. 4.9%, respectively, OR=2.9, p=0.037). As result, the frequency of combination of these variants was almost 3-fold increased in patients with the signs of PE (14.2% vs. 4.9% in "isolated DVT" group, OR=3.2; p=0.002).
Conclusions: Simultaneous presence of PAI-1 4G/4G and TNF-A -308A variants is associated with increased PE risk in young VTE patients. Further studies are needed to confirm this finding.

PB2194 | Carrier Frequencies of Antithrombin-, Protein C-, or Protein S-deficient Variants Estimated Using a Public Database and Expression Experiments

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Background: Genetic deficiencies of antithrombin (AT), protein C (PC), and protein S (PS) are risk factors for venous thromboembolism. The prevalences of heterozygous AT, PC, and PS deficiencies are reported to be approximately 0.02-0.2%, 0.2-0.4%, and 0.03-0.5%, respectively. The Exome Aggregation Consortium (ExAC) browser provides a public database displaying reference data information from over 60,000 exomes.
Aims: The present study is aimed to estimate the frequencies of congenital AT, PC, and PS deficiencies in the worldwide population, in combination with the ExAC database and transient expression experiments.
Methods: In the ExAC database, 133, 157, and 221 variants of *SERPINC1* (encoding AT), *PROC* (PC), and *PROS1*(PS), respectively, were registered as missense and putative loss-of-function variants. We first selected 9 *SERPINC1*, 4 *PROC*, and 14 *PROS1* variants with relatively high allele frequencies (≥0.1% and ≥2 counts in all regions or in each region). In addition, we randomly selected 12 of 122 *SERPINC1*, 15 of 146 *PROC*, and 19 of 192 *PROS1* missense variants. In total, 73 variant and wild-type (WT) recombinant proteins were transiently expressed in HEK293 cells. Their expression levels were assessed by ELISA and the anticoagulant activities were measured by chromogenic and clot-based assays.
Results: All the 21 *SERPINC1* variants showed normal or mildly reduced secretion in the culture media, and 9 of them showed reduced activity. Among the 19 *PROC* variants, 7 and 8 showed impaired secretion and reduced activity, respectively. All the 33 *PROS1* variants showed normal or mildly reduced secretion, and 8 of them showed reduced activity. In reference to their allele frequencies in the database, we estimated the frequencies of congenital AT, PC and PS deficiencies are 0.33%, 0.51%, and 0.44%, respectively.
Conclusions: The present study demonstrated that the frequencies of congenital AT, PC and PS deficiencies may be comparable with each other ranging from approximately 0.3-0.5% worldwide.

PB2195 | *De Novo* Variants in Antithrombin Deficiency: Incidence and Heterogeneous Mechanisms

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Background: Genetic variations, generated by different mechanisms, are responsible of diversity but they are also involved in diseases. There is no a systematic analysis of *de novo* variants in antithrombin deficiency, the first and so far, strongest congenital thrombophilia.

TABLE 1 Cases with SERPINC1 de novo variants.DVT:DVT:Deep venous thrombosis;PE:Pulmonary embolism;CT:Cerebral thrombosis;w:weeks

Case	Sample	Age (years)	Thrombosis (age 1st event, years)	Anti-FXa (%)	Variant	Procedence mutated alelle
P1	Proband	54	DVT(–)	52	c.1201C>T;p. His401Tyr	Father
	Mother	–	No	112	No	
	Father	–	No	111	No	
P2	Proband	26	DVT(15)	26	c.335C>T;p. Pro112Leu	Father
	Father	56	No	86	No	
	Mother	53	No	83	No	
P3	Proband	28	DVT&PE(14)	59	c.1141T>C;p. Ser381Pro	Mother
	Father	–	No	101	No	
	Mother	–	No	99	No	
P4	Proband	33	DVT(19)	39	c.1315C>A;p. Pro439Thr	Unknown
	Father	–	No	130	No	
	Mother	63	No	114	No	
P5	Proband	24	DVT,PE&CT(13)	60	Deletion2MB	Father
	Father	63	No	91	No	
	Mother	59	No	101	No	
P6	Proband	54	DVT(36)	50	c.817A>G;p. Lys273Glu	Unknown
	Father	–	No	98	No	
	Mother	–	No	97	No	
P7	Proband	1	DVT(3 w)	30	Deletion19Kb&p. Val30Glu	Mother
	Father	38	No	92	p.Val30Glu	
	Mother	35	No	72	No	

Aims: To identify and characterize *de novo* variants in *SERPINC1* associated with antithrombin deficiency.

Methods: The study included 370 unrelated patients with antithrombin deficiency. *SERPINC1* was studied by sequencing (Sanger, NGS and/or nanopore) and MLPA. Comparative genome hybridization was performed in some cases. Paternity was determined by genotyping 16 Short Tandem Repeats (PowerPlex® 16). Mosaicism was evaluated by deep sequencing (Ion Torrent). The origin of the mutated allele was determined by haplotyping long-range PCRs of the whole *SERPINC1* gene and nanopore sequencing.

Results: After selecting patients with *SERPINC1* defects (N=244) and those with available parental samples (N=145), we identified 7 cases with severe thrombosis and type I deficiency, who had *de novo* mutations. Paternity was proved in all cases. Five cases had SNVs (no one was CpG), and 2 showed gross gene defects: one with a 2Mb deletion (22 genes) and the second with a 19KB deletion affecting exon 1 and the neighboring gene. In both cases nanopore sequencing revealed repetitive elements (LINEs and ALUs) flanking the deletions. Anticoagulant activity and deep sequencing (>6000 reads) of the mutated amplicon supported a germline variant and discarded paternal mosaicism in all cases. Haplotype analysis revealed the origin of the mutated allele in 5 cases.

Conclusions: The rate of *de novo* variants underlying antithrombin deficiency (4.8%) supports their analysis even in cases with no affected parents. Incidence of *de novo* gross gene rearrangements is remarkable (28%) probably because repetitive elements are abundant in this gene. Mosaicism was discarded, and in most cases variations were probably originated during paternal gametogenesis. PI18/00598 (ISCIII y FEDER); and 19873/GERM/15 (Fundación Séneca).

PB2197 | Structural Basis of the Pro-coagulant Phenotype of Prothrombin Variant Arg553Trp (p.Arg596Trp - Padua 2)

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Background: Thrombin (αT) is the final effector protease in the coagulation cascade, converting fibrinogen into fibrin clots and inducing platelets agglutination via PAR-1 cleavage. αT is a Na^+-activated enzyme and is irreversibly inhibited by antithrombin-III (AT-III). Simioni and colleagues have recently identified the mutation p.Arg596Trp in the prothrombin gene (Padua-2) of heterozygous members from two families, with strong pro-coagulant phenotype [Bulato et al., JTH (2016)]. The plasma of these patients showed significant prolongation of clotting time in thrombin generation assays, compared to healthy subjects, and almost complete resistance to AT-III.
Aims: Provide the structural basis for the pro-coagulant phenotype of patients with Padua-2 prothrombin mutation.
Methods: rPre2-PD2 was cloned and expressed in *E. coli* and, after disulfide renaturation and purification, activated with ecarin to yield rαT-PD2; the purity, chemical identity, disulfide bonds topology of rαT-PD2 was established by SDS-PAGE, RP-HPLC and MS; fibrin clotting parameters, the kinetic constants (k_{cat}, K_m) for the cleavage of the chromogenic substrate S2238, release of fibrinopeptides A and B (FpA, FpB), and inhibition by AT-III were determined as described [Pontarollo et al., JBC (2017)].
Results: For S2238 hydrolysis, the specificity constant (k_{cat}/K_m) determined with rαT-PD2 was 6-fold lower than that estimated for rαT. Likewise, rαT-PD2 releases FpA and FpB by 25- and 7-fold much less efficiently, respectively. Fibrin clotting time (t_c) on isolated was estimated as 22.7min for rαT-PD2 and 0.5min for rαT. Notably, rαT-PD2 was inhibited by AT-III (K_a = 2.6±0.2·10^6 M^{-1} min^{-1}) less efficiently than rαT (K_a = 7.3±0.2·10^6 M^{-1} min^{-1}).
Conclusions: Modelling studies indicate that Arg221a-Trp mutation in the Na+-binding loop disrupts the stabilizing salt bridge with Glu146 in the γ-loop, which may collapse onto the active site and part of exosite-I impairing substrates (fibrinogen) and inhibitors (AT-III) binding. This structural model is fully consistent with biochemical data reported above and previous clinical data.

PB2198 | Pulmonary Embolism and Thrombophilia Gene Mutations: Georgian Population Study

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Background: Venous thromboembolism (VTE) is a common and potentially lethal disorder that manifests mainly as deep venous thrombosis (DVT) and pulmonary embolism (PE) and occurs as a consequence of genetic and environmental risk factors. We aimed to assess the prevalence of inherited thrombophilia in patients with PE and in controls without VTE.
Aims: The aim of the study was to evaluate the association of inherited thrombophilia (Factor V Leiden, Prothrombin G20210A and MTHFR C677T gene mutations) and pulmonary embolism (PE) in patients from Georgian population. To estimate whether family history of thrombosis has positive correlation with these mutations in patients with PE.
Methods: 48 (21 female and 27 male) Georgian patients (with isolated PE or combined with other complications - deep or superficial vein thrombosis, myocardial infarction, ischemic stroke, miscarriages) and 100 control group (Georgian population over 50 without VTE) were genotyped by PCR analyses. Statistical analysis was performed on SPSS v. 21 statistical software. The difference was considered to be significant when $p< 0.05$.
Results: Relationships between PE and Prothrombin (10.42% in patients and 1% in control; $\chi2$ (1, N=148)=7.394, p=.014) and MTHFR (12.5% in patients and 1% in control; $\chi2$ (1, N=148)=9.519, p=.005) mutations were significant. Relationship between PE and FVL mutation (6.25% in patients and 1% in control; $\chi2$ (1, N=148)=3.399, p=.1) was weak. The combined double and triple mutations were seen in 4 cases. Family history of thrombosis was positive in 27 (56.25%) patients, negative in 10 (20.83%) patients and undetermined in 11 (22.92%) patients.
Conclusions: Our study reconfirms role of studied mutations (totally 25%) in PE. Opposite to other studies where FVL mutation is prevalent in pathogenesis of PE, our study revealed significant impact of Prothrombin and MTHFR gene mutations, although FVL (4.57%) is more distributed than Prothrombin gene mutation (3.72%) in Georgian population.

PB2199 | Decreased Expression of Annexin A2 Is Associated with Reduced Cell Surface Fibrinolysis and Venous Thromboembolism

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Background: Annexin A2 (A2) is a calcium-regulated, phospholipid-binding protein that complexes with S100A10 at the cell surface to bind plasminogen and tissue plasminogen activator, thereby increasing the catalytic efficiency of plasmin generation. Disruption of the A2 gene in mice leads to impaired clearance of injury-induced arterial thrombi, and autoantibodies to A2 in human antiphospholipid syndrome are associated with venous thromboembolism (VTE).

Aims: Here, we aimed to define the potential role of A2 dysfunction in VTE.

Methods: In an IRB-approved protocol, we studied 87 consenting healthy adult controls (HCs) and 115 subjects with history of VTE. Subjects were classified as having a positive or negative family history (FH) for VTE, and presence or absence of five inherited thrombophilia risk factors (IRFs; factor V Leiden variant, prothrombin G20210A variant, or deficiency of protein C, protein S, or antithrombin).

Results: Plasma-based clot lysis times were significantly longer in 91 VTE subjects versus 73 HCs (37.5 versus 30.7 minutes, *p=0.0001*). Additionally, mean plasmin generation on the surface of peripheral blood mononuclear cells (PBMCs) was reduced in 60 VTE subjects versus 107 HCs (0.33 versus 0.49 RFU/min^2, *p=0.0021*), and also among 24 VTE subjects with FH, but without IRFs (0.246 RFU/min^2, *p=0.0031*). Semi-quantitative western blotting of PBMC lysates revealed reduced mean A2 expression in 41 VTE subjects versus 19 HCs (0.367 vs 0.689 arbitrary units, *p=0.0216*), and most significantly in 11 VTE subjects with FH, but without IRFs (0.187 arbitrary units, *p=0.0056*). Neither quantitative RT-PCR nor *ANXA2* gene sequencing revealed informative abnormalities, suggesting that post-transcriptional mechanisms accounted for loss of A2 expression.

Conclusions: These data suggest for the first time that impaired cell surface fibrinolysis and loss of A2 expression are associated with VTE, particularly in individuals with FH but without an identified inherited thrombophilia. We conclude that reduced expression of A2 may represent a novel risk factor for thrombosis.

PB2200 | Effects of Body Mass Index on the Human Plasma Proteome - Potential Drivers of Obesity-related Cardiovascular Disease?

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Background: Variation in body mass index (BMI) is associated with cardiovascular health outcomes, but the mechanisms leading from BMI to disease risk are still unclear.

Aims: To use observational and genetically-informed methods to (1) explore the relationship between BMI and the plasma proteome and (2) identify potential drivers of obesity-related cardiovascular disease.

Methods: This study used proteomic data measured by SomaLogic from 2,737 healthy adults from the INTERVAL study to explore the effect of self-reported BMI on 3,622 unique plasma proteins using observational and genetically-informed methods. Linear regression models were used, complemented by one-sample Mendelian randomization (MR) analyses using a genetic risk score (GRS) comprised of 654 single nucleotide polymorphisms (SNPs) from a recent genome-wide association study (GWAS) of adult BMI.

Results: Observationally, BMI showed 1,576 protein associations at $p < 1.4\text{x}10^{-5}$: the strongest being leptin and sex hormone binding globulin (SHBG). The GRS was positively associated with BMI but not with reported confounders. There was MR evidence that BMI (per standard deviation (SD) higher) had an effect on eight proteins, including leptin (0.63 SD, 95% CI 0.48-0.79, $p = 1.6\text{x}10^{-15}$) and SHBG (-0.45 SD, 95% CI -0.65 to -0.25, $p = 1.4\text{x}10^{-5}$). There was strong agreement in the direction and magnitude of observational and MR estimates. There was also evidence that proteins that are most strongly altered by BMI were enriched for genes involved in cardiovascular disease.

Conclusions: We here provide evidence for a profound impact of higher adiposity on the human proteome and suggests that such protein alterations could be important mechanistic drivers of obesity-related cardiovascular and metabolic diseases.

POST-THROMBOTIC SYNDROME

PB2201 | Exploring Alternative Approaches of Using the Villalta Scale to Capture the Post-Thrombotic Syndrome: A Sub-analysis of the ATTRACT Trial

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Background: There is a lack of consistency in how the Villalta scale (VS) is used to denote the post-thrombotic syndrome (PTS).

Aims: Within the ATTRACT Trial database, we aimed to compare 8 approaches of using the VS to define PTS, in terms of their ability to discriminate between DVT patients with poorer vs. better venous disease-specific QOL over follow-up.

Methods: At baseline, 1, 6, 12, 18 and 24 months, we assessed the VS in both legs and measured venous disease-specific quality of life (VEINES-QOL). We compared 8 approaches to classify patients as having vs. not having PTS during 24 months follow-up. For each approach, we calculated the proportion of patients that would be classified as having PTS, and compared the fitted average area under the curve (AUC) of VEINES-QOL scores between the 6 and 24 month visits for patients classified with vs. without PTS. Finally,

TABLE 1 Approach, definition, construct captured

Approach	How defined	Construct
1	Ipsi-VS ≥ 5 at least once from 6 to 24 months follow-up (ATTRACT primary outcome)	PTS
2	Ipsi-VS ≥ 5 on two or more consecutive assessments from 6 to 24 months follow-up	PTS
3	Ipsi-VS ≥ 5 on any two or more assessments (not necessarily consecutive) from 6 to 24 months follow-up	PTS
4	Ipsi-VS ≥ 10 at least once from 6 to 24 months follow-up (ATTRACT secondary outcome)	Moderate/severe PTS
5	Ipsi-VS ≥ 10 on two or more consecutive assessments from 6 to 24 months follow-up	Moderate/severe PTS
6	Ipsi-VS ≥ 10 on any two or more consecutive assessments (not necessarily consecutive) from 6 to 24 months follow-up	Moderate/severe PTS
7	Ipsi-VS minus contra-VS ≥ 5 at least once from 6 to 24 months follow-up	PTS, adjusted for contra CVI
8	Ipsi-VS ≥ 5 at least once from 6 to 24 months follow-up, only assessed in patients with VS < 5 in the contralateral leg at baseline	PTS, only assessed in patients without baseline CVI

ipsi ipsilateral; contra contralateral; CVI chronic venous insufficiency.

TABLE 2 Results for each approach

Approach	N (%) classified with PTS	Difference (SE) in average fitted AUC of VEINES-QOL scores *	P value for difference in AUC, PTS vs. No PTS	Comparison of PCDT vs. No PCDT Adjusted# RR (95% CI)
1	328/691 (47%)	-21.2 (1.5)	<.0001	0.96 (0.82 to 1.11)
2	159/691 (23%)	-23.7 (1.7)	<.0001	0.95 (0.72 to 1.26)
3	200/691 (29%)	-22.0 (1.6)	<.0001	0.94 (0.74 to 1.18)
4	144/691 (21%)	-25.5 (1.8)	<.0001	0.73 (0.54 to 0.98)
5	58/691 (8%)	-31.7 (2.6)	<.0001	0.87 (0.52 to 1.45)
6	73/691 (11%)	-31.0 (2.3)	<.0001	0.97 (0.62 to 1.51)
7	189/691 (27%)	-13.6 (1.8)	<.0001	0.86 (0.68 to 1.09)
8	265/607 (44%)	-19.9 (1.5)	<.0001	0.98 (0.82 to 1.18)

* Adjusted for age, sex, BMI, treatment (PCDT vs. No PCDT), extent of DVT and baseline VS; # Adjusted for extent of DVT and center; AUC area under curve; PCDT pharmacomechnical catheter-directed lysis.

we explored if results of the ATTRACT Trial would have differed if approaches 2-8 had been used as the primary outcome instead of approach 1.

Results:

Conclusions: For the outcome any PTS, approach 1, 2, and 3 had similar ΔAUC; thus, approach 1 is preferred due to greater convenience (only 1 positive assessment needed). For moderate/ severe PTS, approach 5 and 6 had similar and greater ΔAUC than approach 4; thus, approach 6 is preferred due to greater convenience (any 2 positive assessments). Attempting to "adjust" the VS for contralateral CVI or restrict the population to patients without baseline CVI (i.e "pure" PTS rather than preexisting CVI) did not improve ΔAUC, hence approach 1 is preferred to 7 or 8. Finally, results of the ATTRACT trial (used approach 1 for any PTS and approach 4 for moderate/severe PTS) were substantively unchanged if reanalysed using the other approaches.

PB2202 | Leukocyte Gene Expression in Post-Thrombotic Syndrome

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Background: Following deep vein thrombosis (DVT), one in three patients experiences chronic signs and symptoms in the affected leg, referred to as post-thrombotic syndrome (PTS). Inadequate thrombus resolution, resulting in vein damage and venous hypertension, is is considered a key player in the onset of PTS. Since thrombus resolution is guided by leukocytes, a better understanding of their role could benefit preventive treatment. This is the first transcriptomics study to address this subject.

Aims: To identify differences in leukocyte gene expression between patients with and without PTS.

Methods: We performed a case-control study on a prospectively followed cohort consisting of 30 patients with PTS (cases), 30 patients with a history of DVT without PTS (controls) and 30 healthy individuals (HI) matched for age, gender and BMI. The medical ethical committee of the MUMC+ approved this study and all participants gave written informed consent. Based on the integrity of RNA isolated from buffy coats, a subpopulation of 20 participants was selected for subsequent directional mRNA sequencing (~30M reads/sample). Reads were aligned to the human transcriptome and analyzed with DESeq2. Differentially expressed genes (DEGs) with a false discovery rate adjusted p-value< 0.1 were considered significant. Pathogenically relevant DEGs were selected for confirmation by quantitative polymerase chain reaction (qPCR).

Results: Our subpopulation of 6 cases, 7 controls and 7 HI had no significant differences in baseline characteristics. We found thirteen DEGs originating solely from the comparison of cases with controls (Table 1). Four DEGs were identified as pathogenically relevant (Figure 1): an angiogenic growth factor (*HDGFL3*), an antigen-recognizing molecule (*HLA-DPA1*), a thrombo-inflammatory modulator (*PPIA*) and an inflammation regulating receptor (*ADIPOR1*). qPCR could not confirm mRNA-sequencing findings in these four DEGs, a common problem in transcriptomic studies.

Conclusions: Our study uncovered differences in leukocyte gene expression which might provide novel biomarkers or therapeutic targets if validated by future research.

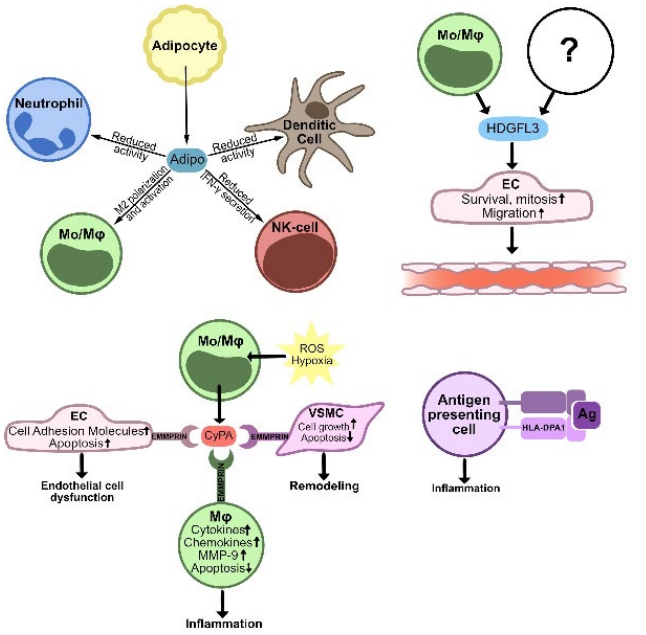

FIGURE 1 Overview of functional pathways for Adipo(nectin) receptor ADIPOR1, HDGFL3, PPIA encoding Cyclophilin (CyPA) and antigen (Ag) recognizing HLA-DPA1

TABLE 1 Identified DEGs (adjusted p-value < 0.1) in a comparison of cases vs. controls

Gene name	Log2 fold change	Adjusted p-value	Gene name	Log2 fold change	Adjusted p-value
HDGFL3	0.61	0.02	RPS14	-0.65	0.09
RPL13A	-0.64	0.09	PPIA	-1.33	0.09
RPL12	-0.75	0.09	KCNQ1OT1	0.92	0.09
AC011462.2	0.80	0.09	ADIPOR1	-1.81	0.09
AC092384.2	0.93	0.09	AL596202.1	1.57	0.09
AF127577.1	-0.71	0.09	RPL41	-0.98	0.09
HLA-DPA1	9.51	0.09			

PB2203 | Measuring Functional Limitations after Venous Thromboembolism: Optimization of the Post-VTE Functional Status (PVFS) Scale

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Background: We recently proposed the post-VTE functional status (PVFS) scale for assessment of patient-relevant functional limitations following an episode of venous thromboembolism (VTE).

Aims: To further develop this PVFS scale.

Methods: Guided by the input of VTE experts and patients, we refined the PVFS scale and its accompanying manual, and attempted to acquire broad consensus on its use. We also quantified the reproducibility of PVFS scale assessment.

Results: A Delphi analysis was performed involving 53 international VTE experts with diverse scientific and clinical backgrounds. In this process, the number of scale grades of the originally proposed PVFS scale was reduced, and descriptions of the grades were improved. After these changes, a consensus was reached on the number/definitions of the grades (Table 1), and method/timing of the scale assessment (Figure 1). The relevance and potential impact of the scale was confirmed in three focus groups totaling 18 VTE patients, who suggested additional changes to the manual, but not to the scale itself. Using the improved manual, the κ-statistics between the PVFS scale self-reporting and its assessment via the structured interview was 0.75 (95%CI 0.58-1.0), and 1.0 (95%CI 0.83-1.0) between independent raters of the recorded interview of 16 focus groups members.

Conclusions: We improved the PVFS scale and demonstrated broad consensus on its relevance, optimal grades, and methods of assessing the PVFS scale among international VTE experts and patients. We improved the PVFS scale and its manual. The interobserver agreement of scale grade assignment was shown to be good-to-excellent.

TABLE 1 Final post-VTE functional status scale as agreed upon by the Delphi panel and patient focus groups

PVFS scale grade	Description
0: No functional limitations	All usual duties/activities at home or at work can be carried out at the same level of intensity. Symptoms, pain and anxiety are absent.
1: Negligible functional limitations	All usual duties/activities at home or at work can be carried out at the same level of intensity, despite some symptoms, pain, or anxiety.
2: Slight functional limitations	Some usual duties/activities at home or at work are carried out at a lower level of intensity or are occasionally avoided due to symptoms, pain, or anxiety.
3: Moderate functional limitations	Usual duties/activities at home or at work have been structurally modified (reduced) due to symptoms, pain, or anxiety.
4: Severe functional limitations	Assistance needed in activities of daily living due to symptoms, pain, or anxiety: nursing care and attention are required.
D: Death	Death occurred before the scheduled assessment.

Full manual for structured interview and patient self-report is available. Providing a reference value (pre-VTE grade) is optional and should refer to the functional status 1 month prior to the VTE diagnosis.

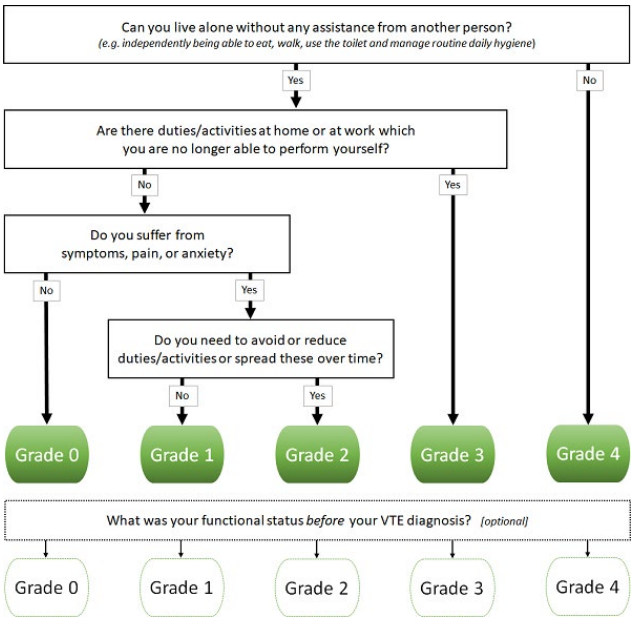

FIGURE 1 Flowchart for patient self-report, one of the two methods for self-reporting of the post-VTE functional status scale

The PVFS scale may become an important outcome measure of functional impairment for quality of patient care and in future VTE trials.

PB2204 | VTE and its complications: A Real World View Daryl Pollock, RN, MN CNS Thrombosis/Haemostasis

D. Pollock

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Background: Venous Thromboembolism (VTE) is an umbrella term which covers Deep Vein Thrombosis (DVT) and Pulmonary embolism (PE). VTE is an issue which is responsible for more deaths than breast ca, road accidents and AIDS combined and each case costs >AUD 10,000 and the costs in NZ are likely to be similar. VTE is preventable in a large number of hospitalised patients. Thromboprophylaxis reduces the risk of DVT/PE by >60% across a broad spectrum of patients, with a very low risk of adverse events.

The prevalence of complications of DVT such as swelling, pain, discolouration and scaling of the affected limb has been discussed as high as 50% and up to 33% of people will have a recurrence of DVT within 10 years.

Aims: The aim of this study is to assess whether there are any clinical indicators that can assist the health professional reduce the chance of complications for people diagnosed with DVT.

Methods: A group of approximately 500 people diagnosed with DVT from 2013 - 2018 in Palmerston North NZ were reviewed to ascertain the incidence of Post thrombotic syndrome (PTS), PE, chronic venous insufficiency and recurrence. This review was undertaken utilising the Clinical Portal (electronic notes system) and clinical notes.

The Villalta Scale was used to grade the severity of PTS and the Vienna Prediction Model was utilised to predict rate of recurrence prior to discontinuing anticoagulation therapy.

Results: Pending.

Conclusions: The results of this study (which is still being evaluated) will determine the incidence of recurrence, whether the risk of recurrence and PTS is different in people with proximal or distal DVT. The number of people left on lifelong anticoagulation for prevention of recurrence will also be discussed.

PB2205 | Tinzaparin Lead-In to Prevent the Post-Thrombotic Syndrome (The TILE Pilot Study): Rationale and Study Protocol

J.P. Galanaud[1]; J. Abdulrehman[2]; A. Delluc[3]; S. Schulman[4]; S. Kahn[5]

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Background: Patients with common femoral or iliac deep-vein thrombosis (DVT) are at very high risk of developing post-thrombotic syndrome (PTS, ≈50%) including moderate to severe PTS (≈28%).

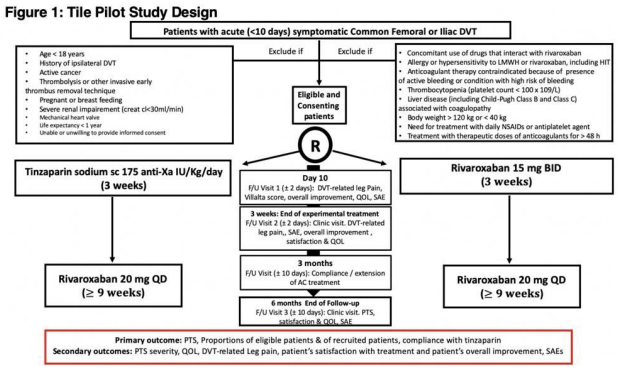

FIGURE 1 The pilot study design

Inflammation during the acute phase of DVT plays an important role in the pathophysiology of PTS. Low molecular weight heparins (LMWHs) have anti-inflammatory properties that may hasten acute symptom resolution after DVT and prevent the development of PTS.

Aims: To determine whether an initial 3-week lead-in course of tinzaparin, a LMWH, followed by rivaroxaban, a direct oral anticoagulant (DOAC), is superior to rivaroxaban alone in resolving acute symptoms and preventing PTS in high-risk DVT patients (symptomatic iliac or common femoral DVT).

Methods: A multicenter open-label assessor-blinded pilot randomized controlled trial (RCT) will be conducted 1) to assess study feasibility; and 2) to estimate the magnitude of difference in effectiveness between tinzaparin followed by rivaroxaban vs. rivaroxaban alone to determine the required sample size for a future large, definitive, multicenter RCT. Pilot study design, eligibility criteria and outcomes are shown in Figure 1. For the pilot, 60 patients will be recruited over 18 months at 4 Canadian centres.

Results: The pilot study will be considered successful if >50% of eligible patients agree to participate, and compliance with tinzaparin is >80%. For the definitive RCT, sample size will be based on the pilot study's results and the primary outcome will be the proportion of patients with PTS at 2 years.

Conclusions: The results of our pilot study will directly inform the feasibility and size of our definitive RCT. Given the health burden, economic burden, and impaired QOL caused by PTS, a non-invasive, safe and time-limited treatment to decrease the risk of PTS and accelerate early symptom resolution is desirable and may be cost-effective and even cost-saving.

PB2206 | The Impact of Successful Ultrasound-Accelerated Catheter-Directed Thrombolysis on the Prevention of Post-Thrombotic Syndrome: A Post-Hoc Analysis of the CAVA-Trial

P. Notten[1,2]; C. Arnoldussen[3,4]; R. Brans[3]; A. deSmet[5]; L. Tick[6]; M. van dePoel[7]; O. Wikkeling[8]; L.-J. Vleming[9]; A. Koster[10]; K.-S. Jie[11]; E. Jacobs[12]; H. Ebben[13]; N. Planken[14]; C. Wittens[15]; H. tenCate[2,16,17]; A.J. tenCate-Hoek[2,16,17]

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Background: The CAVA-trial did not show the anticipated risk reduction for post-thrombotic syndrome (PTS) after early thrombus removal via additional ultrasound-accelerated catheter-directed thrombolysis (UACDT) in patients with acute iliofemoral deep-vein thrombosis (IFDVT). Difficulties in achieving an effective degree of recanalization trough thrombolysis may have influenced these outcomes.

Aims: We aimed to assess whether successful thrombolysis with restored patency did reduce the risk of PTS. In addition, we explored determinants of successful thrombolysis and the impact on health related quality of life (QoL).

Methods: A post-hoc analysis of the CAVA-trial was performed to assess the impact of successful UACDT on the development of PTS. The proportion of PTS at 1 year follow-up was compared between patients with successful thrombolysis and patients that received standard treatment only. In addition, clinical success was determined using the Venous Clinical Severity Score (VCSS) and QoL-measures.

Results: UACDT was initiated in 77 (50.7%) patients, of which 41 (53.2%) procedures were considered successful (inter-rater agreement κ=0.69, 95%CI 0.47-0.83). A total of 75 patients received standard treatment only (49.3%). PTS developed in 48 (41.4%) patients: 15 (36.6%) in the successful thrombolysis group versus 33 (44.0%) in the standard treatment group (p=0.44). Successful thrombolysis was associated with lower VCSS (3.50±2.57 versus 4.82±2.74, p=0.02) and higher EQ5D-scores (40.2±36.4 versus 23.4±34.4, p=0.01). Compared to unsuccessful thrombolysis, successful thrombolysis was associated with a shorter symptom duration at inclusion (p=0.05), and higher rates of performed adjunctive procedures (p< 0.001), and stent placement (p< 0.001).

Conclusions: Successful thrombolysis was not associated with a reduced proportion of PTS 1 year after acute IFDVT compared to patients receiving standard treatment alone. There was however a significant reduction in symptom severity and improvement of generic QoL. Better patient selection and optimization of treatment protocols is needed to achieve the full potential of UACDT for the prevention of PTS.

PB2207 | Hokusai Post-DVT Study: A Follow-up Study on Long-term Outcomes of Deep Vein Thrombosis in Patients Treated with Edoxaban vs Warfarin

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Background: Post-thrombotic syndrome (PTS) is a long-term complication of deep vein thrombosis (DVT) and it is associated with a decreased quality of life (QoL). Unfortunately, therapeutic options are limited and therefore, prevention of PTS is very important. Few studies have indicated that treatment of DVT with direct oral anticoagulant (DOAC) may be associated with a lower risk for PTS development compared to vitamin K antagonist (VKA) treatment. To our knowledge, no studies have been conducted in which edoxaban was compared to VKA. We hypothesize that edoxaban treatment compared to VKA treatment (both preceded by heparin) for DVT leads to a lower incidence of PTS along with an improved QoL.

Aims: To assess the cumulative incidence of PTS after acute DVT and the long-term quality of life following DVT in patients treated with edoxaban in comparison to patients treated with warfarin.

Methods: The present study is a follow up of the original HOKUSAI VTE trial (NCT00986154). We are collecting data from previously enrolled patients at least 5 years after the index DVT. Primary outcome is the incidence of PTS, defined by the Villalta score. The secondary outcome is QoL, assessed by the validated generic (SF-36) and DVT-specific (VEINES-QoL) questionnaires. We will compare these outcomes in both treatment arms. Ethical approval was granted in all participating centers and patients signed informed consent.

Results: Up to now, 22 of the 76 (29%) of the centers that were invited managed to participate and we have currently included 277 patients. The analyses will be performed after completion of data collection (May 2020).

Conclusions: So far, we have included 277 patients. Inclusions will continue until May 2020 and at the ISTH 2020 be able to present our results on PTS incidence and QoL after DVT in patients treated with edoxaban compared to patients treated with warfarin.

PB2208 | Recanalization of Venous Thrombosis by Robotic Assisted High Frequency Pulsed Cavitational Ultrasound Therapy: 2 Weeks Follow-up in a Swine Model

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Background: Post thrombotic syndrome is a frequent complication of deep venous thrombosis and is associated with high morbidity and hospitalization. Pulsed cavitational ultrasound therapy (PCUT) emerged recently as a new technique to destroy remotely soft tissue. We demonstrated that PCUT was able to recanalize non-invasively *in vitro* model of acute venous thrombosis.

Aims: We aim to test the safety and efficacy of venous recanalization by noninvasive PCUT in in *vivo* swine model of acute venous thrombosis.

Methods: All the experiments were performed on White large X Landrace swine. We induced an acute femoral deep venous thrombosis by using an endovascular approach. A 3 cm length occlusive thrombosis was obtained after 2 hours. Swine were divided in three groups: one with PCUT without follow-up (n=11), one with PCUT and follow-up at 14 days (n=8), and a control group also followed for 14 days (n=8). All swine received curative anticoagulation. To achieve PCUT, a 2.25 MHz transducer centered by a linear probe (SL10-2) was used and cavitation was obtained in the center of the vein. A robotic arm was used to automatically move the transducer along the thrombus. Effectiveness was evaluated by echo-Doppler and phlebography. Safety was assessed by Doppler ultrasound of insonified area and by histological analysis.

Results: Among the 27 swine, we obtained 25 occlusive venous thromboses and 2 partial. The median length of the thrombus was 26 +/- 4.4 mm. Acutely, thrombosis recanalization was systematically obtained among the 19 swine with PCUT with median treatment duration of 33 min. After a 14-day follow-up, 75% of recanalisation remains permeable vs 0% of vein permeable in the no therapy group. No embolism or pulmonary infarction was observed in all pigs.

Conclusions: We have demonstrated in vivo the safety and the efficacy of PCUT for non-invasive venous recanalization, persistent after 2 weeks.

PB2209 | Study of Plasma miRNAs and Microparticle miRNAs in Severe Forms of Pulmonary Hypertension

J. Oto[1]; O. Tura-Ceide[2]; V. Sánchez-López[3]; J. Osorio[2]; C. Bonjoch[2]; D. Hervás[4]; E. Arellano[5]; P. Medina[1]; I. Blanco[2]; V.I. Peinado[2]; R. Otero[6]; J.A. Barberà[2]; *J Oto and O Tura-Ceide have contributed equally to this work.

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Background: Pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH) are serious forms of precapillary pulmonary hypertension (PH) with reduced prognosis, despite recent advances in their treatment. Epigenetic mechanisms might be involved in the pathogenesis of these diseases.

Aims: To study the epigenetic mechanisms in severe precapillary PH through the identification of dysregulated microRNAs (miRs) contained in circulating plasma microparticles (MPs) and their differential expression with respect to paired total plasma.

Methods: Thirty patients with PH and 10 healthy subjects were prospectively recruited. Patients with PH were classified in: PAH, surgical CTEPH and non-surgical CTEPH (n=10/each). Plasma samples were collected and MPs were isolated from all subjects. Total RNA was isolated

from both plasma and MPs with the *miRNeasy Mini kit* (Qiagen) and the expression of 179 miRs was quantified using the Human Serum/Plasma Focus, miRCURY LNA miRNA Focus PCR *V5* (Qiagen). Expression levels were normalized with the endogenous and stable miR-425-5p selected using *BestRef*. Statistical analysis of differentially expressed miRs (in plasma and MPs) among all groups was performed using R (v3.5.0).

Results: Fourteen miRs (miR-301a-3p, miR-339-5p, miR-652-3p, miR-331-3p, miR-484, miR-18a-5p, miR-127-3p, miR-133a-3p, miR-629-5p, miR-361-5p, miR-200c-3p, miR-144-5p, miR-34a-5p and miR-10b-5p) contained in MPs and 4 plasma miRs (miR-133a-3p, miR-28-5p, miR-26a-5p and miR103a-3p) were differentially expressed among the different groups. Among them, miR-133a-3p was found to be dysregulated in both MPs and plasma.

Conclusions: Patients with precapillary PH show a differentiated profile of miRs in both plasma and circulating MPs. miR-133a-3p might play a prominent role in PH as it is downregulated in both plasma and MPs in patients with PAH or CTEPH. Funding sources: Instituto de Salud Carlos III (PI15/01085, PI15/00582, CPII15/00002 and CP17/00114), SETH and SEPAR 164/2016.

PB2210 | Post-Thrombotic Syndrome in Patients with Venous Thromboembolism Treated with Dabigatran or Warfarin - a Long-Term Cross-Sectional Follow-up of RE-COVER Study Patients

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Background: Studies suggest that the direct factor Xa inhibitor rivaroxaban reduces the risk of post-thrombotic syndrome (PTS) 2-5 years after deep vein thrombosis (DVT) compared to warfarin, but this has not been evaluated for oral direct thrombin inhibitors.

TABLE 1 Baseline characteristics of patients in the original RE-COVER studies

Variable	Dabigatran N=166	Warfarin N=183	P-value Chi-square or t-test
Study, n(%)			0.5
RE-COVER 1	88 (53.0)	90 (49.2)	
RE-COVER 2	78 (47.0)	93 (50.8)	
Age, index, years, mean (SD)	52.0 (13.5)	53.3 (13.6)	0.8
BMI, index, kg/m^2, mean (SD)	29.1 (6.4)	30.1 (6.1)	0.6
Female sex, n (%)	63 (38.0)	71 (38.8)	0.9
Race, white, n (%)	162 (97.6)	173 (94.5)	0.2
Type of index event, n (%)			0.9
DVT only	107 (64.5)	113 (61.7)	
PE only	43 (25.9)	51 (27.9)	
DVT and PE	16 (9.6)	19 (10.4)	
Cancer at baseline, n (%)	6 (3.6)	7 (3.8)	0.9
Country, n(%)			1.0
Canada	137 (83.7)	150 (82.0)	
Norway	13 (7.8)	16 (8.7)	
Sweden	16 (9.6)	17 (9.3)	

TABLE 2 Post-thrombotic syndrome and health-related quality of life after venous thromboembolism

Variable		N	Dabigatran	Warfarin	p-value Chi-square or t-test
Time from index event to follow-up, years, mean (SD)		**348**	**8.8 (1.4)**	**8.6 (1.5)**	**0.7**
DVT (+/- PE)	**PTS, n (%)**	**253**	**77 (63.1)**	**78 (59.5)**	**0.6**
DVT (+/- PE)	EQ VAS, mean (SD)	243	76.6 (18.1)	75.9 (19.1)	0.9
PE only	PTS, n (%)	90	20 (48.8)	20 (40.8)	0.4
PE only	EQ VAS, mean (SD)	92	78.3 (15.8)	74.9 (14.7)	0.5

Aims: To compare the long-term prevalence of PTS and health-related quality of life (HRQoL) in acute DVT and pulmonary embolism (PE) patients randomized to treatment with dabigatran or warfarin.

Methods: In the RE-COVER studies, patients with DVT and/or PE were randomized to 6 months of dabigatran or warfarin. We did a long-term cross-sectional follow-up study in patients from Canada, Sweden, and Norway. The primary outcome was PTS assessed by the patient-reported Villalta scale (PRV). PTS was defined as PRV ≥ 5 in the leg with DVT or the leg with the highest PRV in patients with PE only. HRQoL was assessed with EQ-5D. The study was approved by the research ethics committees and informed consent was obtained from all patients.

Results: A total of 349 patients were included between December 2015 and November 2018, of which 166 were treated with dabigatran and 183 with warfarin. Baseline variables were balanced between treatment groups (Table 1). Mean time from index event to follow-up was 8.7 (SD 1.4) years. 255 patients had DVT (+/- PE) as index event and 94 had PE only. Two DVT patients and 4 with PE were excluded due to missing PRV. PTS was diagnosed in 155/253 (61.3%) patients with DVT and in 40/90 (44.4%) patients with PE only. The prevalence of PTS and HRQoL scores did not differ between treatment groups in patients with DVT or patients with PE only (Table 2).

Conclusions: In this long-term cross-sectional study, the prevalence of PTS and HRQoL were similar in patients treated with dabigatran and warfarin.

PB2211 | Effectiveness and Safety of Pulmonary Rehabilitation to Reduce Functional Impairment Based on the Post-PE Syndrome

G.J.A.M. Boon[1]; S.M.J. Janssen[2]; S. Barco[3,4]; H.J. Bogaard[5]; W. Ghanima[6]; L.J.M. Kroft[7]; L.J. Meijboom[8]; M.K. Ninaber[9]; E.J. Nossent[10]; P. Symersky[11]; H.W. Vliegen[12]; A. Vonk Noordegraaf[10]; M.V. Huisman[1]; B. Siegerink[13,14]; J.J. Abbink[2]; F.A. Klok[1]

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Background: Half of patients report persistent dyspnea and functional impairment months to years after an episode of acute PE. Within this post-PE syndrome, the most severe cause is chronic thromboembolic pulmonary hypertension (CTEPH), which can be treated by surgery, angioplasty or pharmacotherapy. Most patients however have chronic thromboembolic pulmonary disease or are deconditioned, for which no optimal treatment strategy is currently available.

Aims: To assess the effectiveness and safety of a PE-specific rehabilitation program aimed at reducing post-PE syndrome based functional impairment.

Methods: Consecutive patients with the post-PE syndrome were referred to a specialist rehabilitation center for a 12-week personalized rehabilitation program, after CTEPH was ruled out. Disease-specific quality of life (PEmb-QoL) and maximal workload on ergometry were measured prospectively and the recently developed Post-VTE

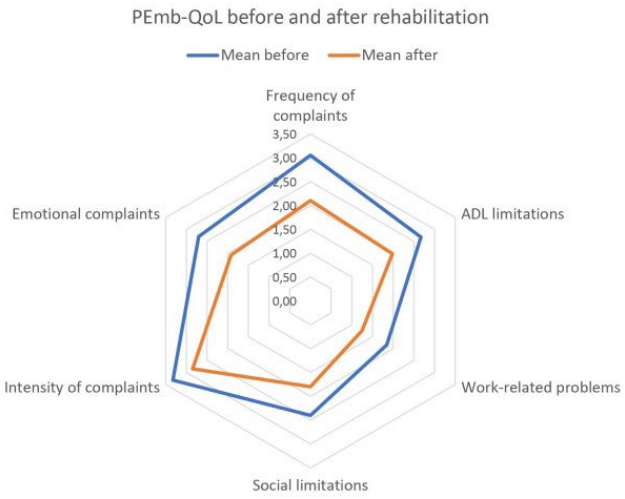
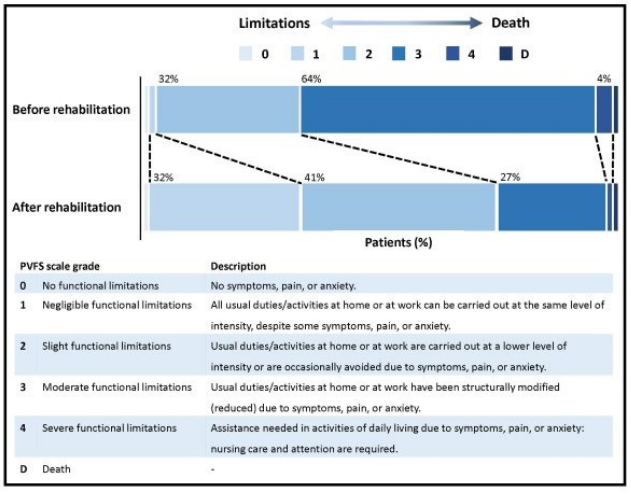


FIGURE 1 Radar chart representing the change in all dimensions of the PEmb-Qol questionnaire before and after the rehabilitation program; lower score



PVFS scale grade		Description
0	No functional limitations	No symptoms, pain, or anxiety.
1	Negligible functional limitations	All usual duties/activities at home or at work can be carried out at the same level of intensity, despite some symptoms, pain, or anxiety.
2	Slight functional limitations	Usual duties/activities at home or at work are carried out at a lower level of intensity or are occasionally avoided due to symptoms, pain, or anxiety.
3	Moderate functional limitations	Usual duties/activities at home or at work have been structurally modified (reduced) due to symptoms, pain, or anxiety.
4	Severe functional limitations	Assistance needed in activities of daily living due to symptoms, pain, or anxiety: nursing care and attention are required.
D	Death	-

FIGURE 2 Assignments of PVFS scale grades before and after completing the 12-week pulmonary rehabilitation program

Functional Status (PVFS) Scale *(Klok et al. Thromb Res 2019)* was assessed post-hoc prior to start and at the end of the rehabilitation program.

Results: 22 patients started rehabilitation: 13 were female (59%) and mean age was 52 years (SD13). Median time since last PE diagnosis was 6.3 months. Median achieved maximal workload during first training was 56% of predicted (15-82%), and improved to 65% (40-165%) during final training (p< 0.003). PEmb-Qol improved after rehabilitation: mean difference was 4.1 points (95%CI 2.3 to 6.0; Figure 1). Rehabilitation resulted in significant improvement on the PVFS scale (p< 0.001) with a number needed to treat of 4 for one patient to improve in functional status (Figure 2). One patient was diagnosed with atrial fibrillation; no other complications occurred.

Conclusions: Patients with post-PE syndrome who completed the PE-specific pulmonary rehabilitation program improved in terms of maximum workload, quality of life and functional status. Our data support offering pulmonary rehabilitation to this patient group, but randomized studies are needed to validate our findings.

PB2212 | Endovascular Management with Iliofemoral Stenting in a Patient with Chronic Venous Occlusive Disease and Antiphospholipid Antibodies

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Background: Iliac vein stenosis is responsible of recurrent deep vein thrombosis (DVT) and of the development of post-thrombotic syndrome (PTS). Endovenous angioplasty, combined with stenting, is becoming popular among vascular surgeons as a safe, effective and minimally invasive technique. Reported patency from recent studies is 90% to 100% for non-thrombotic disease and 74% to 89% for post-thrombotic disease at 3 to 5 years. Although non-thrombotic iliac vein lesions seem not to require postoperative anticoagulation, the question of postoperative therapy for chronic thrombotic stenosis is not resolved.

Aims: We describe the case of a patients with antiphospholipid antibodies (APLA) who had recurrent episodes of deep vein thrombosis and post-thrombotic syndrome of the right lower limb lasting 17 years, despite adequate anticoagulant treatment. Just after delivery, in 2001 patient had a DVT of the right leg. Initially treated with LMWH, she was then treated with warfarin, acenocoumarol and fondaparinux 7.5 mg/day, and continued to recur. In 2014 she started rivaroxaban. After three months she had pulmonary embolism, treated with fondaparinux. Patient recovered, and in february 2017 she was back to warfarin. Three months later, she had a third recurrent thrombosis. Since then she was on fondaparinux, 10 mg/daily. Her leg was swollen and painful.

Fig. 1 – Before the procedure

FIGURE 1

Methods: After reviewing the literature, in 2018 she was submitted to iliofemoral IntraVenous UltraSound (IVUS) assisted procedure. Two stents were inserted (Veniti Vici Venous Stent System, Boston Scientific).

Results: Immediately after the procedure, the leg returned to its regular size and pain disappeared. The patient is still well and under fondaparinux 10 mg/daily treatment.

Conclusions: This procedure requires multidisciplinary expertise when applied to patients who have had recurrent venous thrombosis associated with thrombophilia, either inherited or acquired, in order to optimize quality and length of anticoagulant treatment to avoid re-thrombosis. Controlled randomized clinical studies are needed.

FIGURE 2

PB2213 | Results of a Dedicated Diagnostic Work-up of Patients with Post-PE Syndrome

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Background: After a diagnosis of acute pulmonary embolism (PE), up to 50% of patients report persistent dyspnea and functional limitations. This post-PE syndrome comprises heterogeneous conditions, including chronic thromboembolic pulmonary hypertension (CTEPH) and chronic thromboembolic vascular disease (CTED). Previous studies have suggested that deconditioning (relative unfitness) plays an important role too.

Aims: To evaluate causes of persistent dyspnea in consecutive PE patients referred to the dedicated 'post-PE syndrome outpatient clinic' of the Leiden University Medical Center between 2006 and 2019.

Methods: In this post-hoc study, we evaluated the diagnostic findings in consecutive patients with moderate to severe dyspnea (modified Borg scale ≥3 at rest) after acute PE, who underwent a standardized diagnostic work-up including echocardiography, CT pulmonary angiogram (CTPA), pulmonary function tests and cardiopulmonary exercise testing. In patients with suspected CTEPH, ventilation/perfusion scintigraphy and right heart catheterization were performed.

Results: 56 patients were referred to the 'post-PE syndrome clinic' after adequate anticoagulant treatment; mean time from diagnosis till referral was 39 months (2-476). Ten patients were diagnosed with CTEPH (18%) and CTED was established in 13 patients (23%). Alternative explanations for persistent complaints were found in three patients: two with interstitial lung disease and one with anemia. The 30 remaining patients were found to be deconditioned and/or displayed breathing dysregulation as main explanation for their dyspnea, without having persistent perfusion defects. This was

TABLE 1 Baseline characteristics of the patients with post-PE syndrome subjected to the standardized diagnostic work-up

	All PE patients (n=56)
Age at PE event (mean ±SD)	54 (14)
Male sex (n, %)	22 (39%)
BMI (mean ±SD)	29 (6.3)
Located in main pulmonary artery	14 (25%)
Unprovoked PE *(n, %)*	29 (52%)
Previous VTE (n, %)	15 (27%)
Pre-existing comorbidity (n, %)	
COPD / asthma	10 (18%)
Diabetes mellitus	3 (5.4%)
Coronary artery disease	2 (3.6%)
Heart failure	2 (3.6%)
Active malignancy	1 (1.8%)
Smoking status (n, %)	
Never	30 (54%)
Quit smoking	19 (34%)
	Pack years (mean ±SD): 16 (11)
Still smoking	7 (13%)
	Pack years (mean ±SD): 35 (18)
Type of anticoagulant treatment (n, %)	
DOAC	27 (48%)
VKA	26 (46%)
LMWH	2 (3.6%)
Indefinite anticoagulant treatment *(n, %)*	34 (61%)

Abbreviations: PE, pulmonary embolism; SD, standard deviation; BMI, body mass index; VTE, venous thromboembolism; COPD, chronic obstructive pulmonary disease; LMWH, low-molecular-weight heparin; VKA, vitamin K antagonist; DOAC, direct oral anticoagulant

accompanied by suboptimal treated pre-existing conditions, such as asthma, COPD or sleep apnea in 6/30 patients.

Conclusions: In our cohort of PE patients with persistent moderate to severe dyspnea, nearly half (41%) had either CTEPH or CTED. The other patients had deconditioning and/or breathing dysregulation which may be effectively targeted by multidisciplinary cardiopulmonary rehabilitation since no other treatment is currently available.

PB2214 | Assessment of Radiological Signs of Chronic Thromboembolic Pulmonary Hypertension on CTPAs Performed for Acute Pulmonary Embolism Leads to Earlier Diagnosis of CTEPH

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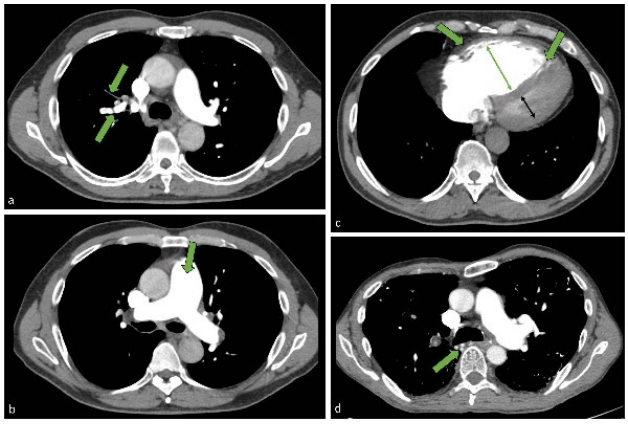
Background: The current diagnostic delay of chronic thromboembolic pulmonary hypertension (CTEPH) negatively affects patients' prognosis. It was shown that expert reading, in retrospect, often reveals radiological signs of CTEPH at the time of acute pulmonary embolism (PE) presentation preceding a CTEPH diagnosis, suggesting already existing CTEPH. Careful computed tomography pulmonary angiography (CTPA) reading may thus help to achieve earlier CTEPH diagnosis.



a) intravascular web and arterial retraction; b) dilated main pulmonary artery; c) flattening of the interventricular septum, RV hypertrophy and RV/LV diameter ratio >1.0; d) dilated bronchial arteries.

FIGURE 1 CTPA image showing the 6 radiological predictors of CTEPH, in addition to RV/LV ratio of >1.0

Aims: To evaluate

1). whether concomitant signs of CTEPH on routine CTPA for suspected PE are reported;
2). whether senior radiology residents instructed to look for CTEPH achieve comparable predictive accuracy to expert radiologists.

Methods: Reports of CTPAs demonstrating acute PE in 50 patients whom later had developed CTEPH were screened by two independent reviewers for documented signs of CTEPH. Furthermore, these CTPA images and those of 50 PE patients who had not developed CTEPH were assessed for the presence/absence of CTEPH by two independent, blinded senior radiology residents ('non-expert radiologists'). This was compared to the consensus reading of 3 expert chest radiologists.

Results: CTEPH or chronic thrombi were mentioned in the original CTPA reports from 14/50 CTEPH patients (28%), while it was recognized by the experts in 36/50 (72%). The two non-expert radiologists had little agreement on their overall judgment of the presence/absence of CTEPH (κ-statistic 0.38), but correctly identified 40 (80%) and 44 (88%) cases. Using a standardized definition of CTEPH (≥3 predefined radiological signs present (Figure 1)), moderate-to-good

TABLE 1 Results of the assessment of radiological signs of CTEPH in controls and cases by two non-expert radiologists, compared to the experts

	Non-expert radiologist 1	Non-expert radiologist 2	Consensus reading by 3 expert radiologists
Overall judgment on the presence or absence of CTEPH			
Sensitivity	80% (95%CI 66-90)	78% (95%CI 64-88)	72% (95%CI 58-84)
Specificity	78% (95%CI 64-88)	56% (95%CI 41-70)	94% (95%CI 83-99)
Presence of ≥3 of 6 predefined radiological predictors of CTEPH			
Sensitivity	40% (95%CI 26-55)	78% (95%CI 64-88)	70% (95%CI 55-82)
Specificity	98% (95%CI 89-99.9)	90% (95%CI 78-97)	96% (95%CI 86-99.5)

agreement was reached between both non-expert radiologists and the experts' consensus (k-statistics 0.46 and 0.61) at slightly lower sensitivities and comparable specificities (Table 1).

Conclusions: Concomitant signs of CTEPH were poorly documented in daily practice, while the majority of CTEPH patients was identified by senior radiology residents after a simple instruction. These findings underline the feasibility of achieving earlier CTEPH diagnosis by more explicit CTPA reading, which may ultimately affect prognosis.

PB2215 | Prevention of Post-thrombotic Syndrome with Elastic Compressive Stockings

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Background: Post thrombotic syndrome (PTS) is a frequent chronic and long-term complication on the deep vein thrombosis (DVT), which brings a higher risk for vein ulceration, the last and the most difficult presentation of the PTS.

Aims: To evaluate the efficiency of the compressional therapy with graduated elastic compression stockings (ECSs), with the patients first or repeated symptomatic DVT.

Methods: 52 patients were diagnosed and treated from DVT in the outpatients department in ITM. All the patients were protected by wearing ECSs class 1 or 2 30 months after DVT.

Results: 52 patients were analysed (38 female/14 male) with symptoms connected with PTS and wearing ECSs. 15 (5 male) of them had symptoms connected with PTS, (11 (3 male) were wearing the ECSs occasionally), six (two male) had brown-reddish coloring on the skin, and 31 had no symptoms connected with PTS. Vein ulceration has not been noticed.

Conclusions: Despite the progress in treatment of DVT in the last decades the incidence of PTS is not decreased. There is no golden standard in diagnosis, because the PTS is a syndrome. The daily usage of ECSs can significantly prevent worsening of the established PTS. ECSs as a long-term, unique and the most efficient form of prevention of PTS, now it is contested for its preventing characteristics, but it is still respected for its role in decreasing the PTS symptomatology.

I strongly recommend ECSs (for preventing the chronic complications of PTS, not for treatment of the symptoms - Grade 2B, ACCP, 10th Edition), but in the future we should focus on identification of patients with DVT and high risk for PTS to strictly detect the efficiency of ECSs in order to prevent or treat PTS, with a personalized treatment and of course with total collaboration from the patients.

PB2216 | Patency and Clinical Outcomes of Venous Recanalization in the Treatment of Postthrombotic Syndrome

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Background: Postthrombotic syndrome (PTS) is a chronic but preventable complication after deep vein thrombosis (DVT). It can seriously affect patients quality of life and has a major impact on health care costs. PTS is a consequence of inadequate venous circulation due to chronic venous obstruction, valvular insufficiency and increased exposure to inflammatory mediators. Recent technical innovations now make it possible to carry out recanalization of acute venous obstructions with percutaneous transluminal angioplasty and stenting and more recently but less studied in chronic obstructions

Aims: To assess immediate and at 6 months efficacy and safety of recanalization of chronic venous obstructions.

Methods: All consecutive patients treated by recanalization of a chronic venous obstruction with PTS between 01/01/2015 and 01/12/2019 in our center were included and files retrospectively reviewed. Results are mean±SD.

Results: There was 63 (71%) women among the 89 patients included. Age was 43±14.1 years and body mass index 26±2.5 kg/m². All patients had had DVT at least 3 months previously and 68 (76%) still presented iliofemoral occlusive sequelae, despite well-conducted anticoagulation. Villalta score was 9±4.1, and 8 (9%) had venous ulcer.

Recanalization succeeded in 88 patients and 84 received at least 1 stent per procedure. 64 (73%) patients received direct oral anticoagulant and 24 VKA after recanalization. On top 78 patients received also anti-platelet therapy.

48 patients had the 6 months follow-up. Primary patency rate was 85%, Villalta score was 3.2±3.8. Ulcer healed in 1 patient, and reduced in size in 2 patients.

We retrieved 3 minor bleedings immediately after recanalization, but no major bleeding and no death at 1 and 6 months.

Conclusions: Recanalization of chronic venous obstruction appears to be an interesting therapeutic option in PTS leading to symptoms reduction without any major complication in a single-center large cohort.

PB2217 | Quality of Life (QOL) Assessment Using VEINES/QOL Score for Deep Vein Thrombosis (DVT) Patients in Uruguay

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Background: Post-thrombotic syndrome (PTS) develops approximately 20-60% of patients after DVT and affects the overall QOL negatively, reducing working time because physical limitations and emotional stress due to fear of recurrence. DVT associated with hospitalization was the leading cause of disability-adjusted-life-years (DALYs) lost in low and middle income countries.

Aims: To evaluate QOL of patients with DVT treated in Hemostasis and Thrombosis Unit (UHYT), in Hospital de Clinicas (HC), Uruguay.

Methods: We performed a transversal cohort study in patients attending UHYT, between 2019 -2020. We applied VEINES score surveys in patients over 18 years and took epidemiologic data from medical history. Individual consent and approval from the ethics committee was taken.

Results: 60 patients with DVT were surveyed. Median age: 58 years. Men vs. women, 0.9 vs.1.

Thrombotic events: 73% DVT alone, 27% pulmonary embolism (PE) associated. DVT: 60% left limb, 40% right limb. Recurrence was seen in 45%. Warfarin was used in 60% of cases. 73% developed PTS. Symptoms are shown in figure 1.

Patients with PTS showed higher VEINES/QOL scores. We found an association between women and higher VEINES/QOL scores and in working patients (table 1). Symptoms got worse during activity. Social limitations were both sitting and standing activities. Localization of DVT, anticoagulant therapy, and recurrence did not show association with a worse QOL. 95% of patients who changed anticoagulation to DOACs showed an improvement in their QOL.

Conclusions: The incidence of PTS in our population was higher than reported in the international bibliography. As we expected, PTS has a negative impact on the QOL of patients, showing the need to empower patients in the prevention of the primary event of DVT, focusing mainly on thromboprophylaxis with worldwide awareness campaigns.

TABLE 1 Results

VEINES/QOL	Media	SD	p value
Women	11.9	193.7	0.003
Men	54.5	8.2	
PTS	21.3	168.5	0.001
no PTS	57	10.6	
	PTS	No PTS	
Working	17	2	0.049
Unemployed	24	13	

PB2218 | Quality of Life Outcomes after Iliac Vein Stenting for Chronic Deep Venous Obstruction: A Tertiary Centre Experience Using the VEINES-QoL/Sym

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Background: Post-thrombotic syndrome is a debilitating illness with significant impact on quality of life. Iliac vein stenting to treat deep venous obstruction in post-thrombotic syndrome is growing in popularity.

Aims:

- To measure change in venous disease-specific quality of life after iliac vein stenting for chronic deep venous obstruction.
- To identify factors associated with improvement post-stenting.

Methods: We performed a retrospective analysis of VEINES-QoL/Sym questionnaires completed at baseline and follow-up after iliac vein stenting (6-36 months). Item responses were re-coded using the intrinsic method to calculate a score out of 100. Additional patient data were obtained from electronic records.

Results: We identified 188 patients who had completed one or more VEINES-QoL/Sym at baseline or follow up (6- 12- 24- 36 months). Median follow-up was 12 months, 127 (68%) were female and 149 (79%) were treated for post thrombotic disease. Sixty patients (31%) required re-intervention and 6 (3%) had occluded stents that could not be recanalized.

Mean VEINES-QoL/Sym score at baseline was 36.75 [32.37-41.12]. Significant improvement was observed post-operatively and sustained at 6-,12-,24- and 36-month follow up (61.47 [56.64-66.31], 59.03 [53.69-64.37], 57.35, [51.13-63.58] 59.36 [50.55-68.16] p=< 0.05). Median Villalta score decreased from 14 at baseline to 8.5 at 6 months, 7.5 at 12 months, 7 at 24 months and 6 at 36 months follow-up (p=< 0.05).

A subgroup analysis of patients with patent stents found that those with ≤ 35% in-stent stenosis on duplex ultrasound at follow-up had improved VEINES-QoL/Sym scores compared to patients with >35% stenosis (62.04 vs 51.51, p=< 0.05).

Conclusions: Venous-disease specific quality of life improved significantly after deep venous stenting, and this was sustained at 6- 12 -24- and 36 months follow-up.

THROMBOPHILIA

PB2220 | Are We Choosing Wisely? Heritable Thrombophilia (HT) Testing in a District General Hospital in the UK

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Background: There are no updated guidelines on HT testing. Interpretation and clinical relevance of results pose difficulty for clinicians who frequently seek advice from haematologists who in turn consult the British Society of Haematology guidelines on HT from 2010.

Positive results can cause anxiety for patients and their families. Further testing is frequently required followed by a review increasing burden on healthcare overall in an area where clinical evidence is lacking.

In our laboratory, HT test kit comprises protein C, S, AT deficiency and APC resistance at a cost of £59.13.

Aims: Identification of categories prompting an HT screen.

Impact on management.

Cost.

Methods: All HT requests at Peterborough City Hospital from 1.1.2018 to 31.12.2018 were reviewed for indication, age, effect on management and cost.

Results: We received 424 requests. 418 were analysed. 256 (61.2%) were not indicated.

The remaining were for "soft indications"; 42 (10%) for unprovoked VTE in patients under 50, 38 (9%) for miscarriages and early pregnancy loss, 38 (9%) in patients with family members with thrombosis, one arterial thrombosis under 50, and one had no clinical details. 28 tests came back positive. 3 patients with miscarriages or early pregnancy loss tested positive for FV Leiden which impacted on management (low molecular weight heparin would be prescribed).

Two unprovoked VTE patients with protein S deficiency and AT deficiency received lifelong anticoagulation based on the results. In the latter case, first degree relatives were tested.

The remaining 23 abnormal results did not affect management.

Conclusions: 98.8% of tests had no impact on management. Total cost was £24,716.34.

Inappropriate tests cost £15,137.28. Tests in the "soft indications" category cost £9519.93.

We plan to screen all requests and limit testing to FV Leiden in miscarriages and pregnancy loss and HT screen only in selected patients with unprovoked VTE.

Potential savings are £24420.69 (98.8% of cost).

PB2221 | Evaluation of Platelet Indices in Patients with Splanchnic Vein Thrombosis

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Background: Splanchnic vein thrombosis (SVT) is a rare and lethal form of venous thrombosis. The role of platelet indices (PIs) apart from mean platelet volume (MPV) is not well studied among venous thrombosis especially in SVT.

Aims: The present study was aimed to assess PIs as a potential risk factors for SVT.

Methods: This was a prospective case control study. A total 100 cases of SVT and 80 controls were screened for routine thrombophilic risk factors along with PIs [Platelet count (PC), plateletcrit (PCT), platelet distribution width (PDW), & MPV]. The data were compared using student t test.

Results: Among the PIs, the mean platelet count was significantly lower in patients (169 $x10^9$/L) than the controls (255 $x10^9$/L) (p< 0.001), the mean value of PCT was significantly lower in-patients (0.164%) than the controls (0.226%) (p< 0.001) and PDW was significantly higher in cases (17.19%) than in the control group (16.82) (p=0.004). Surprisingly, MPV, though relatively higher in cases (10.19 fL) as compare to controls(10.02fL), did not show statistical significance (p=0.863) In addition, 16 patients were found to have a positive thrombophilic risk factor (confirmed after repeat testing). Among these 8 cases had inherited risk factors and 8 had acquired one. When the idiopathic group (without any laboratory risk factor derangement; total number: 62) were compared with controls, the statistically significant PIs were the platelet count (p=0.002), PCT (p=0.016) and PDW (p=0.039), however the MPV did not showed significant correlation (p=0.868).

Conclusions: The PDW and PCT together with PC were found to significantly differ in SVT cases than in controls. The MPV as compared to earlier studies, though higher in cases, was statistically insignificant. It may be worthwhile to determine these PIs particularly in patients where the laboratory thrombophilic parameters are normal.

PB2222 | What Kind of Thrombophilia in Reunion Island?

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Background: Venous thromboembolic disease is a multifactorial disease. The incidence varies across continents, lower in Asia, compared to Europe. Reunion Island was an uninhabited island, which became French in 1638, with different waves of population arrivals, of various origins (Malagasy, African and Indian) with a lot of interbreeding.

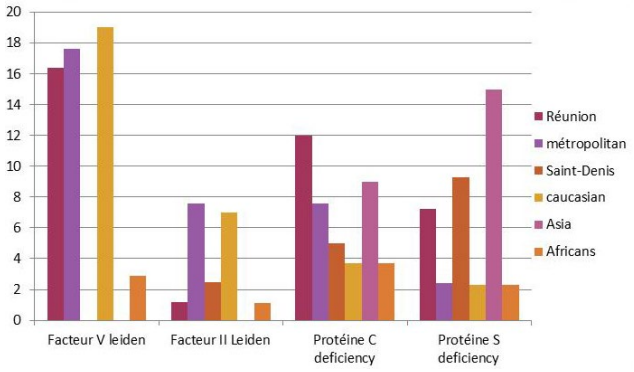

FIGURE 1 Comparison of percentage and prevalence of thrombophilia in Reunion Island and in different ethnicity

Aims: To analyse the differents factors of thrombophilia in Reunion Island, and compare to Europe and Asian.

Methods: We performed a retrospective study, from 2005 to 2018, in an Angiology department in Saint-Paul, including all patients who have a personal history of venous thrombosis (VT) or who are part of a family survey, with a complete thrombophilia screening, and, we have a retrospective study of thrombophilia for differents pathologies, in hospital in Saint-Denis in 2018-2019.

Results: 48 patients were included, 42 following a VT(calf, proximal, pulmonary embolism, 1 cerebral, 2 superficial) and 6 in a family survey. 31 patients have Reunion origin and 17 Metropolitan origin. For thrombophilic factors, we find 1 mutation of Factor II G20210A heterozygous (FII), 15 inhibitor deficiency, and 13 mutations of V Leiden heterozygote (FVL) in Reunion patients and 3 mutations (FII), 4 of inhibitor deficiency, and 6 (FVL) in metropolitan patients, for the first. In the second, we have 239 research of FII and 6 were positive (2 not after VT) and 934 research of inhibitors deficiency and 134 were positive.

Conclusions: If we compare the incidences of the different thrombophilia of Reunion Islanders with other populations, we find a similar frequency of inhibitors deficiency as in the Asian population and similar frequency of FVL like caucasian. On the other hand, we find few FII, only heterozygous. Maybe, this mutation is not in relation with thrombosis like in Asian country, and the mutation of Arg596 could be present, like many others that have been identified in Asian.

PB2223 | High Prevalence of the Hereditary Anticoagulant Deficiency in Chronic Thromboembolic Pulmonary Hypertension (CTEPH)

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Background: As a hereditary thrombotic risk factor, the role of hereditary thrombophilia in the pathogenesis of chronic thromboembolic pulmonary hypertension (CTEPH) still remained unresolved, especially in East Asian patients. The previous studies have focused on two small Caucasian cohorts, ignoring the ethnic specificity of thrombophilia, which may have led to bias in their results.

Aims: We aimed to analyze the association between the clinical characteristics of CTEPH and hereditary thrombophilia.

Methods: We conducted a retrospective cohort study. Hereditary thrombophilia was diagnosed through the screening tests for protein C, protein S, antithrombin III, and factor V Leiden and prothrombin G20210A mutations. Data on clinical, angiographic and hemodynamic characteristics were collected.

TABLE 1 Clinical characteristics of CTEPH patients by thrombophilia status

	Overall CTEPH (n = 315)	Thrombophilia CTEPH (n = 30)	Non-Thrombophilia CTEPH (n = 285)	p-value*
Demographic characteristics				
Age (yrs, mean ± SD)	53.2 ± 14.8	50.5 ± 15.9	53.5 ± 14.7	0.296
Female (n, %)	146 (46.3)	8 (26.7)	138 (48.4)	0.023
BMI (kg/m^2)	24.2 ± 4.3	22.8 ± 4.3	24.3 ± 4.3	0.072
Clinical parameters				
DVT history (n, %)	149 (47.3)	20 (66.7)	129 (45.3)	0.026
Recurrent DVT (n, %)	11 (3.5)	4 (13.3)	7 (2.5)	0.002
NYHA function class (n, %)				0.535
II	120 (38.1)	13 (43.3)	107 (37.5)	
III/IV	195 (61.9)	17 (56.7)	178 (62.5)	
NT-proBNP (pg/ml, mean ± SD)	1919 ± 2295	2379 ± 2217	1872 ± 2298	0.253
6MWD (m, mean ± SD)	386 ± 125	442 ± 146	381 ± 122	0.032
Blood type O, n (%)	51 (16.2)	5 (16.7)	46 (16.1)	0.941
Vena cava filter (n, %)	49 (15.6)	5 (16.7)	44 (15.4)	0.860
Hemodynamics				
RAP (mmHg, mean ± SD)	8.2 ± 4.8	8.3 ± 4.3	8.2 ± 4.9	0.973
sPAP (mmHg, mean ± SD)	83.6 ± 22.4	77.7 ± 22.1	84.2 ± 22.3	0.130
dPAP (mmHg, mean ± SD)	31.5 ± 8.8	28.9 ± 9.5	31.8 ± 8.7	0.085
mPAP (mmHg, mean ± SD)	50.1 ± 12.5	46.2 ± 13.1	50.5 ± 12.4	0.073
PAWP (mmHg, mean ± SD)	10.0 ± 2.9	9.2 ± 2.9	10.1 ± 2.9	0.101
CI (L/min/m^2, mean ± SD)	2.5 ± 0.6	2.6 ± 0.5	2.5 ± 0.7	0.323
PVR (Wood units, mean ± SD)	9.6 ± 4.6	8.1 ± 3.9	9.7 ± 4.7	0.083
SaO_2 (%, mean ± SD)	89.9 ± 5.3	90.1 ± 3.6	89.8 ± 5.4	0.789
SvO_2 (%, mean ± SD)	62.3 ± 8.1	63.9 ± 6.9	62.2 ± 8.2	0.282
Distribution of pulmonary artery lesion (n, %)[&]				
Unilateral pulmonary artery occlusion	12 (3.8)	3 (10.0)	9 (3.2)	0.058
Left pulmonary artery Level I and II	77 (24.9)	15 (51.7)	62 (22.1)	< 0.001
Right pulmonary artery Level I and II	111 (35.9)	14 (48.3)	97 (34.6)	0.145
Total Level I and II	135 (43.7)	20 (69.0)	115 (41.1)	0.003
Treatment (n, %)				
PEA	90 (28.6)	12 (40.0)	78 (27.4)	0.145
BPA	129 (41.0)	14 (46.7)	115 (40.4)	0.503

6MWD = six-minute walk distance; BMI = body mass index; BPA = balloon pulmonary angioplasty; CI = cardiac Index; dPAP = diastolic pulmonary artery pressure; DVT = deep vein thrombosis; mPAP = mean pulmonary artery pressure; NT-proBNP = N-terminal fragment of pro-brain natriuretic peptide; NYHA = New York Heart Association; PAWP = pulmonary artery wedge pressure; PEA = pulmonary endarterectomy; PVR = pulmonary vascular resistance; RAP = right atrial pressure; SaO_2 = arterial oxygen saturation; sPAP = systolic pulmonary artery pressure; SvO_2 = mixed venous oxygen saturation.

* The p value was from the statistical comparison of means/percentages between Thrombophilia and Non-Thrombophilia group;

& Only 309 patients were examined with pulmonary angiography, with 29 patients in Thrombophilia CTEPH group and 280 patients in Non-Thrombophilia CTEPH group.

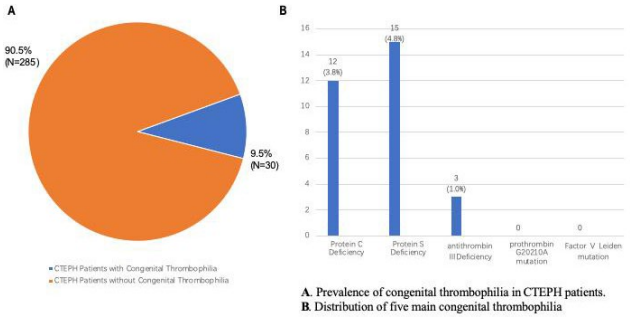

FIGURE 1 Prevalence of five congenital thrombophilia in Chinese patients with CTEPH

Results: Between May 2013 to April 2019, 315 patients with CTEPH were enrolled. Thirty (9.5%) were diagnosed with hereditary thrombophilia, including 12 protein C deficiency (3.8%), 15 protein S deficiency (4.8%), 3 antithrombin III deficiency (1.0%). None factor V Leiden and prothrombin G20210A mutations was defined (Figure 1). Compared with the non-thrombophilia group, patients with hereditary anticoagulant deficiency occurred less likely among females (26.7% vs. 48.4%, p=0.023), more frequently among those with a history of deep vein thrombosis (66.7% vs. 45.3%, p=0.026), especially recurrent thrombosis (13.3% vs. 2.5%, p=0.002). The age and NYHA classification were similar between two groups. While for patients with hereditary anticoagulant deficiency, their exercise tolerance was better evaluated by six-minute walk distance (442 ± 146 vs. 381 ± 122, p=0.032), the same as the haemodynamic profiles.The pulmonary artery lesions in patients with hereditary anticoagulant deficiency were more proximal, especially in left pulmonary artery (Table 1).

Conclusions: The prevalence of hereditary anticoagulant deficiency in Chinese CTEPH patients was significantly high. This association may suggest the special role of vein thrombosis in CTEPH etiology.

PB2224 | Use of Direct Oral Anticoagulants in Congenital Thrombophilias

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Background: Direct oral anticoagulants (DOACs) inhibit either factor Xa or thrombin in the coagulation cascade providing similar efficacy for venous thromboembolism (VTE) prophylaxis and treatment as compared with traditional Vitamin K Antagonist (VKA). Efficacy of DOAC therapy in patients with congenital thrombophilias in the real-world setting is an area of ongoing interest and clinical relevance.

Aims: Describe a single Center's experience in use of DOACs for VTE prophylaxis in patients with congenital thrombophilias.

Methods: Retrospective record review of patients at the Gulf States Hemophilia and Thrombophilia Center of Houston, Texas on either rivaroxaban or apixaban VTE prophylaxis with a diagnosis of congenital thrombophilia (Natural Anticoagulant Deficiency, Prothrombin gene mutation (FII mutation), Factor V Leiden mutation (FVL), Mixed Double -Zygosity).

Results: Chart review included 31 patients diagnosed with a congenital thrombophilia (6% (2)- Antithrombin deficiency, 16% (5)- Protein S, 10% (3)- Protein C, 6% (2)- Heterozygous FII mutation, 30% (9)- Hetero FVL, 16% (5)- Homo FVL mutation, 16% (5)- Mixed Double-Zygotes). A total of 19 (61%) on rivaroxaban average therapy duration to date of 3.18 years (6 year max, 1 year minimum). Twelve (39%) on apixaban average therapy duration to date of 2.25 years (6 year max, 1 year minimum). Of the 31 patients 4 (13%) had reported recurrence on rivaroxaban (venous thromboembolism- 2 noncompliant, recurrent iliac artery occlusion-2 on single agent therapy). Reported recurrent VTEs occurred within the first two years of rivaroxaban therapy. There were no reported major bleed events on either DOAC therapy.

Conclusions: These results support the use of DOACs for the prevention of VTE in patients with congenital thrombophilia. Although this a small cohort, additional studies in this population on DOAC therapy is merited.

PB2225 | Positive Thrombophilia Study in the Context of Starting Hormonal Treatment in the Gender Dysphory: Experience in a Single Center

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Background: Doing the thrombophilia test prior to the start of hormonal therapy in patients with "male to female" gender dysphoria is quite accepted because the history of venous thromboembolic disease (VTE) and the existence of thrombophilia contraindicates the initiation of estrogenic treatment. On the other hand, in patients with "female to male" gender dysphoria who are going to receive treatment with testoterone it is controversial since there is no evidence that links it to an increased thrombotic risk.

Aims: To describe our experience in the clinical management of patients with gender dysphoria with thrombophilia.

Methods: During 2018 and 2019, a total of 27 studies of thrombophilia were carried out in our center in people with gender dysphoria, the results and attitude adopted according to it were analyzed.

TABLE 1

Thrombophilia	Anticoagulation / antiplatelet therapy
Factor II mutation	No
Lupus anticoagulant positive	No
Factor V Leiden mutation	Aspirin

TABLE 2

Thrombophilia	Anticoagulant / antiplatelet therapy
Lupus anticoagulant positive and anticardiolipin antibody IgM positive	Sintrom
Factor II mutation	Estrogen treatment not performed
Protein C and S deficit	Sintrom
Lupus anticoagulant positive	No
Protein S treatment	Estrogen treatment not performed

Results: 70.37% (19 patients) had a negative study, with 29.63% (8 patients) presenting a positive test. Of these 8 patients, 3 were "female to male" and were going to receive testosterone therapy. The characteristics of these patients are shown in Table 1.The remaining patients (5) were to receive estrogen therapy. The characteristics of these patients are summarized in Table 2.

None of the patients who received hormone therapy suffered VTE.

Conclusions: Doing the thrombophilia test prior to the start of estrogenic hormonal treatment is necessary since it determines the clinical attitude. When reporting the risk of VTE that is conferred by estrogenic hormonal treatment in patients with thrombophilia, sometimes they decline to receive such treatment. In patients who did receive it, anticoagulation was chosen to minimize the risk of VTE.

More studies are needed to define the role of the thrombophilia study in patients who are going to require testosterone therapy since there is no clear evidence that links it to VTE and therefore the indication of anticoagulation / antiplatelet therapy is much discussed.

PB2226 | Increased Levels of Procoagulant Factors and the Risk of Venous Thromboembolism

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Background: Despite remarkable advances in understanding its pathophysiology, idiopathic venous thromboembolism still represents 30-40% of all cases. In recent years, increased levels of procoagulant factors have been shown to correlate with an increased VTE risk though assessing their exact contribution remains a challenge.

Aims: In this case-control study, we analysed the association between the plasma levels of Factors VIII (FVIII), IX (FIX), XI (FXI) and fibrinogen, and the development of a first episode of VTE.

Methods: The study was conducted at the Thrombotic and Haemorrhagic Diseases Unit of Padova University Hospital. We enrolled four hundred and eight patients with either a first objectively confirmed episode of proximal lower limbs deep vein thrombosis (DVT) or a pulmonary embolism — isolated or associated with proximal lower limbs DVT — who subsequently underwent venous blood sampling to assess the plasma levels of FVIII, FIX, FXI and fibrinogen. We also enrolled ninety-eight age- and sex-matched controls among the patients' spouses, partners and carers, who underwent the same blood tests.

Results: For all factors considered, the OR was higher when comparing individuals with the highest plasma levels (i.e. >3rd quartile) with reference individuals (i.e. ≤1st quartile). The highest VTE risk was observed for elevated FVIII levels (>3rd quartile vs. ≤1st quartile), with an OR adjusted for age and sex of 22.0 (95% CI 9.4-51.5). For the remaining factors, the aforementioned analysis adjusted for age, sex yielded markedly reduced ORs. We did not observe significant ORs for factors IX and XI, whereas for elevated fibrinogen levels (>3rd quartile vs. ≤1st quartile) we observed an OR of 3.3 (95% CI 1.4-7.9).

Conclusions: Our results indicate a strong association between increased FVIII levels and VTE risk, with a dose-response relationship. Furthermore, our findings suggest that increased fibrinogen levels ought to be considered as an independent risk factor for VTE.

PB2227 | Primary Aortic Thrombus with Multiple Visceral Embolisation: A Presenting Manifestation of Chronic Myelomonocytic Leukemia

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Background: Aortic thrombi are uncommon and are typically associated with significant atherosclerosis or presence of an aneurysm. Patients with chronic myelomonocytic leukemia(CMML) are well known to be associated with arterial thrombosis, involving medium-sized and small-sized arteries. However, a large artery(aortic) thrombus has rarely been reported in patients with CMML. Todate there is no consensus on the management of this condition. Herein we describe a rare case of aortic thrombus with multiple visceral thromboembolism, subsequently diagnosed to have CMML.

Aims: We aim to raise the awareness of rare presentation of CMML as aortic thrombus with visceral thromboembolism, and summarise the pathophysiology as well as review the literature on current treatment options available.

Methods: We report here a Chinese gentleman who presented with fever and abdominal pain. Physical examination was unremarkable except for upper abdominal tenderness. A CT scan abdomen showed large mid-thoracic descending aortic mural thrombus and multiple splenic and renal infarcts. He was started on aspirin and enoxaparin. An extensive workup for the etiology of aortic thrombus was negative for infectious, cardiogenic, vasculitic and thrombophilic cause of arterial thromboembolism. Follow-up showed persistent anaemia with leucocytosis and thrombocytosis. A bone marrow study done revealed features suggestive of CMML.

Results: Patient was initially started on aspirin and enoxaparin, which was subsequently transitioned to rivaroxaban. He was also started on hydroxyurea, with which his haemoglobin, leucocyte and platelet counts have remained stable. He has remained well with no further thromboembolic episodes.

Conclusions: Aortic mural thrombi are rare in the absence of atherosclerosis or aneurysm. A thorough evaluation is necessary to determine the cause of thrombosis. Although anti platelet therapy is recommended for patients with uncomplicated aortic thrombus, presence of embolic phenomenon warrants treatment with anticoagulation. In addition, ethology-specific treatment may render these patients less thrombogenic, and patient may not need to be be continued on long term anticoagulation.

PB2228 | Thrombophilia Testing in Patients with Arterial Thrombosis in Routine Clinical Practice

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Background: Inherited thrombophilias are well-established predisposing factors for venous thromboembolism. However, their role in arterial thombosis (AT) remains uncertain. Nevertheless, many patients with AT undergo thombophilia testing (TT), even though there is a lack of evidence regarding how a positive test result should influence treatment.

Aims: To describe the experience regarding TT in the setting of AT in our center and to asses whether this test affects clinical management of these patients.

Methods: In this retrospective observational single-center study, we reviewed consecutive patients who underwent TT ordered due to an AT over 2019 in our center.

Data was analyzed with IBM SPSS software, version 24. Results are expressed as counts and percentages for categorical data and as median for continous variables.

Results: A total of 70 patients were included, with a median age of 53 years. Most patients were young or middle-aged (less than 65 years), althought some older patients (n=5) were included in selected cases without classic cardiovascular factors and with a positive family history. 81% of the TT were ordered due to a stroke or transient ischemic attack. Baseline characteristics of the patients are summarized in table 1.

We found 2 patients with a prothrombin heterozygous gene mutation, 2 with a Factor V Leiden heterozygous mutation and 17 with a single positive test for antiphospholipid syndrome (APS) (either lupus anticoagulant or antiphospholipid antibodies), although most were not confirmed (Table 2).

Of the 21 (30%) patients with, at least, one positive test result, clinical management was altered in a significant way in only 2 patients (both changed to long-term anticoagulation for APS).

Conclusions: Our findings are consistent with previous studies and suggest current testing practices at our center are not clinically helpful, as there is a low likelihood of changes in therapy influenced by the results of TT (excluding APS).

TABLE 2 Thrombophilia testing results

Test	Positive result	Negative result	Missing
Protein C	0	68	2
Protein S	0	68	2
Antithrombin	0	69	1
Factor V Leiden mutation	**2 (both heterozygous)**	67	1
Prothrombin G20210A mutation	**2 (both heterozygous)**	67	1
Lupus anticoagulant/ antiphospholipid antibodies	**17**	51	2

PB2229 | A New Global Assay for Detection of Protein C Pathway-Associated Thrombophilia Using Thrombomodulin-Mediated Clot Waveform Analysis

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Background: Protein C deficiency (PC-def), protein S deficiency (PS-def), anti-phospholipid syndrome (APS), and high level of factor VIII (high-FVIII) cause absolute or relative dysfunction of PC pathway. Although a few screening assays for these thrombophilia are available, such as thrombin-generation-based assay (Thrombopath®) and APTT-based assay (ProC® Global), clot waveform analysis (CWA) which is a simple and quantitative global assay for monitoring bleeding disorders such as hemophilia has little been used for this purpose.

Aims: To establish a screening assay using a modified-PT-based CWA for detecting dysfunctional PC pathway.

Methods: CWA was performed on ACL-TOP® (IL-Japan). Sample plasma was incubated with recombinant tissue factor (rTF), synthesized phospholipid (PL) in the presence or absence of recombinant thrombomodulin (rTM). Clot formation was initiated with $CaCl_2$ and absorbance change was monitored. The peak of the first derivative curves (maximum coagulation velocity) was measured.

Results: Optimal concentrations of reagents (rTF 1 pM, PL 12 μM, rTM 20 nM, $CaCl_2$ 10 mM) were determined in pooled normal plasma

TABLE 1 Baseline characteristics of the patients

Sex	Male: 62.9% (n=44); female: 37.1% (n=26)
Age	Median= 53 (IQR: 44-59)
Family history	None: 45.7% (n=32): Arterial thrombosis: 38.6% (n=27); venous thrombosis: 4.3% (n=3); both arterial and venous thrombosis: 4.3% (n=3)
Cardiovascular risk factors (CVRF)	None: 28.6% (n=20). One CVRF: 35.71% (n=25); two: 25.71%; three: 5.71%; four: 4.28%. Smoking (current or past): 50% (n=35) Dyslipidemia: 25.71% (n=18) Hypertension: 15.71% (n=11) Diabetes mellitus: 14.28% (n=10) Obesity: 10% (n=7) Others: 5.7% (n=4)
Thrombotic history	Stroke: 85.7% (n=60); myocardial infarction: 10%; venous thrombosis: 11.4%; recurrent arterial events: 15.7% (n=11); recurrent venous events: 4.3% (n=3)

Fig1. Peak and peak ratio in rTM-mediated CWA

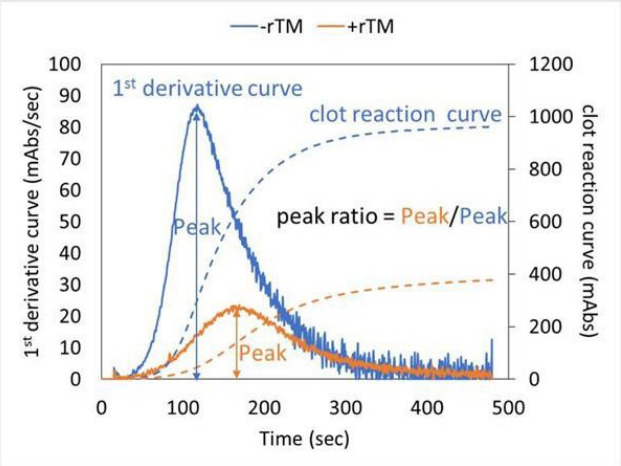

FIGURE 1

Fig2A. Peak and peak ratio in plasma of dysfunctional PC pathway

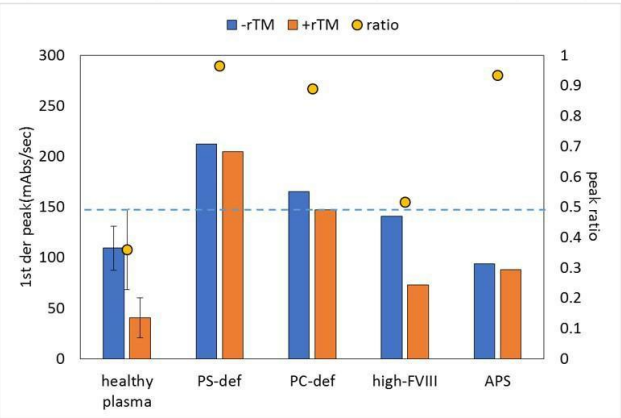

Fig2B. Peak and peak ratio in PC- or PS-deficent plasma spiked with pooled normal plasma

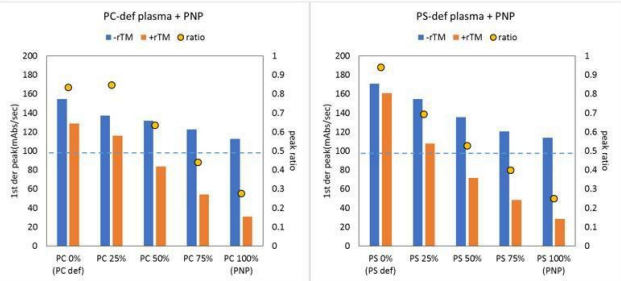

FIGURE 2

(PNP) so that the peak ratio with or without rTM reached ~0.3, which mean that added rTM reduced the peak down to 30% [Fig 1]. The peak ratio in 35 healthy plasmas was 0.36±0.13 (mean±SD), hence the cut-off value was set as 0.49. Plasma of PC-def, PS-def, high-FVIII (300 IU/dl), and APS showed higher peak ratio than cut-off value (0.79, 0.97, 0.52, 0.93, respectively) [Fig 2A]. PC-def, PS-def, high-FVIII showed higher peak values reflecting hypercoagulation (182, 207, 141 mAbs/sec, respectively) than healthy plasmas (109±22). PC-def or PS-def plasma mixed with PNP (25, 50, 75, 100%) showed dose-dependent reduction in peak ratio (PC-def; 0.85, 0.64, 0.44, 0.28 or PS-def; 0.69, 0.53, 0.40, 0.25, respectively). Therefore, peak ratio exceeded the cut-off value at 50% or less concentration of PC/PS [Fig 2B].

Conclusions: Thrombomodulin-mediated CWA successfully distinguished PC pathway-associated thrombophilia from normal individuals.

PB2230 | Thrombophilia Testing in Algerian Pediatric Patients

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Background: Thrombophilia refers to the propensity to develop thrombosis caused by acquired or hereditary risk factors. Thrombosis in childhood are relatively frequent and multifactorial with an estimation of 0.07 to 0.49/ 10000 child/year.

Despite combination of risk factors in pediatric patients, evaluation for thrombophilia should also be performed.

Aims: This work aims to show the contribution of thrombophilia assessment in pediatric patients with thrombosis.

Methods: We studied 14 cases of pediatric thrombosis with positive thrombophilia testing. It is about 9 boys and 5 girls (sex ratio H / F = 1.8) with an average age of 4 years.

4 of 14 patients had thrombosis in perinatal period (0-1 months), 8 of 14 during childhood (2-12 years) and 2 of 14 during adolescence (12-16 years).

Arterial thrombosis are predominant (*n=8*) mainly ischemic stroke. Venous thromboembolic events (*n=6*) are represented by venous thrombosis of the lower extremities (*n=4*) and portal cavernoma (*n=2*).

We performed thrombophilia testing compound determination of inhibitors activity (antithrombin, proteins S and C) and antiphospholipid antibodies detection.

Results: Thrombophilia testing shows inherited disorders (10 of 14). We report 4 cases of protein S deficiency, 3 cases of protein C deficiency and one case of antithrombin deficiency. Also, we found 2 cases of Activated Protein C Resistance. In 60% of cases, the inherited character has been confirmed by family investigation.

Acquired thrombophilia was found in 4 patients (29%), represented by pediatric antiphospholipid syndrome. For one patient, in front of neonatal ischemic stroke, the presence of maternal antiphospholipid antibodies has been evoked. Persistent Lupus Anticoagulant antibodies at least 12 weeks have been reported for all these patients.

Conclusions: This study shows that children's thrombosis can be explained by thrombophilia testing in accordance with ISTH recommendations in 2002. This diagnosis determines the long-term outcomes and management of these patients and allows identification of asymptomatic family members.

PB2232 | The Prothrombin Belgrade Mutation Causing Antithrombin Resistance Does Not Affect Fibrin Clot Formation

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Background: Prothrombin Belgrade mutation is a result of the c.1787G>T mutation in the prothrombin gene, which leads to the substitution of Arg596 by Gln. This mutation is located in the antithrombin binding site and leads to the impaired inactivation of thrombin by antithrombin and antithrombin resistance, resulting in a thrombotic phenotype. Previous studies have shown the complex mechanism of this mutation, manifested with higher endogenous thrombin potential, lower prothrombin activity with normal prothrombin levels in carriers' plasma.

Aims: Considering that Prothrombin Belgrade mutation mechanism is still not fully elucidated, our aim is to determine the effect of this mutation on fibrin clot formation and its lysis.

Methods: Recombinant wild type and mutated prothrombin was generated by transient transfection of HEK293T cell line. Using recombinant proteins and prothrombin deficient plasma, samples that correspond to plasma of a non-carrier, heterozygous and homozygous carrier was reconstituted. Reconstituted plasma samples with the addition of thrombin, tissue plasminogen activator (t-PA), phospholipids and calcium-chloride were used in OHP assay (Overall Hemostasis Potential) to determine the kinetic profiles of coagulation (Overall Coagulation Potential, OCP) and fibrinolysis (Overall Fibrinolytic Potential, OFP). Fibrin clots formed in reconstituted plasma samples with the addition of fluorescent fibrinogen, were analyzed by confocal microscopy in order to determine the fluorescence intensity and the density of fibrin network. In addition, fibrin clots prepared using OHP assay protocol, were observed using electron microscopy in order to determine the thickness of individual fibrin fibers.

Results: There was no significant difference between OHP, OCP and OFP parameters between tested reconstituted plasma samples ($p>0.05$). Fibrin network density and fibrin fiber thickness between non-carrier, heterozygous and homozygous carrier reconstituted plasma did not differ significantly ($p>0.05$).

Conclusions: Our results indicate that Prothrombin Belgrade mutation does not affect fibrin formation or its lysis.

PB2233 | Is May-Thurner Syndrome a Forgotten Cause of Deep Vein Thrombosis?

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Background: May-Thurner syndrome (MTS), also known as Cockett's syndrome or iliac vein compression syndrome, is a rare cause of iliofemoral deep venous thrombosis (DVT). An anatomic anomaly produces chronic compression of the left common iliac vein by the overlying right common iliac artery when it passes between the right common iliac artery and the spine. Prolonged compression on the vein potentiates thrombus formation by impairing the intima and by leading to the development of membranes within the lumen that may decrease and/or block venous flow.

Aims: Presentation of 4 cases of MTS.

Methods: Collection of clinical data in SClínico® application.

Results: Patient 1 is a 54-year-old male with signs of DVT. Computed tomography scan (CT-scan) confirmed MTS. Despite ongoing anticoagulation with rivaroxaban, he developed a post-thrombotic syndrome and is waiting for endovascular treatment. Patient 2, a 15-year-old female, initiated oral contraceptive 1 month before presenting with left DVT complicated with bilateral pulmonary thromboembolism. CT-scan confirmed MTS. She remains asymptomatic and hypocoagulated with warfarin (Time in Therapeutic Range >80%). Patient 3, a 19-year-old obese female, maintained leg pain after 6-month anticoagulation for DVT despite absence of DVT signs in duplex ultrasound. Magnetic resonance imaging (MRI) confirmed MTS. Rivaroxaban was restarted and she's currently asymptomatic. Patient 4, a 30-year-old female, presents acute pain and left leg swelling in her puerperium. Her workup revealed left-sided acute DVT secondary to MTS, confirmed by MRI.

Conclusions: MTS may be underdiagnosed, therefore all clinicians treating DVT should be aware of it. Anticoagulation, clinical follow-up and non-invasive imaging play critical roles in the diagnosis and prevention of relapse and complications associated with MTS. Despite none of our patients underwent stenting, guidelines nowadays recommend venous stenting to relieve symptoms and prevent relapse. Patient 4 emphasizes that DVT during puerperium should not only be attributed to hypercoagulability secondary to pregnancy.

PB2234 | Relevance of Thrombophilia Screening in Adult Renal Transplant Recipients

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Background: Thrombosis after kidney transplantation may result in catastrophic outcomes. Thrombophilia has been implicated in post-transplant thrombosis. However, data concerning the impact of thrombophilia on thrombotic risk in renal graft recipients are inconclusive.

Aims: Evaluate whether identifying patients with thrombophilia during pretransplant laboratoring screenig predicted post-transplant outcomes.

Methods: A single-center prospective study between 2011 and 2017 was performed. Pretransplant thrombophilia screening including antithrombin (AT), protein C (PC), protein S (PS) and activated

protein C resistance (APCR) was performed in patients with end-stage of chronic renal disease.

Renal transplant recipients were included. Details of recipients characteristics (cardio-vascular risk factors, type of nephropathy, history of thrombotic events, dialysis modality, surgical difficulties, immunosuppression modality), details of donor characteristics (age, type of donor, side of renal graft, warm ischemia time) were documented. Post-operative thrombotic outcomes were recorded up to one year post renal transplantation. Folders with missing data were excluded.

Results: Among 120 screened patients, 70 were transplanted. Only 35 kidney transplant recipients were reviewed. Median age was 36 years old [24-61]. Sex ratio was 2.2. Thrombophilia was found in 11% patients (N = 4): 2 cases with PC deficiency, one case with PS deficiency and one case with APCR.

A post-transplant thrombotic event occurred in 7 patients (20%); the renal transplant was involved in two patients, whereas, the others events were: 3 fistula thrombosis, 1 arterial occlusion and 1 superficial vein thrombosis of the upper limb. Thrombotic events occurred in 7 patients with no thrombophilia and none of patients with thrombophilia (P = 0.562). Post-operative thrombotic events were not significantly associated to both recipients and donors clinical characteristics.

Conclusions: Our data suggest that the utility of universal, comprehensive preoperative thrombophilia testing is not beneficial in determining risk of post-operative thrombosis occurrence. Thrombophilia testing may be considered in a select population with a history of pretransplant thrombotic event.

PB2235 | Homozygous Antithrombin Budapest 3 Deficiency Causes Inferior Vena Cava System Agenesis

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Background: Agenesis of inferior vena cava is a rare congenital malformation with unknown etiology, although the potential role of thrombotic events during embryogenesis has been suggested.

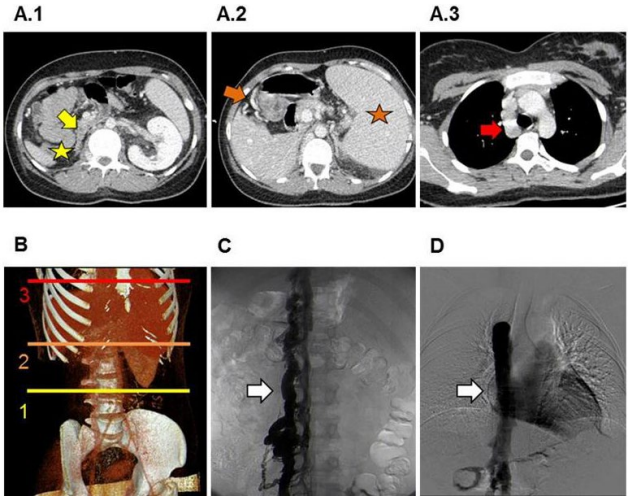

FIGURE 1 Axial CT (A), 3D scan (B) and flebograhy (C,D) supporting inferior vena cava agenesis (yellow arrow) and compensatory mechanisms

TABLE 1 Characteristics of homozygous carriers of the Budapest 3 variant evaluated by CT analysis

Patient	Cohort	Gender	Age	Anti-FXa (%)*	Antigen (%)	First thrombotic event (age)	Recurrence	Atresia	Other anatomic disorder	Other thrombophilia
1	Spain	F	27	18%	70%	DVT (24)	Yes	IVC	Absence of right kidney and right adrenal gland	No
2	Spain	F	47	24%	70%	DVT (30)	No	No	No	No
3	Spain	F	43	17%	69%	DVT (39)	No	No	No	No
4	Spain	M	69	9%	69%	No thrombosis	No	No	No	No
5	Spain	M	39	15%	81%	DVT (16)	No	IVC	No	FVLeiden +/-
6	Spain	M	34	8%	75%	DVT (30)	No	LIV	Double IVC	No
7	Spain	M	86	28%	ND	DVT (87)	Yes	IVC	No	No
8	Hungary	F	54	12%	80%	DVT (15)	No	IVC*	Hypoplasia of both iliacal veins	No
9	Hungary	F	27	14%	84%	DVT (16)	Yes	IVC	Hypoplasia of vena iliaca communis	No
10	Hungary	M	25	23%	84%	DVT (16 days)	Yes (5a)	IVC*	Hypoplasia of vena iliaca communis	FVLeiden +/-
11	Hungary	M	11	16%	80%	No thrombosis	No	IVC	No	No
12	Hungary	F	31	ND**	ND	DVT (29)	No	IVC	No	FVLeiden +/-
13	Hungary	F	26	25%	70%	DVT (25)	No	IVC	No	No
14	Hungary	F	74	15%	76%	DVT (20)	No	IVC	No	No
15	Hungary	M	33	16%	84%	No thrombosis	No	No	No	No
16	Hungary	M	29	14%	ND	DVT (24)	Yes	No	No	No
17	Hungary	F	27	17%	76%	DVT (22)	Yes	No	No	No
18	Hungary	M	50	12%	ND	DVT (35)	Yes	No	No	No
19	Hungary	F	26	16%	88%	DVT (10)	No	No	No	No
20	Hungary	M	21	16%	80%	DVT (14)	No	IVC	No	No
21	Hungary	M	27	17%	90%	DVT (21)	Yes	IVC	No	No

*DVT: Deep Venous Thrombosis; IVC: Inferior Vena Cava; LIV: Left Iliac Vein; ND: no data; * lower limb venography and cavography; anti-FXa activity, reference interval: 80-120%*

Aims: The identification of inferior vena cava agenesis (Figure 1) in a case with early idiopathic venous thrombosis and antithrombin deficiency caused by the homozygous c.391C>T variant in *SERPINC1* (p.Leu131Phe, antithrombin Budapest 3), the most severe viable thrombophilia, encouraged to evaluate the potential role of this very rare homozygous type II deficiency in this vascular abnormality.

Methods: Two large cohorts (Spain, N=313 and Hungary, N=281) of unrelated patients with congenital antithrombin deficiency (biological and genetically characterized) were enrolled. Image analysis included computed tomography (CT) and cavography/ phlebography.

Results: We recruited the worldwide largest cohort of patients with homozygote Budapest 3 variant (N=59, 10 from 7 Spanish families and 49 from 40 Hungarian families). In 12 out of 21 cases with available CT, inferior vena cava agenesis was identified, and four of them had additional anatomic defects: hypoplasia of one or both iliac veins (N=3) and renal and adrenal agenesis (N=1). Moreover, one additional case had atresia of the left iliac vein. Compensatory mechanisms, such as dilation of azygos/ hemiazygos veins or double inferior vena cava, were observed (Table 1).

Conclusions: This study shows a strong association between a specific genetic defect, homozygous *SERPINC1* c.391C>T, responsible for a very severe thrombophilic state, with an embryonic congenital

malformation, inferior vena cava system agenesis, identified in 62% of carriers. The impaired control of thrombin generation might facilitate intrauterine or perinatal thrombosis in this localization. Moreover, the high risk of thrombosis described in patients with this congenital malformation might be explained by the underlying thrombophilia. These data encourage the search for severe thrombophilic states and to consider the use of antithrombotic prophylaxis at risk situations in patients with these vascular defects.

ISCIII&FEDER-PI18/00598;FundaciónSéneca-19873/GERM/15.

PB2236 | Inherited Deficiency of Antithrombin, Protein C or Protein S Is Associated with an Increased Risk of Cancer at a Younger Age

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Background: Hypercoagulability is part of the aggressive biology of cancer playing an essential role in thrombosis formation and tumor growth. Whether or not carriers of major inherited thrombophilia have a higher predisposition to cancer is still unknown.

Aims: To evaluate whether carriers of inherited defects of antithrombin, protein C or protein S exhibited a higher risk for the development of cancer as compared to non carriers.

Methods: In this prospective family cohort study we enrolled a total of 506 subjects (45% men; median age at the enrollement 38 years), belonging to 84 different kindreds.

Results: A total of 4210 and 5353 patient observation-years were considered in carriers and non-carriers, respectively. During the observation-years, sixteen tumors developed in carriers (6.7%) as compared to eighteen in non-carriers (7.7%). Overall, annual incidences of cancer were 0.4% (95% CI, 0.2 to 0.6) in carriers and 0.34% (95% CI 0.2 to 0.5) in non-carriers, respectively (RR, 1.0 [95% CI 0.99 to 1.0]).

Because the risk of cancer varies with age, we performed a time-dependent analysis. By considering only family members less than 60 years of age, during the observation-years, nine tumors developed in carriers (3.6%) as compared to three in non-carriers (1.2%). Overall, annual incidences of cancer were 0.2% (95% CI, 0.07 to 0.35) and 0.06% (95% CI 0.007 to 0.12) in carriers and non-carriers, respectively. Carriers of inherited defects less than 60 years of age showed a RR for cancer of 3.81(95% CI, 1.03 to 14.1) as compared to non-carriers.

Conclusions: In this large prospective cohort study, family members who were carriers of major thrombophilia due to clotting inherited inhibitor deficiencies presented with a higher risk of developing cancer before the age of 60 as compared to non-carriers. Further studies are needed to confirm this data.

PB2237 | Mandor's Disease: A Manifestation of Rare Conditions

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Background: Mondor's disease is a rare entity including superficial thrombophlebitis located in the thoracoabdominal wall. It is usually a benign condition but it can reveal a neoplastic lesion or a thrombophilia.

Aims: The aim of this study is to present two unusual etiology of Mondor's disease.

Methods: Two new cases are presented.

Results: A-39-year-old male patient followed for acute articular rheumatism was hospitalized for thrombosis of the right thoraco-epigastric vein. There was no thoracic trauma, a heavy load, or an intense physical effort. The examination of the breasts, the thoracic radiography and the abdominal-pelvic ultrasound were normal. The digestive endoscopy showed only a gastritis. Biology showed a decreased rate of antithrombin III. The decision was to treat the patient by Vitamin K antagonist (VKA) for prolonged duration.

A 69-year-old patient with diabetes and hypertension was hospitalized for thrombosis of superior epigastric vein. Constitutional thrombophilia screening was negative.

The mammography was normal. The thoraco-abdominal CT-Scan showed an agenesis of the lower vena cava. The patient was treated with heparin VKA and a local treatment. The abdominal thrombophlebitis decreased within 6 weeks.

Conclusions: Mondor's disease affects more frequently women than men. To our knowledge, only 50 male cases were reported. The peculiarities of our observations reside in the occurrence of the disease in a man and that it was a manifestation of even less frequent condition such as Inferior vena cava agenesis and Antithrombin III deficiency.

PB2238 | Deep Vein Thrombosis Associated with Protein S Deficiency

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Background: Protein S is a vitamin K-dependent serum protein that has a pivotal role in the anticoagulant system. The major function of protein S is as a cofactor to facilitate the action of activated protein C on its substrates, activated factor V (FVa) and activated factor VIII (FVIIIa). Deficiency of protein S is associated with increased risk of deep vein thrombosis (DVT).

Aims: To describe a case report of deficiency protein S presenting as DVT.

Methods: A 45 year male had unilateral swelling of left lower limb with sudden onset painful for last five days. There was history of diabetes mellitus in the past. Venous Doppler showed hypoechoic thrombus within the lumen of left greater saphenous veins, common femoral, superficial femoral, popliteal and tibialis anterior signifying occlusion. Investigations revealed high D-dimer levels (28.87 µg/mL), protein S levels 35%, and HbA1c levels 7.3%. Antithrombin III and protein C levels were normal. The patient was admitted and started on fondaparinux 2.5 mg subcutaneous injection once daily for 2 months along with oral anti diabetes theraphy.

Results: The present case asserted to the evidence that protein S deficiency is associated with thrombosis. Inheritable forms are rare, usually due to large deletions in the PS gene (PROS1). Without molecular analysis, it is difficult to decided whether PS deficiency is acquired or hereditary. Hereditary forms usually associated with DVT rather than arterial thrombosis. In our case patient had DVT in left leg revealed by Doppler ultrasound examination. Theraphy is mainly antithrombotics and long term anticoagulants. During the initial phase, heparin as intravenous unfractioned heparin or as subcutaneous low molecular weight heparin (LMWH) should be continued for a minimum of five days.

Conclusions: Fondaparinux treatment 2.5 mg subcutaneous injection once daily for 2 months giving a good response in patient DVT associated with Protein S deficiency.

PB2239 | Relation between the von Willebrand Factor and ADAMTS13 with Venous Thrombosis Risk. Results from the Retrove Project

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Background: High von Willebrand factor (vWf) levels are a known risk factor of venous thrombosis (VT). ADAMTS13 is the regulator of vWf. Recently, our group has described that low ADAMTS13 levels are associated with VTrisk in women. Also, Pagliari, *et al.* reported that ADAMTS13 activity levels < 86% are associated with a moderate risk of VT.

Aims: To replicate our previous results with an extended population (800 individuals). Also, to evaluate if the interaction of low ADAMTS13 levels and high vWf levels increases VT risk.

Methods: Patients consisted of 400 individuals with VT recruited by the RETROVE Project (Riesgo de Enfermedad TROmboembólica VEnosa). Also, 400 healthy controls were included. Controls were not matched for sex or age due to the design of the project. We determined antigen ADAMTS13 and vWf by ELISA. The VT risk was calculated by logistic regression. We estimated the crude and adjusted for the age odds ratio.

Results: We found an association between low ADAMTS13 levels and VT in women, but when OR was adjusted by age, the statistical significance was lost. High vWf levels were associated with VT risk (OR adjusted: 8.48 (5.48-13.12 95%CI) p= 0.0001) (Table 1). The

TABLE 1

			ADAMTS13>0.59µg/mL	ADAMTS13≤0.59µg/mL	OR (95% CI)	OR adjusted	TOTAL
10th Percentile ADAMTS13	ALL	Controls	356(89%)	44 (11%)*	1.69 (1.13-2.55) P=0.012	0.94 (0.60-1.47) NS	400
10th Percentile ADAMTS13	ALL	Patients	331 (82.8%)	69 (17.3%)*			400
10th Percentile ADAMTS13	Women	Controls	187 (90.8%)	19 (9.2%)*	1.83 (1.01-3.4) p=0.049	1.15 (0.59-2.26) NS	206
10th Percentile ADAMTS13	Women	Patients	172 (84.3%)	32 (15.7%)*			204
10th Percentile ADAMTS13	Men	Controls	169 (87.1%)	25 (12.9%)	1.57 (0.91-2.76) NS	0.76 (0.40-1.42) NS	194
10th Percentile ADAMTS13	Men	Patients	159 (81.2%)	37 (18.8%)			196
			vWf<183%	vWf≥183%			
90th Percentile vWf	ALL	Controls	359 (67.4%)	41 (15.4%)*	11.32 (7.75-16.54) p=0.0001	8.48 (5.48-13.12) p=0.0001	400
90th Percentile vWf	ALL	Patients	174 (32.6%)	225 (84.6%)*			399

TABLE 2

		vWf-ADAMTS13			
		vWf<183%	vWf≥183%	OR (95%CI)	TOTAL
ADAMTS13≤0.59μg/mL	Controls	35(79.5%)	9 (20.5%)*	14 (5.75-37.31) p=2.62e-08	44
ADAMTS13≤0.59μg/mL	Patients	15(21.7%)	54 (78.3%)*		69
ADAMTS13>0.59μg/mL	Controls	324 (91.0%)	32 (9.0%)*	11 (7.17-16.61) p=0.001	356
ADAMTS13>0.59μg/mL	Patients	159 (48.2%)	171 (51.8%)+		330
*statistical significance results	NS: non significance results				

correlation between ADAMTS13 and vWf levels with age was: R=-0.291 and R=0.480 respectively (p< 0.01).

Also, high ORs were found with high vWf levels plus low or high ADAMTS13 levels, suggesting that vWf levels were the main responsible for VT risk (Table2). We calculated the interaction between low ADAMTS13 levels and high vWf levels with VT, non-significance results were obtained (OR= 1.48 (0.53-4.15 95% CI) (NS).

Conclusions: In the RETROVE project, the interaction between low ADAMTS13 levels and high von Willebrand factor levels seems not to be related to VT risk.

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PB2240 | Characterisation of Antithrombin p.Thr147Ala, the First Common Founder Variant from African Origin Responsible for Inherited Antithrombin Deficiency

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Background: Hereditary antithrombin deficiency is a rare autosomal dominant disorder predisposing to recurrent venous thromboembolism (VTE). To date, only two founder variants have been described, both in Caucasians.

Aims: To characterize a new recurrent *SERPINC1* variant, p.Thr147Ala, identified in people from African ancestry.

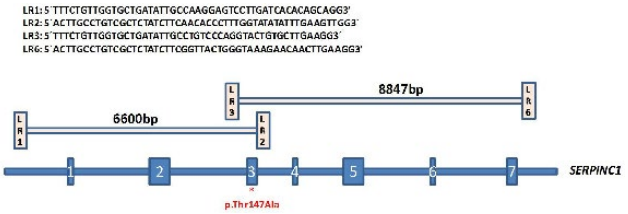

FIGURE 1 Representation of SERPINC1: exons, introns and localization of p.Thr147Ala variant. Primers sequences used, and 2 amplicons localizations are shown

TABLE 1 SNVs linked to the p.Thr147Ala variation (italic bold) in SERPINC1. #According to GRCh37/hg19 * Minor allele frequency according to 1000 Genomes

dbSNP ID	Coordinate (chrom 1)#	Change	Localization	Codon	Missense change	MAF* (%) Caucasian	MAF* (%) African
rs2227611	173875467	A>G	Intron variant	--	--	0.3	27.6
rs138511779	173877033	C>T	Intron variant	--	--	0.0	26.0
rs1799876	173878471	A>G	Intron variant	--	--	33.1	83.5
rs5878	173878832	T>C	Exon 5	337	Q (synonymous)	34.7	84.4
rs5877	173878862	T>C	Exon 5	327	V (synonymous)	34.0	83.7
rs2227610	173879178	T>C	Intron variant	--	--	0.0	2.0
rs2227610	173881122	T>C	Exon 3	147	T/A	0.0	1.0
rs941989	173881871	C>T	Intron variant	--	--	34.2	84.4
rs2227601	173882730	T>C	Intron variant	--	--	12.3	68.5
rs2227597	173884739	C>G	Intron variant	--	--	12.1	44.8
rs2227596	173884793	T>C	Intron variant	--	--	15.9	78.7
rs2227594	173885481	C>T	Intron variant	--	--	12.1	44.8
rs2227593	173885536	G>T	Intron variant	--	--	12.1	44.9
rs2227590	173886037	G>A	Intron variant	--	--	12.1	46.9

According to GRCh37/hg19
* Minor allele frequency according to 1000 Genomes.

Methods: The study included 10 patients from African origin living in Belgium. Eight of them experienced VTE, stroke or obstetric complications.

Antithrombin was functionally and biochemically characterized using patient plasma and a recombinant model. *SERPINC1* (14480bp) was amplified by 2 long-range-PCRs (LR-PCR) (Figure 1) and sequenced with nanopore technology (MinION). SNVs were analyzed by Illumina Variant Studio and Integrative Genomics Viewer. Haplotype was verified by studying the reads carrying p.Thr147Ala by specific bioinformatic algorithms using *grep* command in Linux.

Results: All patients carried the same variant, p.Thr147Ala (rs2227606), being, absent in Caucasians and Asians but present in 1% of Afro-Americans, according to ExAC, 1000 Genomes, and gnomeAD.

Anti-FXa activity was reduced (61.8±9.3%) while anti-FIIa activity and antigen levels were normal (100.9±10.2% and 91.2±14.9%, respectively). On crossed-immunoelectrophoresis under high ionic strength conditions, an increase of antithrombin fractions with reduced heparin affinity was observed, not detected in physiological conditions. Purified recombinant p.Thr147Ala showed a reduced anti-FXa activity (61.6%±1.6%) but normal heparin affinity. Structural modelling revealed that the variant abolished three hydrogen bonds of Thr147, one of them involving Arg49, a residue directly interacting with heparin.

Nanopore sequencing revealed a common haplotype containing 13 intragenic markers (Figure 2), confirmed by *grep* analysis.

Conclusions: Nanopore sequencing efficiently and rapidly determines the haplotype of a genetic variant without requiring family studies or cloning strategies. Our study describes the first pathogenic founder variant from African origin, p.Thr147Ala, responsible for a mild antithrombin type II HBS deficiency. This study contributes to the knowledge about population-specific thrombotic risk factors.

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Wetenschappelijk Fonds Willy Gepts

PB2241 | Thrombophilia and Thrombotic Complications in Armenia

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Background: The incidence of hereditary thrombophilia varies considerably in different countries and populations. Data on the epidemiology of thrombophilia and thrombotic events in Armenia is very limited or lacking.

Aims: The aim of this study was to analyze the incidence of thrombophilia in Armenian population for the period of 2009-2019 and to evaluate the frequency of thrombotic complications and recurrent thrombosis among patients with thrombophilia.

Methods: The data for this study have been derived from the ambulance cards, hospitalization journals, and medical records of the Registry of the Armenian Hemophilia and Thrombophilia Center, Hematology Center after Prof. R.H.Yeolyan. Demographic data were obtained from the Statistical Committee of the Republic of Armenia.

Results: For the whole group of patients the median age was 28.7 years, range [16-60] During the study period 189 primary patients with FV homozygote, 71 with FII homozygote thrombophilia were registered. 97 (37%) patients had both FV and FII homozygous mutations. The average annual incidence for patients with FV homozygote mutation was 0.6±0.10 and FII homozygous mutation 0.23±0.06 per 100 000 population. 47 (18%) patients had recurrent thrombosis. The average annual detectability from the general population was 0.01%. Thrombotic complications of different locations were observed in 98 (38%) patients. Regarding heterozygous mutations, there were 840 (0.29±0.01) FV carriers and 258 (0.89±0.01 per 100 000 population) FII carriers detected.

Conclusions: Our Center is the only hemostasis and thrombosis institution in our country and to the best of our knowledge this is the first report to describe the epidemiological situation of hereditary thrombophilia and recurrent thrombosis in Armenian population.

PB2242 | Venous Thrombosis-dominant Congenital Dysfibrinogenemia Presenting with Cerebral Venous Sinus Thrombosis

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Background: Congenital dysfibrinogenemia (CD) is a rare autosomal dominant disorder characterized by normal levels of circulating fibrinogen with low functional activity due to missense mutations in one of the fibrinogen genes. The majority of these patients remain asymptomatic; however, the estimated cumulative incidence of thrombosis at age 50 years is 30%.

Aims: To describe a patient with CD diagnosed after 2 major episodes of venous thrombosis.

Methods: Retrospective review of electronic medical records to describe the clinical manifestations and diagnostic evaluation.

Results: 44-year-old Asian man presented after a fall with loss of consciousness, and was diagnosed with a large cerebral venous sinus

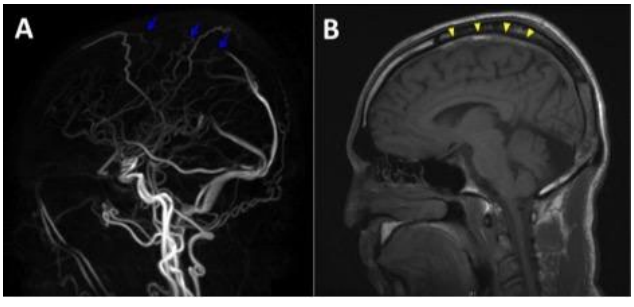

FIGURE 1 (A) MRV showing limited flow in the middle third superior sagittal sinus. (B) Brain MRI showing T1 hyperintense thrombus

TABLE 1 Results of laboratory tests in patient and his mother

Laboratory Tests	Patient	Mother (**tests done while on apixaban for atrial fibrillation)
PT (sec)	13.9 (normal 9.7 - 12.8)	17.0 (normal 10.2 - 12.6)
aPTT (sec)	30.8	34.8
TT (sec)	61.0	70.6
Reptilase time (sec)	N/A	47.0
Fibrinogen, activity (mg/dL)	< 50	62
Fibrinogen, antigen (mg/dL)	304	381
Thrombophilia Testing	Antithrombin level normal; Protein C and Protein S activities normal; Lupus anticoagulant negative; Cardiolipin antibodies and Anti-β2 glycoprotein antibodies negative; Activated Protein C resistance normal; Homocysteine level normal; PNH screen negative; JAK2 V617F negative; CALR negative; Factor VIII level normal; Factor IX level normal; Factor XI level normal	N/A

thrombosis (Figure 1). He received unfractionated heparin and was discharged on warfarin, which he continued for 1 year.

Two years after stopping anticoagulation, he developed massive left leg swelling and cyanotic discoloration after running a marathon. A compression ultrasound showed extensive common and external iliac vein thrombosis. He received tissue-plasminogen activator and a venous stent. His initial coagulation tests are listed on Table 1. Post-procedure fibrinogen was < 50 mg/dL and he received 3 units of cryoprecipitate. He was discharged on rivaroxaban 20 mg daily. There was no family history of thrombosis or bleeding disorders. Thrombophilia screening was negative (Table 1). Fibrinogen activity repeated 6 months after was 53 mg/dL and was verified one month later. He had prolonged thrombin time (TT) and his fibrinogen antigen was normal. Based on these results he was diagnosed with dysfibrinogenemia. Genotyping revealed FGG missense mutation of c.901C>T. Patient has remained on rivaroxaban 10 mg daily with no recurrent thrombosis or bleeding events.

Patient's asymptomatic mother was tested and she was also found to have dysfibrinogenemia (Table 1). Genotyping is in progress.

Conclusions: CD should be considered in young patients presenting with unprovoked recurrent major venous thrombosis even in the absence of a positive family history.

PB2243 | The Presence of Paraproteinaemia in Patients with Antiphospholipid Syndrome - A Single Centre Case Series

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Background: Case reports have described the presence of antiphospholipid (aPL) antibodies in patients with clonal lymphocytic and plasma cell disorders. We have anecdotally noted that on investigation of patients with antiphospholipid syndrome (APS) who had recurrent thrombosis despite high-dose anticoagulation with vitamin K antagonists, there was the presence of a paraprotein in some patients.

Aims: To review the clinical phenotype and management of patients with APS with an associated identified paraprotein.

Methods: The presence of a paraprotein was assessed in patients in our APS registry. Clinical records were reviewed for thrombosis, anticoagulation management and assessment for an underlying lymphoproliferative disorder.

Results: Nine patients were identified. All patients had a thrombotic event with the following initial sites: 3 cerebral, 3 arterial limb, 2 deep vein thrombosis and 1 splanchnic. Six patients (67%) had recurrent thrombotic events on anticoagulation. 2 of 6 women had previous obstetric complications (both pre-eclampsia). 4 patients (44%) has triple positive aPL serology. All patients received warfarin as anticoagulation with 8 patients receiving high-intensity dosing due to thrombosis recurrence/arterial disease. 5 patients had a diagnosis of MGUS, 2 had smouldering myeloma and 2 had lymphoplasmacytic lymphoma (LPL). Two patients with LPL received multiple lines cytoreductive therapy due to recurrent thrombosis despite INR targets of 3-4 with subsequent rises in paraprotein. One patient also had

4-weekly plasma exchanges. Despite treating the underlying paraprotein with a partial disease response, one patient with LPL had on-going arterial thromboses with high-dose anticoagulation and had major bleeding with ibrutinib. No patients had hyperviscosity syndrome.

Conclusions: Patients with recurrent thromboembolism on anticoagulation with APS may have an associated paraprotein disorder. Further evaluation of the epitope for these paraproteins needs to be evaluated for possible aPL activity. Whether treatment with cytoreduction is required to reduce the risk of recurrent thrombosis, needs consideration and further research.

PB2244 | Risk of Recurrence after a First-lifetime Venous Thrombosis: Impact of Inherited Thrombophilia

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Background: The optimal duration of anticoagulation after a first episode of venous thrombo-embolism (VTE) relys on the risk of both recurrence and bleeding. However, factors associated with clinically relevant increased risk of recurrence are not fully established. Even though inherited thrombophilia (IT) has been shown to increase the risk of a first VTE, it is still controversial whether IT enhances the risk of recurrent VTE.

Aims: To evaluate the impact of IT on the risk of recurrence after a first-time VTE.

Methods: We conducted a retrospective single-center observational study. Over a period of 6 years (2011-2016), patients with first-lifetime VTE screened for IT including natural anticoagulants deficiencies and resistance to activated protein C were enrolled.

Results: 102 patients with an average age of 37.8 years and sex ratio (M/F) of 0.78 were included. Proximal deep venous thrombosis was the most frequent localization observed in 34.3% of patients followed by thrombosis at unusual site (21.6%) and distal deep venous thrombosis (12.7%). The rate of IT was 25.5%. The mean duration of anticoagulation was 11.8 months. Fourteen (53.8%) patients with IT pursued long-term anticoagulation. After a mean follow-up of 24.3 months after anticoagulation withdrawal, VTE recurred in 7 (9.7%) patients. Among them, one patient had IT.

IT did not significantly increase the risk of recurrence in both univariate and multivariate analysis (HR= 0.4; IC= [0.05-3.32]; p=0.4, adjusted HR= 1.14; IC= [0.17-10.27]; p= 0.91).

Conclusions: We concluded that IT is not a recurrence predictor after a first VTE. Thus, it seems reasonable to rationalize IT indications in VTE and reserve it for situations associated with therapeutic consequences.

PB2245 | Thrombophilic Abnormalities Screening in Elderly Women Patients with Venous Thrombosis

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Background: Speaking of biological risk factors for venous thromboembolism in the elderly women who are at high risk of thrombosis, still are few data available. Thrombophilia is considered one of the risk factors for venous thrombosis.

Aims: The aim of the present study, was to assessed the prevalence of PAI1, MTHFR C667T mutation, MTHFR A1298C mutation, factor V Leiden R506Q mutation and G20210A prothrombin gene variant, in women patients over 65 years who were referred to us for thrombophilia testing and who present venous thromboembolic disease.

Methods: Sixty four women patients were included in the group.

Results: For PAI 1 we found 20 patients as normal, 20 were found heterozygous carriers and 12 homozygous carriers. Regarding factor V Leiden R506Q mutation we found 52 patient as normal, and 12 heterozygous carriers and for MTHFR C667T mutation there were 24 normal, heterozygous carriers 33 and 7 patients homozygous carriers. MTHFR A1298C mutation was normal for 35 patients, heterozygous carriers for 27 and 2 homozygous carriers. G20210A prothrombin gene variant was normal for 63 patient and only 1 was heterozygous carrier.

Conclusions: The identification of a thrombophilia can lead to overestimating the risk of thrombosis. thrombophilia testing is rarely useful following venous thromboembolism, possible to identify the risk of recurrence in some patients in whom the thromboembolic event was unexplained.

PB2246 | Hypoplasminogenemia - A Forgotten Diagnosis: A Case Series of Genotype and Phenotype Correlation

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Background: Congenital plasminogen deficiency is a rare condition caused by mutations in PLG (plasminogen). PLG deficiency type 1pts (patients) have pseudomembraneous lesions (especially homozygous pts). There is a lack of data concerning heterozygous patients (PLG deficiency type 2, dysplasminogenemia) with residual activities around 50-60%. Most of the pts are asymptomatic, but a risk for thromboembolic occlussions is described.

Aims: To detect if pts are really asymptomatic or have e.g. a raised thrombotic risk.

Methods: By help of a register we could detect 12 patients presenting in our centre since 2016 (not randomized to thrombotic or bleeding complications).

Results:

- 10 female, 2 male patients
- median age 39.8 years
- median plasminogen activity %: 59%, minimum 16- max, 67%
- 4 thrombotic events, 4 missed abortions
- 1 bleeding complications
- note: 1 with het. FV-Leiden-Mutations
- genetics: ACMG class 3, 4, 5

Conclusions: Plasminogen deficiency may be worth to be tested in pts with thromboembolic events. Like described before, it can be combined with FV-Leiden-Mutation. Further, multicentre studies are useful to collect more data of the heterozygous pts.

Other items for considerations: No off-label drug, no funding agency is involved, no clinical study just an anonymous register.

PB2247 | Efficacy and Safety Profile of Direct Oral Anticoagulants in Consecutive Patients with Severe Hereditary Thrombophilia: A Single-centre Cohort Study

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Background: Severe hereditary thrombophilia increases the risk to develop venous thromboembolism (VTE) even recurrent.

Aims: To evaluate efficacy and safety profile of direct oral anticoagulants (DOACs) in patients with VTE and severe thrombophilia.

Methods: Consecutive adult patients with a first acute VTE between 2014-2019 and confirmed severe thrombophilia treated with DOACs for at least 3 months were enrolled. Severe thrombophilia patients treated for at least 3 months with heparin or vitamin K antagonists [VKA]) between 2007-2019 acted as controls. Endpoints were incidence rate (IR) of VTE recurrence, bleeding complications, post-thrombotic syndrome (PTS) in the two cohorts.

Results: We enrolled 72 cases (mean age 48 ± 16 years, F 51%, protein [P]C 13, PS 23, antithrombin 10, homozygous factor V Leiden (FVL) or prothrombin mutation 11, compound heterozygous 13, FVL pseudohomozygous 1, antithrombin resistence 1) and 82 controls (mean age 44 ± 18 years, F 53%, PC 24, PS 26, antithrombin 13, homozygous FVL or prothrombin mutation 6, compound heterozygous 11, FVL pseudohomozygous 2). 21 patients initially treated with VKA and then with DOACs (PC 4, PS 5, antithrombin 3, homozygous FVL 2, compound heterozygous 3, FVL pseudohomozygous 3, antithrombin resistance 1) were also considered. Among cases, 3% were treated with dabigatran, 70% with rivaroxaban, 19% with apixaban and 8% with edoxaban. We documented 3 (3.2%) VTE recurrences in cases and 4 (3.8%) in controls, with IR of 2.23 per 100 p-y and 2.16 per 100 p-y, respectively, and RR 1.03 [95%CI 0.19-4.98]. We found 7 (7.5%) minor or clinically relevant bleeding complications in cases and 3 (2.9%) in controls, IR 5.2 per 100 p-y and 1.6 per 100 p-y, respectively, RR 3.21 [95%CI 0.84-15.2]. Similar incidence of post-thrombotic syndrome was observed between groups.

Conclusions: DOACs showed an efficacy and safety profile comparable with heparin/VKA in severe thrombophilic patients. A slight increase in menorrhagia was observed.

TABLE 1 VTE recurrence according to thrombophilia type and bleeding complications

	Thrombophilia and DOACs	Thrombophilia and heparin/AVK
VTE recurrence	Deep vein thrombosis in homozygous FVL during rivaroxaban	Deep vein thrombosis in compound heterozygous during warfarin
	Superficial vein thrombosis in protein C deficiency during rivaroxaban	Splanchnic vein thrombosis in protein S deficiency during heparin
	Deep vein thrombosis in FVL pseudohomozygous during apixaban	Deep vein thrombosis in protein C deficiency during warfarin
		Superficial vein thrombosis in protein S deficiency during heparin
Bleeding	1) Hospitalization for menorrhagia (clinically relevant) 2) Hospitalization for metrorrhagia (clinically relevant) 3) Menorrhagia (minor)	2 patients with recurrent conjunctival hemorrhage (minor)
	Iron deficiency anemia with the need for transfusion support (clinically relevant)	Hematuria (minor)
	Hospitalization for hematuria (clinically relevant)	
	Hemorrhoids hematochezia (minor)	
	Gingivorrhagia and hematuria (minor)	

PB2248 | Thrombophilic Mutations in Macedonian Women with Adverse Pregnancy Outcome

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Background: Adverce pregnancy outcome is multifactorial condition. Many studies reported the possible relationship between thrombophilia and adverse pregnancy outcome especially pregnancy loss. Inherited thrombophilias (mutations of the Factor V Leiden (FVL) G1691A gene, the methylentetrahydrofolate reductase (MTHFR) C677T gene and Factor II gene Prothrombin G20210A) are risk factors for thromboembolic events.
Aims: The aim of the study was to examine the representation of thrombophilic mutations in women with pregnancy loss and healthy controls.
Methods: In this case-control study we evaluated 79 women, divided in two groups. The study group included 43 women with history of pregnancy loss (missed abortion, blighted ovum, miscarriage in the first or second trimester, foetus mortus in utero). The control group included 36 women, age matched, who gave birth to at least one healthy baby without obstetric complications. All of the women included in the study have signed the informed consent approved by the Ethical Committee of the Medical faculty in Skopje. Presence of gene mutations for prothrombin G20210A, FVL and MTHFR C677T was examined in both groups.
Results: Prothrombin G20210A heterozygous was found in 23.5% of the study group vs. 5.5% of the control group; FVL heterozygous was found in 23.5% of the study group vs. 2.8% of the control group; MTHFR homozygous was found in 48.8% of the study group vs. 5.5.% of the control group with a significant statistical difference ($p < 0.05$).
Conclusions: The presence of thrombophilic mutations may predispose to pregnancy loss.

PB2249 | Upregulation of Factor XI in End Stage Renal Disease Is Associated with Increased Thrombin Generation and D-dimer

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Background: Vascular access is the most commonly observed complication in end stage renal disease (ESRD). Increase coagulation factors, decreased levels of inhibitors along with vascular dysfunction contribute to the thrombotic complications in ESRD.
Aims: The purpose of this study is to profile ESRD patients for factor XI, d-dimer and thrombin generation parameters.
Methods: Citrated blood samples from 95 ESRD patients undergoing hemodialysis were collected from the dialysis unit. Control samples were comprised of 50 healthy male and female plasma samples was obtained from George King Biomedical (Overland park, KS). Samples were analyzed for d-dimer and factor XI antigen levels by using a commercially available ELISA methods. In the ESRD cohort endogenous thrombin generation profile was also measured by using a Fluor kinetic method. Such parameters as peak thrombin (PT), area under the curve (AUC) and lag time (LT) were measured. Individual results were compiled and expressed as group means ± SD.
Results: ESRD patients showed significantly elevated levels of factor XI (1.43 ± 0.6 IU/ml) in contrast to the normal (0.91 ± 0.19 IU/ml). D-dimer levels were also markedly elevated in the ESRD group (1059 ± 1355). Thrombin generation parameters showed wide variations with the cumulative mean of (151 ± 97 nM) in the ESRD group, which were lower in comparison to normal plasma (171.5 ± 26.4 nM). Significant differences in the LT and AUC parameters were also noted.
Conclusions: These results clearly suggest that ESRD patients exhibit endogenous activation of coagulation which may be related to increase factor XI levels. This may be related to activation of the intrinsic pathway of coagulation. Therefore, targeting factor Xi in these patients using various different approaches provides an optimal option in the control of thrombogenesis.

VISCERAL VEIN THROMBOSIS

PB2250 | Epidemiology and Clinicopathology of Latent versus Active JAK2-Myeloproliferative Neoplasms (MPN) in Splanchnic Venous Thrombosis

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Background: Splanchnic vein thromboses (SVT) often cause Budd-Chiari syndrome (BCS). JAK2-MPN is the most common cause of SVT/BCS. Endothelial cells harboring JAK2 mutations have been demonstrated in patients with BCS which may contribute to the overall prothrombotic propensity.
Aims: This study attempts to describe the epidemiology and clinicopathology of latent JAK2-MPN (LMPN), where JAK2 mutation is detected without clinical evidence of MPN, versus active JAK2-MPN (AMPN) where JAK2+ MPN is present, in patients with SVT/ BCS.
Methods: We compiled a pooled database of 90 cases with SVT/BCS. Kaplan-Meier survival curves, Cox proportional-hazards model, and Log-rank tests were used to assess the influence of demographic and clinical factors on survival.
Results: The SVT/BCS cohort consisted of 65% LMPN and 35% AMPN with median ages of 39 and 42 years respectively. The peak incidence of SVT/BCS seems to occur between ages 36-48 for

LMPN and 24-36 for AMPN. Females were twice more predominant than males. The AMPN group consisted of 41% Polycythemia Vera (PV), 22% Essential Thrombocythemia, 28% Myelofibrosis (MF) and 3% others. There was no difference in the type of the splanchnic vein involvement or the number of coexisting acquired or inherited conditions between the two groups. Survival of patients with BCS did not differ by their MPN status or sex. 21% of LMPN progressed to AMPN (57% ET, 21.5% PV, and 21.5% MF). The median time to progression was 55 months among those that progressed. While the median time to progression was longer for males (63 vs 39 months) this difference did not reach statistical significance.

Conclusions: This study describes for the first time the epidemiologic and clinical characteristics of patients with SVT/BSC by their MPN status. It reveals that the majority of LMPN do not progress to AMPN, indicating that LMPN prothrombotic propensity is independent of the hematologic AMPN clinical expression.

PB2251 | Association of Portal Vein Thrombosis and Clinical Outcomes in Cirrhotic Patients: A Nested Case-Control Study

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Background: There are limited data with regards to the prevalence, risk factors and clinical outcomes in cirrhotic patients who have Portal vein thrombosis (PVT).

Aims: The aims of this study was to investigate the prevalence and risk factors of PVT in cirrhotic patients. In addition, we sought to investigate the association between the PVT and clinical outcomes in cirrhotic patients.

Methods: A single center nested case-control study was conducted between January 2006 and December 2018. Consecutive patients who were diagnosed with cirrhosis were screened. Cases were selected if patients had a subjective evidence of PVT. Controls were randomly selected in the same cohort using an 1:1 ratio, matching by age, sex and date of the diagnosis of cirrhosis.

Results: A total of 6,071 cirrhotic patients were included in this study. The prevalence of PVT in cirrhotic patients was 2.7% (95% confidence interval [CI] 2.2%-3.1%).. On the multivariable analysis, the independent risk factors of PVT in cirrhosis were Child-Pugh B or C (odds ratios [OR]=3.20, 95%CI: 1.77-5.76, P< 0.001), history of variceal bleeding (OR=2.61 95%CI: 1.33-5.11, P=0.01) and hepatocellular carcinoma (HCC) (OR=10.46, 95%CI: 5.97-18.33 P< 0.001). Among patients PVT who were left untreated, 59.6% had a progression of PVT. Patients with PVT and HCC had the significant higher risk of death when compared with cirrhotic patients who did not have PVT or HCC with a hazard ratio [HR] of 6.59, 95%CI 4.60-9.46, Figure A. A median follow-up period of patients in this cohort was

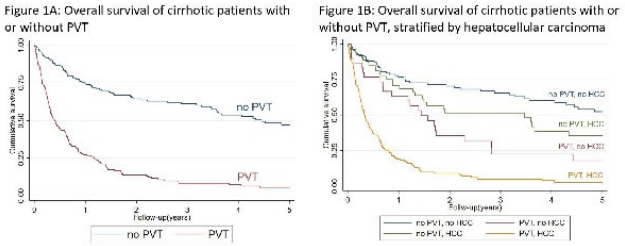

FIGURE 1 Overall survival

2.2 years. Figure B demonstrates the overall survival of cirrhotic patients according to HCC and PVT status.

Conclusions: The findings of this study suggest that patients with advance cirrhosis (Child-Pugh B/C or history of variceal bleeding) or those with HCC should be systematically investigated for PVT. Among patients with PVT who are not anticoagulated, majority of these patients have progression of PVT.

PB2252 | Case report: Renal Vein Thrombosis Associated with Oral Contraceptive Use and Posterior Nutcracker Syndrome

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Background: Nutcracker Phenomenon (NCP) complicated with visceral venous thromboembolism (VTE) is a rare occurrence. Although, peripheral VTE is commonly associated with Oral Contraceptive Pill (OCP) use, visceral VTE secondary to OCP use and in the presence of Posterior Nutcracker syndrome is rarely reported.

Aims: We describe a unique case of a young female who presented for the first time with Left Renal Vein (LRV) thrombosis in association with two risk factors: use of OCP and Posterior Nutcracker Syndrome (PNCS).

Methods: We present the case of a 37-year-old, Saudi female with no known comorbidities (apart from history of severe Gastro Esophageal Reflux Disease (GERD)), who presented to the emergency department with a history of left flank pain for the past three days. The pain was sudden in onset, sharp and constant,relieved partially with paracetamol. She denied fever or any urinary symptoms. She admit using combined oral contraceptive pills for the last 10 years. Enhanced CT scan abdomen and pelvis revealed LRV follows a retroaortic course and appears significantly dilated with intraluminal filling defect extending from renal hilar veins till retroaortic part of LRV which appears compressed at that site and associated with multiple perirenal and periureteric collaterals. These features all suggest PNCP associated with LRV thrombosis.

With the symptomatology of this patient and the CT findings, the case is proven to be PNCS complicated with LRV thrombosis.

Results: We reported a very rare association of LRV thrombosis, posterior nutcracker syndrome and use of oral contraceptive pills.

Conclusions: Visceral thrombosis, in the form of left renal vein thrombosis, should be suspected even with the long term use of OCP beyond 5 years and the presence of other risk factors such as PNCP.

PB2254 | Anticoagulant Therapy for Splanchnic Vein Thrombosis: A Systematic Review and Meta-Analysis

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Background: Treatment of splanchnic vein thrombosis (SVT) remains controversial.

Aims: In this systematic review and meta-analysis, we evaluated the efficacy and safety of anticoagulant therapy in patients with SVT that was unprovoked or associated with transient or persistent risk factors.

Methods: MEDLINE, EMBASE, and clinicaltrial.gov were searched up to December 2019. Studies reporting the rate of SVT recanalization or progression, recurrent VTE, major bleeding, and overall mortality were included. Pooled proportion and risk ratio (RR) with corresponding 95% confidence intervals (CI) were calculated using a random-effects model.

Results: A total of 7,969 patients were included in 98 studies (95 observational studies and 3 randomized controlled trials). During anticoagulation, 58% (95% CI, 51-64) of patients achieved partial or complete recanalization, while 5% (95% CI, 3-7) experienced SVT progression. Recurrent VTE, major bleeding, and overall mortality occurred in 12% (95% CI, 9-16), 9% (95% CI, 7-12), and 11% (95% CI, 9-14) of patients, respectively. The clinical and study-related variables included in subgroup analysis and mixed-effect model explained only a very small part of the inter-study heterogeneity.

In patients in whom anticoagulation was withheld, the rate of SVT recanalization, SVT progression, recurrent VTE, major bleeding, and overall mortality were 22% (95% CI, 15-31), 16% (95% CI, 8-28), 14% (95% CI, 10-21), 16% (95% CI, 13-20), and 25% (95% CI, 20-31), respectively. Forty-nine studies which compared anticoagulation with no treatment showed significant higher recanalization rates (RR 2.39; 95% CI, 1.66-3.44) and lower rates of thrombosis progression (RR 0.24; 95% CI, 0.13-0.42), major bleeding (RR 0.73; 95% CI, 0.58-0.92), and overall mortality (RR 0.45; 95% CI, 0.33-0.60) in patients receiving anticoagulation.

Conclusions: Anticoagulant therapy improves SVT recanalization and reduces the risk of thrombus progression, without increasing major bleeding rates. The incidence of recurrent VTE remains substantial also in anticoagulated patients.

PB2255 | A Retrospective Cohort Study on Splanchnic Vein Thrombosis

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Background: Clinical manifestations and optimal management strategies in patients with splanchnic vein thrombosis (SVT) are not well characterized.

TABLE 1 Outcomes of treatment in patients with SVT

	Anticoagulation	No Anticoagulation	Primary SVT	Secondary SVT
Thrombosis status				
Complete resolution	37%	29%	30%	38%
Partial resolution	26%	-	18%	27%
Unchanged	31%	71%	46%	29%
Worsening	6%	-	6%	6%
Major Bleeding	6%	10%	8%	6%
Any Bleeding	10%	30%	8%	12%
Death	13%	20%	3%	17%

Aims: To describe our experience and treatment outcomes in patients with SVT.

Methods: We conducted a retrospective cohort study including all newly diagnosed SVT evaluated at the Thrombosis Clinic of our institution between January 2007 and December 2018. Patient demographics, clinical and treatment information were collected using standardized forms. Efficacy outcome was thrombosis resolution, and safety outcomes included death and occurrence of bleeding. Groups were compared using t-test or Fisher's exact tests as appropriate.

Results: We included 155 patients (60% males) with a mean age of 56.2 (18-87). Local risk factors were present in 118 (76.1%) patients (secondary SVT), whereas 37 (23.9%) had unknown or only systemic/thrombophilia (primary SVT). Abdominal cancers (31%), surgery (20.6%) and liver cirrhosis (19.4%) were common local risk factors. Thrombophilia screening was conducted in approximately 50% of patients. Factor V Leiden or Prothrombin G20210A mutations were observed in 7.1% of patients whereas 14.4% were *JAK2*V617F mutation positive. Most common manifestations at onset was abdominal pain (56.1%), whereas incidentally found SVT was 44.6%. Portal vein thrombosis was observed more in primary cases (91.9% vs. 69.5%, p=0.012). Anticoagulation was used in 93.5% cases whereas localized therapy was used only in 5.8%. Indefinite anticoagulation was used more frequently in primary SVT (62.2% vs. 41.5%, p=0.045). Outcomes of treatment in patients with SVT are shown in the table. No statistical difference between patients with and without local risk factors or with or without anticoagulation was observed in either thrombosis resolution, death, or bleeding.

Conclusions: This study suggests that in SVT patients anticoagulation results in partial or complete thrombosis resolution in a significant proportion of patients with an acceptable bleeding risk and that outcomes are similar between patients with or without local risk factors.

PB2256 | Portal Vein Thrombosis Revealing Paroxysmal Nocturnal Hemoglobinuria

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquiredhematologic condition characterizedwithvariousclinical manifestations.

Aims: We report here the case of PNH revealed by portal thrombosis in a 28 yearold man to emphasize on such a complication and how to manage it.

Methods: Case report.

Results: A 28 year-old male with no medicalhistorywasadmitted to the emergency room for severe abdominal pain evolving for 3 days. On examination he was a febrile with marked pallor, scleralicterus, abdominal distension and diffuse epigastric tenderness associated with splenomegaly. Laboratory investigations showed pancytopenia with severe regenerative normocyticanemia. Serum bilirubin was increased with predominant indirect bilirubin component associated to elevated Lactate dehydrogenase (LDH) level. Direct antiglobulin test was negative. A computerized tomography (CT) scan wasperformedshowing acute right and left portal vein thrombosis. PNH was suspected confirmed with flow cytometry.

Anticoagulation therapy using low molecular weight heparin was started associated with folic acid and blood transfusion.

Treatment with monoclonal antibody Eculizumab could not be used because of its non availability.

A slight improvement was initially noted with relief of his abdominal pain and decrease of LDH but few days later he presented a sub-occlusive syndrome with dyspnea and acute hemolysis. He was admitted than in the intensive care unit where he dead because of massive pulmonary embolism.

Conclusions: PNH is a rare acquired hematologic disorder with various clinical manifestations. Thrombosis can be the first manifestation of this disease. Prognosis has significantly improved owing to Eculizumab.

PB2257 | Essential Thrombocythemia Complicated with Portal Vein Thrombosis and Liver Cirrhosis - A Case Report

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Background: Essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by excessive, clonal platelet production and increased risk of thrombotic complications.

Aims: Case report of a patient with ET complicated with chronic portal vein thrombosis (PVT) with liver cirrhosis, and treatment approach.

Methods: A 27-year-old man was admitted in the clinic for diagnostic work-up and therapy because of high platelet count (Plt) 908x10^9/l. He had 4 year history of hepatosplenomegaly, esophageal varices gr.III, and high Plt. The patient was recently confirmed with chronic PVT with collateral circulation, portal hypertension, liver cirrhosis ChildA, ascites, and was considered for TIPS. At the time of admission he was not taking any platelet reduction or anticoagulant therapy.

Results: Blood count showed Hb 148g/l, WBC 6.61x10^9/L, Plt 716x10^9/l; blood smear was normal. ET was diagnosed based on bone marrow biopsy which showed megakaryocytes proliferation without fibrosis, and positive JAK2V617F mutation. IPSET score showed high risk of thrombosis and because of comorbidities complete tests of hemostasis were performed. Thromboelastography (TEG) revealed a hypocoagulation state with increased fibrinolytic activity (r=14.6min,k=5.4min,CI=-9.6), moderately decreased

synthesis of clotting factors with normal synthesis of coagulation inhibitors; INR 1.35, APTT 41.1s, fibrinogen 2.35, APC-R 292.6s, AT-III 91, proteinC 62%, proteinS 78%. TEG showed normal platelet function, 100% aggregation and 0% inhibition with ADP and arachidonic acid. Coagulation abnormalities were presumably from patient's liver disease and with the presence of varices the risk of bleeding was considered substantial. Cytoreduction therapy with hydroxyurea 1g/d was started, with aspirin 100 mg/d, but the patient was not given anticoagulation treatment, as chronic PVT was asymptomatic and combined with cirrhosis. One month later the patient had stable Plt 300x10G/l and was referred to gastroenterologist for further treatment.

Conclusions: Chronic PVT complication with cirrhosis in a patient with ET requires detailed investigation of hemostasis and careful, complex treatment approach.

PB2258 | Budd Chiari Syndrome: Beyond 5 Years of Follow up

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Background: Budd Chiari Syndrome (BCS) incidence is 0.5-1 case per million per year. 55-75% are women. There is an underlying disease in the 75% of the cases. Fifty percent of BCS is associated to Philadelphia negative myeloproliferative neoplasms (MPN), being policitemia vera the most frequent one. Forty percent of MPN are JAK2 positive. One third of the BCS are caused by thrombophilia.
The severity of clinical presentation depends on extension and velocity of vein occlusion. Anticoagulation is the main treatment. If clinical manifestations are severe or if there is not response to anticoagulation, it is mandatory to perform endovascular intervention or surgery.

TABLE 1 Patients characteristics

	N 6
Budd Chiari Syndrome evolution	
Acute	2
Chronic	1
Not specified	3
Symptomatic at diagnosis, n	6
Negative Philadelphia myeloproliferative neoplasms , n	5
Polycytemia Vera, n	2
Not specified, n	3
Jak2 Positive, n	5

Table 1- Patients characteristic

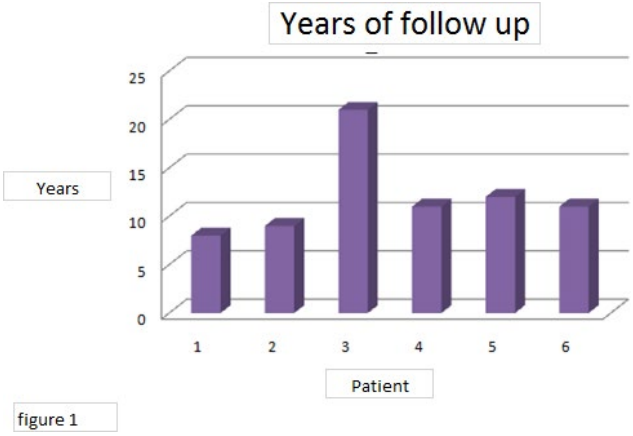

FIGURE 1 Years of follow up

Aims: To describe clinical characteristics, hematological diseases associated and treatment in patients with BCS with more than 5 years of follow up.

Methods: Retrospective review of 6 patients with BCC treated at a university center in Buenos Aires with a follow up of more than 5 years

Results: Six patients were evaluated. Three patients were women (table 1). The median of age at diagnosis was 25 years (IQR 19-30 years) (figure 1). Jak2 positive MPN were present in 5 patients, all of them were treated with hydroxyurea and anticoagulation. Five patients were treated with invasive procedures: 5 transyugular intrahepatic portosystemic shunt (TIPS), 1 angioplasty, 2 thrombus aspirations. None of the patients presented complications during the procedure and all of them are alive. The median of follow up was 11 years (IQR 8-21). In 3 cases, TIPS stenosis developed after a median time of 26 months (IQR 12-74). Angioplasty with TIPS revision and Stent collocation was successfully performed in all these patients.

Conclusions: The combination of anticoagulation and invasive procedures is an interesting option to treat these patients, achieving excellent evolution. It is mandatory to treat the underline disease as well. A multidisciplinary approach is important to guarantee success.

VTE DIAGNOSIS

PB2260 | Dynamic Upper Limb Ultrasound in Idiopathic Upper Extremity Deep Vein Thrombosis

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Background: Dynamic upper limb ultrasound (DULUS) is often undertaken in the investigation of thoracic outlet syndrome (TOS) to assess compression of thoracic outlet structures, such as the subclavian vein (SCV) in venous TOS (VTOS), subclavian artery (SCA) in

arterial TOS and brachial plexus in neurogenic TOS. DULUS measures peak systolic velocities (PSV) in the subclavian artery (SCA) at various degrees of abduction as well as assessing SCV compression. However, there is no data on expected values in subjects with antecedent idiopathic upper extremity deep vein thrombosis (IUEDVT).

Aims: To describe DULUS findings in upper limbs with and without antecedent IUEDVT.

Methods: We reviewed all DULUS performed between January 2014 and May 2019 at Monash Health, Melbourne. At our institution, SCA PSV at neutral, 45°, 90° and 130° abduction, military brace (MB) and reverse stop (RS) positions are recorded. We expressed the PSV obtained at these positions over neutral as the PSV ratio (Figure 1). Other measures included assessment for SCV compression at 90° abduction.

Results: DULUS on 27 upper limbs were included (9 males, mean age 37 years [range 26-54]). Nine sides had antecedent IUEDVT and 18 had not. Age and gender were not different between the two groups. 10 patients had right-sided symptoms. We found SCA PSV ratios at 90° and 130° abduction were higher in the IUEDVT-affected sides compared to non-IUEDVT affected sides (Figure 2). Nearly all cases demonstrated some degree of SCV compression at 90° abduction.

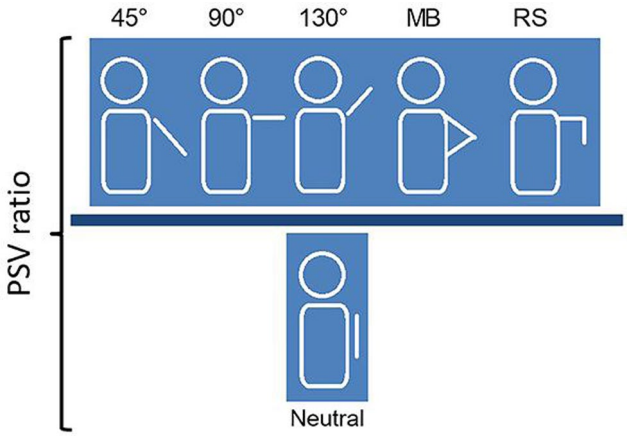

FIGURE 1 Subclavian artery peak systolic velocity ratio

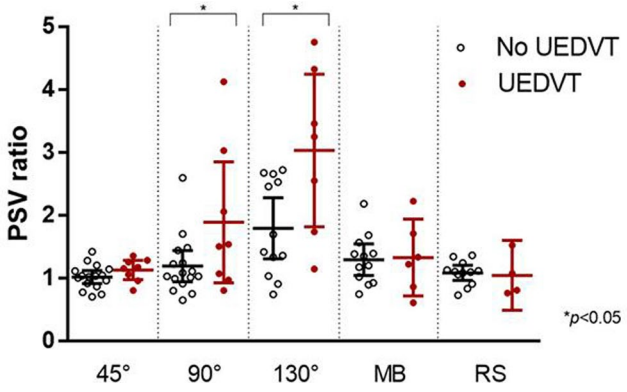

FIGURE 2 Subclavian artery PSV ratio at different positions

Conclusions: On DULUS, upper limbs with antecedent IUEDVT had higher SCA PSV ratios at 90° and 130° abduction than non-IUEDVT sides but compression of the SCV was commonly observed at 90°in both groups. The clinical significance of these observations is uncertain; whether these findings correlate with subsequent or prior IUEDVT requires further study.

PB2261 | D-Dimer is Useful in the Assessment of Suspected Recurrent Venous Thromboembolism in Patients on Rivaroxaban or Apixaban

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Background: Rivaroxaban and apixaban are increasingly being used to treat venous thromboembolism (VTE). Whilst on these agents, the use of D-dimer in the prediction of recurrent VTE (rVTE) is yet to be established.

Aims: To assess the efficacy of D-dimer in the assessment of suspected symptomatic rVTE (deep vein thrombosis [DVT] or pulmonary embolism [PE]) in patients on rivaroxaban or apixaban for previous VTE.

Methods: Chart review of patients on rivaroxaban or apixaban for previous VTE presenting with suspected rVTE from March 2016 to January 2020 at Monash Health, Melbourne. Only patients who had an anti-Xa drug level (IL Test on the ACL TOP analyser), concurrent D-dimer (IL Test D-dimer HS on the ACL TOP analyser) and objective imaging were included. Two haematologists and a radiologist reviewed indeterminate rVTE cases. The primary outcome was accuracy of D-dimer in predicting rVTE utilising our standard D-dimer threshold, 0.23mg/l.

Results: Of 57 potentially eligible patients, 5 were excluded due to inability to confirm rVTE based on available clinical and radiological evidence (See Table 1 for included patient characteristics). Suspected rVTE occurred 5.1 months post index VTE (median, range 0.03-121) with anti-Xa

TABLE 1 Patient characteristics

Characteristics	N = 52
Age (median, range, years)	51 (17-85)
Male (%)	46
Apixaban (%)	37
Apixaban level at suspected rVTE (median, range, ng/ml)	100 (14-403)
Rivaroxaban level at suspected rVTE (median, range, ng/ml)	102 (10-538)
Time from index VTE (median, range, months)	5.1 (0.03-121)
Confirmed rVTE (%)	21
Greater than 30-day follow-up period post suspected rVTE (% of not confirmed VTE)	90

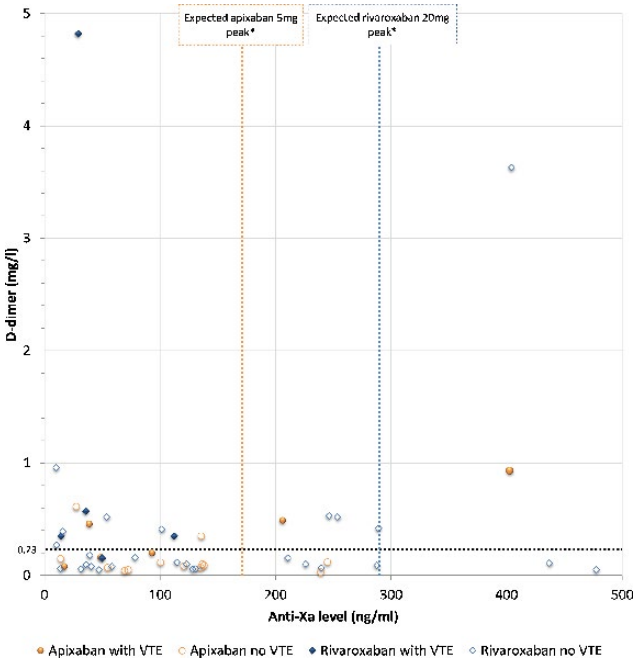

FIGURE 1 Anti-Xa and D-dimer results in patients with suspected rVTE

levels and D-dimer shown in Figure 1. Subtherapeutic anti-Xa levels (< 30ng/ml) were detected in 17%. Suspected rVTE comprised DVT, PE or both in 31%, 65% and 4% respectively. rVTE was subsequently confirmed in 21% of which 73% were PE. D-dimer sensitivity was 64% (95%CI 31-89) and specificity was 73% (95%CI 57-86%), with 55% of false positives from suspected rVTE within one month of index VTE.

Conclusions: In patients with suspected rVTE who are taking rivaroxaban or apixaban with detectable drug activity, the IL Test D-dimer is not universally suppressed and the routine laboratory threshold of 0.23mg/l may retain its utility.

PB2262 | Serial Ultrasounds in Deep Vein Thrombosis Diagnosis, a Single Centre Retrospective Review

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Background: Diagnosis of Deep Vein Thrombosis (DVT) involves the combination of a clinical prediction model, D-dimer testing and radiographic imaging. Practice guidelines recommend initial diagnostic test with compression duplex ultrasound of the lower extremities in patients with a high clinical pretest probability for DVT. A negative initial ultrasound is further stratified with a d-dimer assay and/or one week follow up ultrasound.

Aims: To investigate the local pattern of practice of performing compression ultrasound one-week following initial presentation in patients with suspected DVT whose initial ultrasound study is negative. The project quantifies the number of one-week ultrasound studies performed

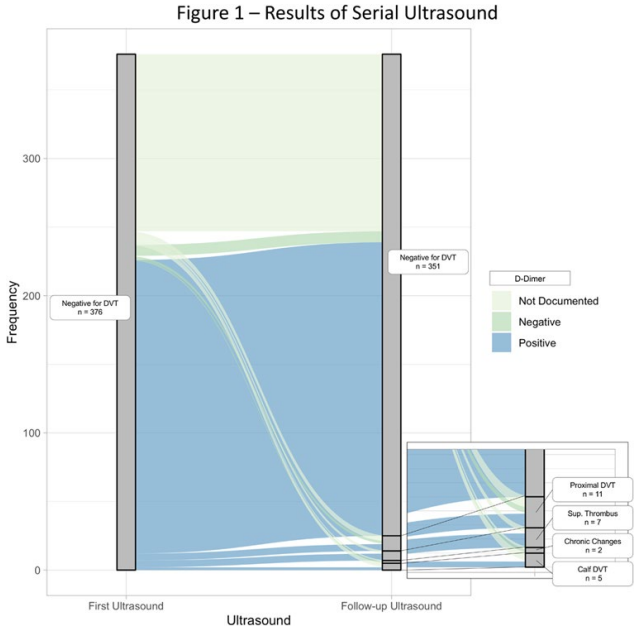

FIGURE 1 Results of serial ultrasounds performed between Jan 2012 and Dec 2016 in the Nova Scotia Health Authority Central Zone

at hospitals in the Nova Scotia Health Authority Central Zone and identifies the proportion of one-week ultrasounds that convert to positive.

Methods: Patients who received two compression ultrasounds of the extremities within a timeframe of 5 to 10 days between January 2012 and December 2016 were screened.

Results: 639 sets of paired ultrasounds of the extremities were screened. Paired ultrasounds with discordant laterality or of the upper extremities were excluded. 376 pairs of studies with negative initial ultrasounds from 362 patients were included for analysis. Of these 376 studies, 2.9% (95% CI: 1.1 - 4.8%) from 11 patients were then found to have a proximal DVT at 1 week follow up. 7 studies reported superficial thrombophlebitis, 5 as below knee DVT and 2 as chronic thrombotic changes at one week follow up. Stratification by d-dimer testing is shown where available. Results are shown in Figure 1.

Conclusions: In this group of patients who underwent serial ultrasonography of the lower extremities for suspected DVT, 11/376 with negative initial ultrasounds were positive for DVT at one week. The recommended practice of performing serial ultrasounds allows for detection of the development of DVT in a selected population.

PB2263 | Ten Years of Venous Thromboembolism Events in a Large Teaching Hospital: Changing Patterns of Disease

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Background: University Hospitals Plymouth is a 900 bedded teaching hospital designated a venous thromboembolism (VTE) exemplar site in 2009 reflecting work in reducing hospital acquired thrombosis (HAT).

TABLE 1 Venous Thromboembolism events 2010 to 2019

Year	Total VTE	All DVT (%)	All PE (%)	HAT DVT (%)	HAT PE (%)
2010	649	329 (51)	320 (49)	85 (42)	116 (58)
2011	782	325 (42)	457 (58)	73 (31)	165 (69)
2012	793	311 (39)	482 (61)	71 (33)	146 (67)
2013	805	342 (42)	463 (58)	66 (38)	106 (62)
2015	835	340 (41)	495 (59)	60 (31)	131 (69)
2016	852	384 (45)	468 (55)	64 (38)	105 (62)
2017	836	329 (39)	507 (61)	56 (32)	119 (68)
2018	825	338 (41)	487 (59)	65 (35)	121 (65)
2019	769	315 (41)	454 (59)	51 (28)	132 (72)

Aims: The study was to look at the impact of national guidance on VTE prevention comparing hospital acquired with community acquired events and compare number of deep vein thrombosis (DVT) with pulmonary embolism (PE) events.

Methods: All VTE events from targeted investigations comprising CT pulmonary angiograms, SPECT-CT scans and Doppler ultrasounds of the upper and lower limbs were collected between January 1st 2010 to the 31st December 2019. Positive events are reviewed to see if they meet the HAT criteria.

Results: In 2010 there were 649 total VTE events with 329 DVT's and 320 PE's. Of these 201 (31%) were HAT events, comprising 85 DVT and 116 PE; a significantly higher number of PE compared to DVT (p=0.005). Over 10 years total VTE has increased by 120 to 769 (18%). Over the same period total DVT's diagnosed has remained relatively constant with 315 in 2019, a 4% decrease. In 2019 454 PE's were diagnosed, giving an increase of 42% (from 320) over ten years showing steady annual increases. In 2010 PE and DVT diagnoses were similar numbers but by 2019 the split of PE compared to DVT was 59:41 compared with 49:51 previously. For HAT events DVT numbers have fallen from 85 events (42%) in 2010 to 51 (28%) in 2019 whilst total HAT has also reduced.

Conclusions: The overall reduction in HAT is likely to be associated with improved awareness of thrombosis and increased VTE prevention in hospitalised patients. Total PE increases may be explained by more sensitive CT scanners able to detect smaller thromboses. We have previously shown HAT reduction is significant and the challenge is to further reduce these numbers.

PB2264 | Elevated von Willebrand Factor Antigen Levels and Ristocetin Cofactor Activity are Risk Factors in Predicting Deep Vein Thrombosis

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Background: Deep vein thrombosis (DVT) is a significant cause of morbidity and mortality, especially a sudden death due to acute pulmonary embolism. Elevated levels of coagulation factor VIII have been associated with increased risk for venous thromboembolism (VTE). The role of Von Willebrand factor (VWF) in VTE is unclear.

Aims: In this study, we investigated the association of raised levels of VWF antigen (VWF:Ag) and VWF ristocetin cofactor activity (VWF:Rco) in patients with Deep vein thrombosis (DVT).

Methods: 60 patients of VTE and 60 age and gender matched controls were tested for levels of VWF:Ag and VWF:RCo by ELISA,fluorescence resonance energy transfer(FRET)was used to detect the activity of ADAMTS13. The study conformed to the ethical guidelines of 2004 Declaration of Helsinki and was approved by the institutional Ethics Committee at the First Affiliated Hospital of Soochow University, China. The informed consent was obtained from all normal individuals and patients.

Results: Plasma VWF:Ag and VWF:Rco in VTE patients (mean±SD,771.8%±6.13 and153.3%±0.40) were significantly higher than those in controls (107.0%±0.19 and101.5±0.07, all $P < 0.0001$). The mean levels of ADAMTS13 activity (86.52%±0.14) in DVT patients was significantly lower in comparison with those in controls(97.45%±0.15,p=0.0011).The plasma VWF level was increased in DVT patients. In Pearson correlation coefficient, the VWF:Ag in DVT patients was positively correlated with plasma D-dimer (ρ=0.948). According to survival analysis, the AUC of D-dimer and VWF:Ag was respectively 0.974 and 0.988.

Conclusions: Both elevated VWF:Ag and VWF:Rco levels may be a thrombotic risk relationship with occurrence of DVT. It may be used as a new marker to value the clinical significance of VWF antigen (VWF:Ag), VWF ristocetin cofactor activity (VWF:Rco) and ADAMTS13 (A13) activity in predicting an ongoing thrombotic metabolism.

PB2265 | Is There a Predictive Value of High Mean Platelet Volume in Early Diagnosis of Venous Thromboembolism in the Emergency Department?

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Background: Platelet activation has an established role in the pathology of venous thromboembolism (VTE). The role of mean platelet volume (MPV), as a biomarker of platelets activation is not clear in the setting of VTE. Although several studies found a positive correlation between mean MPV and VTE, one recent study (the Parma study) - found a negative correlation between MPV and VTE (Lippi G, Buonocore R, Cervellin G. The mean platelet volume is decreased in patients diagnosed with venous thromboembolism in the emergency department. Semin Thromb and Hemost 2016;42:632-5).
Aims: The purpose of our study was to evaluate the role of MPV in differentiation of VTE from non-VTE, in the Emergency Department (ED), when the clinical presentation in both categories is of VTE, and to try to establish what the MPV size limits are in our hospital, when true VTE is diagnosed.
Methods: We conducted a retrospective case-controlled study of 316 VTE consecutive patients who presented to the ED of Galilee Medical Center, Nahariya, Israel. The control group was composed of 124 consecutive patients who presented to the ED with a picture of clinical VTE but without proven VTE.
Results: Higher MPV values were also found in the control group. The correlation of MPV values and VTE was examined with univariate logistic model. It was found that as MPV increases, a decrease in VTE incidence occurs (Odds ratio (OR) = 0.530, p< 0.001).
Conclusions: Statistical analysis of the acquired results indicates that MPV value cannot aid in the differential diagnosis of real VTE or in patients with VTE-like clinical picture presenting to the ED.

PB2266 | Optimizing Magnetic Resonance Direct Thrombus Imaging for Differentiating Acute from Chronic Splanchnic Vein Thrombosis

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Background: Timely diagnosis and treatment of splanchnic vein thrombosis (SVT), which includes portal vein, mesenteric vein, splenic vein and hepatic vein thrombosis, is crucial to prevent morbidity and mortality. However, currently available imaging tests are not able to accurately differentiate acute from chronic SVT, which is essential for adequate management. Magnetic resonance direct

Figure 1A-F. Axial computed tomography (CT) and magnetic resonance image (MRI) of the abdomen of a patient diagnosed with acute thrombosis in the portal and mesenteric veins; A. Contrast-enhanced CT shows a large luminal filling defect in the portal vein (arrow). B. MRI, 3D T1 TFE image shows a high signal intensity in the portal vein compatible with acute thrombus (arrow). C. MRI, 3D T1 Dixon FFE (water-only) image shows a high signal intensity in the portal vein (arrow). D. Contrast-enhanced CT shows filling defect in mesenteric vein (arrow). E. MRI, 3D T1 TFE image shows a high signal intensity in the mesenteric vein compatible with acute thrombus (arrow) F. MRI, 3D T1 Dixon FFE (water-only) image shows a high signal intensity in the mesenteric vein (arrow). *Note:* acute splanchnic vein thrombosis (SVT) is defined as an acute onset (< 2 weeks) of symptomatic SVT diagnosed with doppler ultrasonography, CT venography or MRI.

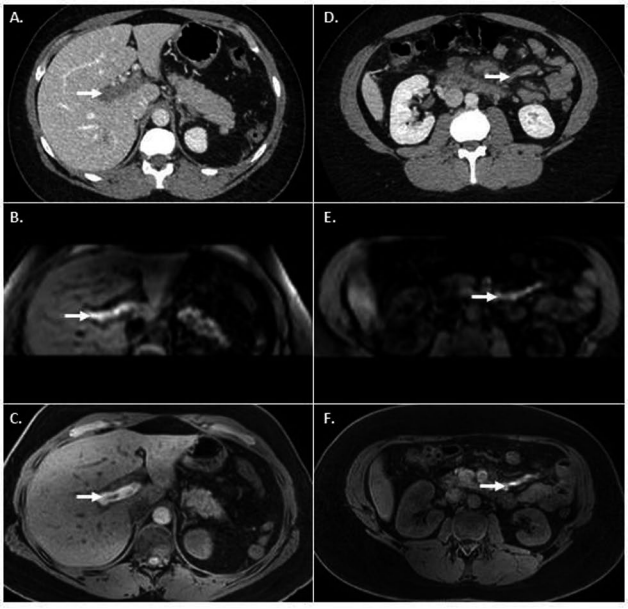

FIGURE 1

Figure 2A-B. A. Computed tomography (CT), coronal image of the abdomen in portal-venous contrast phase after intravenous contrast administration, showing large luminal filling defect caused by chronic thrombus in the portal vein (arrow). B. Magnetic resonance imaging (MRI), coronal 3D T1 Dixon FFE image of the same patient showing isointense signal intensity in the portal vein (arrow). On MRI, signal intensity of the filling defect in the portal vein is not increased, indicating chronic thrombosis. *Note:* chronic splanchnic vein thrombosis (SVT) is defined as non-symptomatic chronic SVT, defined by SVT with unchanged chronic thrombi on 2 serial imaging tests with at least 3 months interval.

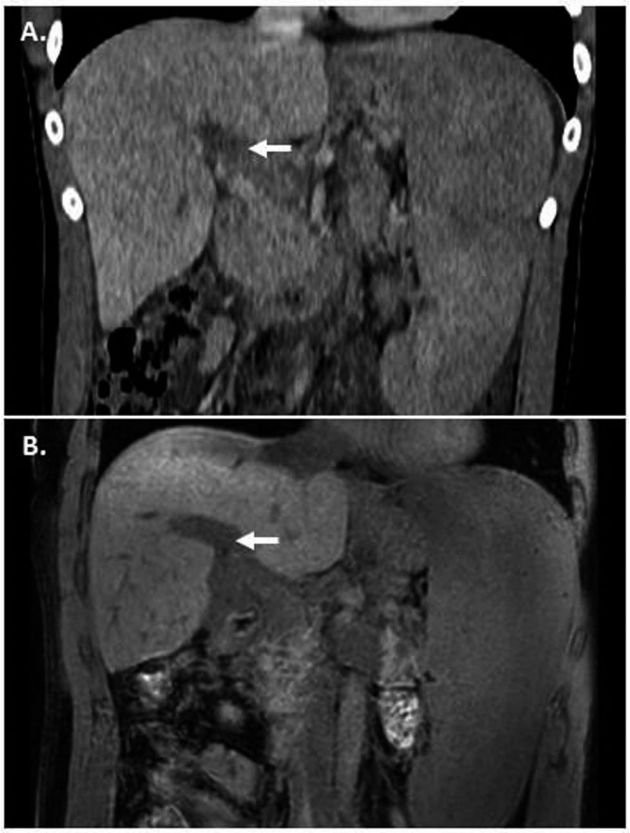

FIGURE 2

thrombus imaging (MRDTI) has been shown to accurately differentiate acute from chronic deep vein thrombosis in the leg and may also be of value in the diagnostic management of SVT.

Aims: This study describes the first optimization phase of the Rhea study (NTR 7061). Our aim was to select and optimize non-contrast-enhanced MRDTI sequences to differentiate acute from chronic SVT.

Methods: Literature was searched for MRDTI sequences, which could be of use for imaging of the splanchnic veins. The most promising sequences were tested in a healthy volunteer in several stages, followed by one patient with acute SVT and two patients with chronic SVT, all diagnosed on (repetitive) contrast-enhanced computed tomography venography, to optimize the MRDTI sequences. All images were evaluated by an expert panel. The Rhea study was approved by all local IRBs and patients provided written informed consent.

Results: Several MRDTI sequences were identified and tested; 3D T1 TFE and 3D T1 Dixon FFE were found to be the most optimal MRDTI sequences for imaging of SVT. The expert panel was able to confirm the diagnosis of acute SVT on the combined two MRDTI sequences (Figure 1) and to exclude acute SVT in the two patients with chronic SVT (Figure 2).

Conclusions: The combination of 3D T1 TFE and 3D T1 Dixon FFE was identified to be the most optimal for SVT imaging. These sequences will be used in the clinical part of the Rhea study in which their test performance on differentiating acute from chronic SVT will be determined.

PB2267 | Diagnosing Deep Vein Thrombosis in Patients with Suspected Symptoms and a Concurrent Malignancy: An Individual Participant Data Meta-Analysis

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Background: A previous individual participant data meta-analysis showed that the Wells-rule and D-dimer testing cannot exclude suspected deep vein thrombosis (DVT) in cancer patients.

Aims: The objective of this study was to explore reasons for this reduced diagnostic accuracy and to optimize the diagnostic pathway for cancer patients suspected of DVT.

Methods: Individual patient data from 13 studies in patients with clinically suspected DVT were used to compare DVT prevalence, and the predictive information of individual Wells-rule items, between patients with and without cancer. Subsequently, updating techniques were applied to improve the utility of the Wells-rule and D-dimer testing specifically for cancer patients.

Results: Among the 10,002 suspected DVT patients, there were 834 patients with cancer. The median prevalence of DVT in these patients with cancer was 37.5% (interquartile range [IQR] 30.8 - 45.5),

while it was 15.1% (IQR 11.5 - 16.7) in patients without cancer. Diagnostic performance of individual Wells-rule items and D-dimer testing was similar across patients with and without cancer, with the exception of 'immobility' and 'history of DVT'. The newly developed Wells-cancer rule showed a pooled c-statistic 0.80 (95% CI 0.75 to 0.83) and good calibration. However, using this model, still only 4.3% (95% CI 3.0 to 5.7) of the suspected patients with cancer could be identified with a predicted DVT post-test probability of less than 2%.

Conclusions: DVT is present in about 40% of cancer patients with symptoms or signs of DVT. Likely due to this high prevalence, clinical models followed by D-dimer testing fail to rule-out DVT efficiently in patients with cancer. Direct referral for compression ultrasonography appears to be the preferred approach for diagnosis of suspected DVT in patients with cancer.

PB2268 | Comparative Study between Thromboelastography and Conventional Coagulation Tests in Deep Vein Thrombosis

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Background: Deep vein thrombosis (DVT) mainly occurs as a result of coagulation disorder in the large veins of the legs. It could reduce or abolish blood flow, and/or changes to the vessel wall. Thromboelastography (TEG) is a novel platform for evaluating coagulation status, and could monitor the whole process from coagulation to thrombosis then to thrombolysis.

Aims: To investigate the consistency between TEG and conventional coagulation tests (CCTs) in DVT.

Methods: A total of 142 patients with DVT were involved. The correlation and consistency analysis between TEG and CCTs were assessed by the Spearman correlation and Kappa value. Receiver operating characteristic curve was selected to analyze the predictive value of TEG for abnormal results of CCTs.

Results:

(1). The correlation between TEG and CCTs were shown in Table 1;

(2). R and PT value, R and TT value, K and PLT value, K and FIB value, Angle and FIB value, MA and PLT value, MA and FIB value were accordant in valuing the hypocoagulable state of DVT patients, respectively;

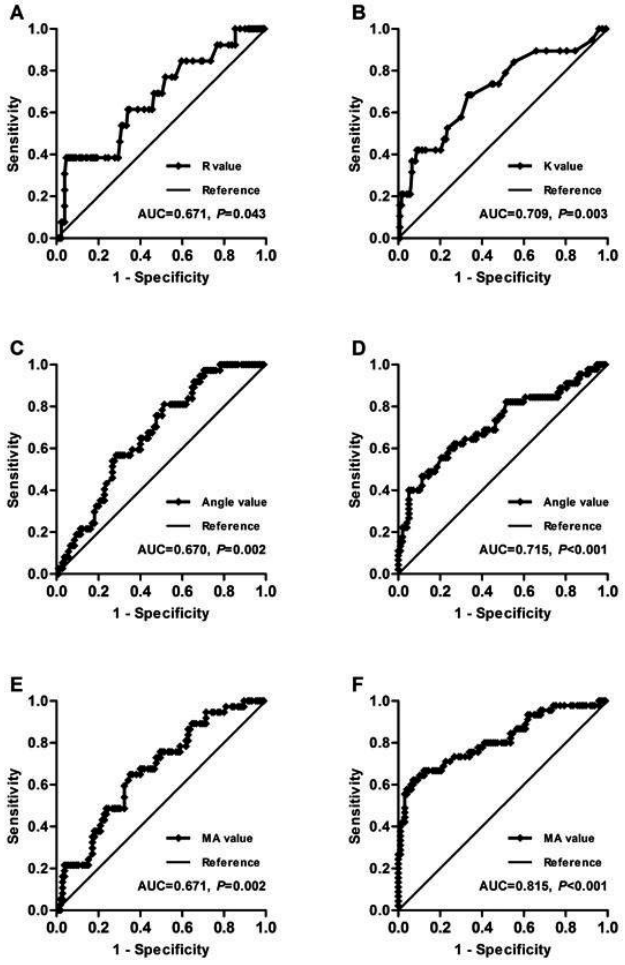

FIGURE 1 The predictive value of thromboelastography to evaluate abnormal results of CCTs by ROC curve

(3). R and APTT value, R and TT value, K and FIB value, Angle and TT value, Angle and FIB value, MA and PLT value, MA and FIB value were accordant in assessing hypercoagulable status of DVT patients, respectively;

(4). For detecting PT>14s, the area under the curve (AUC) of R was 0.671; For detecting PLT< 125×10^9/L, the AUC of K was 0.709; For detecting TT< 14s, the AUC of Angle and MA were 0.670, 0.671, respectively; For detecting FIB>4g/L, the AUC of Angle and MA were 0.715, 0.815 (Figure 1).

TABLE 1 Correlation analysis between thromboelastography and conventional coagulation tests parameters in Deep vein thrombosis

Parameters	R	R	K	K	Angle	Angle	MA	MA
	r	P	r	P	r	P	r	P
PLT	-0.071	0.401	-0.434	<0.001	0.437	<0.001	0.530	<0.001
PT	0.072	0.392	-0.012	0.883	-0.012	0.885	0.081	0.341
APTT	0.289	<0.001	0.087	0.302	-0.094	0.267	0.067	0.428
TT	0.337	<0.001	0.375	<0.001	-0.353	<0.001	-0.348	<0.001
FIB	-0.078	0.354	-0.503	<0.001	0.508	<0.001	0.679	<0.001

Conclusions: There was weak correlation and consistency between TEG and CCTs in DVT patients. The TEG have good predictive value in evaluating the abnormal results of CCTs. Combing the advantages of two methods will better reflect the coagulation status of DVT patients.

PB2269 | A Systematic Review and Meta-Analysis on Diagnostic Delay in Pulmonary Embolism

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Background: Prompt and early recognition of pulmonary embolism (PE) is paramount, given the associated mortality and morbidity if left untreated. However, this can be challenging due to non-specific signs and symptoms.
Aims: The purpose of this study was to gain insight into the prevalence of diagnostic delay and identify determinants that are associated with this delay. Therefore we systematically reviewed the literature and performed a meta-analysis on diagnostic delay in PE.
Methods: We performed a systematic literature search (Medline and Embase) to identify articles reporting on delay in diagnosing PE. Diagnostic delay was defined as the time between the onset of symptoms until confirmation of the PE diagnosis by diagnostic testing. The primary outcome of our study was diagnostic delay, which was expressed as either a mean delay or a percentage of patients with diagnostic delay. Secondary outcomes were determinants for such delay. Modified criteria based on the QUADAS-2 tool were used to assess the risk of bias. We performed random effect meta-analyses to calculate a pooled estimate for mean delay (including a 95% prediction interval) and to explore heterogeneity in subgroups.
Results: The literature search yielded a total of 8518 studies, of which 22 were included in our final analysis. The pooled estimate of the mean diagnostic delay was 6.3 days (95% PI 2.5 to 15.8 days). The percentage of patients having more than seven days of delay varied between 18% and 38%. Studies evaluating determinants associated with diagnostic delay show ambiguous and sometimes even conflicting results, with for no determinant having a clear association with or without delay.
Conclusions: Delay in diagnosing PE occurs frequently. Patients have experienced symptoms for nearly one week before diagnostic confirmation.

PB2270 | An Assessment of the Cost-Effectiveness of Whole-Leg Ultrasound Scans Compared to Proximal Leg Vein Ultrasound Scans in the Diagnosis of Acute Deep Vein Thrombosis

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Background: Guidelines from the National Institute of Clinical Excellence (NICE) suggest proximal leg vein ultrasound scans (USS) for patients with a suspected acute deep vein thrombosis (DVT), with a Wells score of 2 or more. If negative, a rescan of the proximal leg veins in 6-8 days is suggested. The guidelines recommend research into the cost-effectiveness of whole-leg USS compared with the current approach in the diagnosis of acute DVT.
Aims: To assess the cost-effectiveness of a whole-leg USS compared with proximal leg vein USS in the diagnosis of acute DVT.
Methods: We collected data on USS performed for the diagnosis of DVT. The data included the years 2002, 2003 and 2009, when proximal leg vein USS were primarily undertaken (~10% of scans by vascular sonographers did scan to the calf veins). We also collected data for years 2014 and 2015, in which whole-leg scans were primarily undertaken. The cost of an USS is estimated at £200. Our population is around 0.5 million.
Results: See *Table 1*. In 2002, 2003 and 2009 there were 1794 rescans performed which detected 72 DVT's of which 19 (26%) were

TABLE 1

Year	2002	2003	2009	2014	2015
Total number of first scans performed	852	989	1087	959	1000
Total number of positive DVT found (%)	203 (23.8)	223 (22.5)	223 (20.5)	175 (18.2)	180 (18.0)
Total number of calf DVT detected	17	16	16	9	27
Total number of rescans performed	576	665	553	202	183
Total number of positive DVT found (%)	28 (4.9)	30 (4.5)	14 (2.5)	13 (6.4)	9 (4.9)
Total number of calf DVT detected	7	5	7	2	3

calf DVTs. For the year 2014 and 2015, only 385 rescans were performed which detected 22 DVT's of which 5 (23%) were calf DVT's. We calculated the cost of rescanning per annum to be £119,600 when primary scanning just involves the proximal veins (£4983 per +ve scan); whereas it is £38,500 when primary scanning involves the proximal and distal leg veins (£3500 per +ve scan).

Conclusions: We showed that performing a whole-leg scan as an initial diagnostic method does not drastically alter the percentage of DVTs detected at rescan, but it does allow a cost benefit. The reduction in the number of rescans performed suggests whole-leg scanning is a more cost-effective primary option.

PB2271 | Predictive Accuracy of Computed Tomography Pulmonary Perfusion Imaging for Adverse Outcome in Patients with Acute Pulmonary Embolism

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Background: Computed tomography pulmonary angiography (CTPA) is the current imaging modality of choice for the diagnosis of acute pulmonary embolism (PE). Computed tomography pulmonary perfusion (CTPP) imaging is a novel imaging technique that can provide information on lung perfusion in addition to the CTPA based information on extent and location of pulmonary emboli. How this information may be used to improve the outcome of patients with acute PE is unknown.

Aims: We aimed to evaluate the predictive value of CTPP on short and long-term clinical outcome of patients with acute PE.

Methods: This was an observational study in 100 consecutive patients with CTPA confirmed acute PE in whom CTPP was performed as part of routine clinical practice (Figure 1). We evaluated the association between the perfusion defect score (PDS), assessed according to the Chae method [Chae et al, AJR 2010], symptoms of PE at admission, need for reperfusion therapy and/or oxygen therapy, PE-related mortality and persistent symptoms of pain or dyspnoea during 3-month follow-up.

Results: Six of the 100 patients (6%) were hemodynamically unstable at presentation, 5 patients (5%) received systemic thrombolysis and 60 patients (60%) were admitted to the hospital. The PDS was associated with hemodynamic status (15% higher PDS (95%CI 4.7-26%) in those who were unstable at presentation), need for reperfusion therapy (12% higher PDS, 95%CI 0.81-23%) and PE-related mortality (22% higher PDS, 95%CI 4.8-40%; Table 1). The PDS was not associated with presenting symptoms, nor did it predict the need for oxygen therapy or long-term persistent pain or dyspnea.

Figure 1. A. Computed tomography pulmonary perfusion (CTPP), axial and B. coronal image in a patient with an acute thrombus in the right (sub)segmental pulmonary artery (encircled) with moderately reduced to profoundly reduced/absent lung perfusion in the right lower lobe. C. CTPP evaluation for perfusion defects including a schematic drawing of axial images at 6 different levels dividing the lung in 10 lung segments.

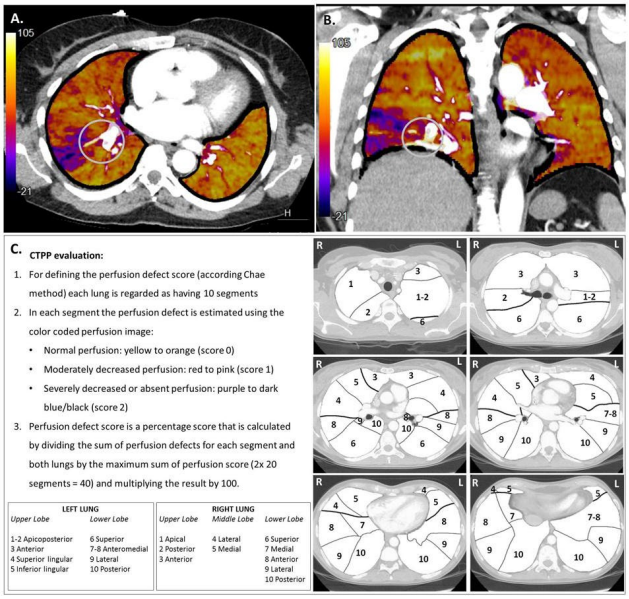

FIGURE 1

TABLE 1 Perfusion defect score (PDS) in 100 acute pulmonary embolism (PE) patients and correlation to presenting symptoms and prognosis

	Prevalence - no (%)	*Mean (SD) PDS in % in patients with:*	*Mean (SD) PDS in % in patients without:*	*Difference (95% CI)*
Symptoms at presentation and 7-day outcome				
Chest pain	25 (25)	32 (12)	26 (13)	5.5 (-2.5 to 13)
Pleural pain	55 (55)	28 (14)	27 (12)	1.5 (-3.7 to 6.7)
Dyspnea	82 (82)	28 (13)	24 (12)	3.7 (-3.0 to 11)
Hemoptysis	6 (6)	27 (14)	27 (13)	-0.74 (-12 to 10)
Hemodynamic instability	6 (6)	42 (13)	27 (13)	15 (4.7 to 26)*
Hospital admission	60 (60)	28 (14)	27 (11)	0.92 (-4.4 to 6.2)
Intensive care admission	8 (8)	35 (19)	26 (12)	8.3 (-0.72 to 17)
Reperfusion therapy	5 (5)	38 (15)	26 (12)	12 (0.81 to 23)*
Need for supplemental oxygen therapy > 24 hours	25 (25)	28 (15)	27 (12)	1.4 (-4.5 to 7.3)
Severe pain requiring IV pain medication > 24 hours	6 (6)	23 (12)	27 (12)	-4.6 (-15 to 5.8)
Need for vasopressor therapy	3 (3)	34 (32)	27 (12)	7.6 (-6.8 to 22)
PE related death	2 (2)	49 (19)	27 (12)	22 (4.8 to 40)*
3-month outcome				
Overall mortality	6 (6.6)	27 (21)	27 (12)	-0.05 (-11 to 11)

Conclusions: Based on our findings, CTPP may be best applied in the initial risk stratification of patients with acute PE, but seems to have limited value in predicting long-term outcome.

PB2272 | The Use of ADJUST (age-adjusted) D-Dimer Cut-off in the Assessment of Individuals Over 50 Years Old with Suspected Deep Vein Thromboses Can Decrease the Need for Compression Ultrasonography: Initial Results of a Prospective Primary Care Study

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Background: As D-dimer (D-d) levels rise with age, a higher proportion of older individuals with suspected deep vein thrombosis (DVT) may have a D-d above the conventional cut-off. This can result in an increased number of unnecessary Compression Ultrasound Scans (CUS), many of which will be normal. In a previous retrospective study, we have shown that the use of an age-adjusted D-d cut-off (age x10; ADJUST D-dimer) in individuals >50 years could decrease the need for CUS by 17% with no false negatives.

Aims: To assess prospectively whether the use of ADJUST D-d could decrease the number of CUS required in individuals >50 years with suspected DVT without increasing false negative results.

Methods: We changed our routine DVT assessment protocol in the light of our previous retrospective study. We continued to use the 2-level Wells score (WS). D-dimer was measured quantitatively using a Point of Care test (Cardiac D-dimer, Roche). Proximal CUS was carried out as per the NICE (National Institute for Health and Care Excellence) protocol. Patients with DVT excluded had a 90-day telephone follow up to identify false negatives.

Results: Between May 2019 and January 2020, 196 individuals >50 years with suspected DVTs were assessed - comprising 85% of all patients seen. Using ADJUST D-d 8 initial CUS were saved in those with a non-high WS and 18 second scans in those with a raised WS, an overall 13% reduction in CUS. There were no venous thromboembolic events in the 16/26 patients who currently have had 90 day follow ups.

Conclusions: The initial results of this prospective study confirm that the use of ADJUST D-d can safely decrease the need for CUS in individuals >50 years in a primary care setting. This improves service efficiency and cost-effectiveness - an advantage in a population with a high proportion of older patients with suspected DVTs.

PB2274 | A Practical Comparison of a PE Diagnostic Approach: Is All Wells in the World?

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Background: In the ambulatory setting, several well-validated prediction tools exist to risk stratify patients with suspected pulmonary embolism (PE). This allows for more appropriate use of diagnostic computed tomography pulmonary angiography (CTPA), in order to address the issue of overuse and over-diagnosis. There is sparse evidence for the application of these tools among inpatients. As such, we hypothesized a lower diagnostic yield of CTPA in this group.

Aims: To determine the diagnostic yield of CTPA for PE in ambulatory patients compared to inpatients in a Canadian tertiary care center. Secondary outcomes include the incidence of: Wells score, D-dimer and appropriate CTPA use; and the comparative predictive value of the Wells score and D-dimer at our institution.

Methods: We performed a retrospective cohort study of all patients who underwent CTPA at the Jewish General Hospital in Montreal, Canada between January 1st and June 30th 2013. Patient demographics, presenting symptoms, and explicit use of the Wells score and D-dimers were collected. Post-hoc Wells scores were generated from available clinical data for all patients to estimate their pre-CTPA risk categorization.

Results: A total of 464 emergency department (ED) patients and 192 inpatients underwent CTPA during the study period. The yield was similar between populations (14.1% vs. 14.4%, p=0.9). There were twice as many subsegmental PEs amongst inpatients (33.3% vs. 14.9%, p=0.044). Only 3.2% of cohort patients had a Wells score recorded. Patients with a generated Wells low probability score were significantly less likely to have a positive CTPA (7.7% vs. 17.4%, p< 0.001). No patients in our cohort with a negative D-dimer had a positive CTPA.

Conclusions: The yield of CTPA is similar in the inpatient and ED populations. This may be partially explained by the suboptimal implementation of pretest probability algorithms and D-dimer use in the ED. Further work is needed to elaborate their role amongst inpatients.

PB2275 | Outcome of the Symptomatic Subsegmental Isolated Pulmonary Embolism: The SSPE Padua Study

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Background: The true incidence of isolated subsegmental pulmonary embolism (ISSPE), either incidental or symptomatic, as well as its clinical importance and the best treatment, if any, are still unclear.

Aims: To assess the incidence and clinical outcome of symptomatic ISSPE.

Methods: from Jan 2016 to Jan 2019 we selected consecutive symptomatic patients for suspected PE who underwent a multidector row computed tomography pulmonary angiography (CTPA). In order to identify the patients with an ISSPE two blinded thoracic radiologists reviewed the images independently. In case of disagreement, the opinion of a third radiologist was obtained. In patients with confirmed ISSPE, clinical characteristics at diagnosis, type and duration of anticoagulant therapy, VTE recurrences, bleeding episodes and VTE-related mortality within 6 months after the diagnosis were

TABLE 1

Table 1

Characteristic of events			
Composite outcome	**Overall**	**Anticoagulant treatment**	**Untreated**
N (%)	13 (14.1%)	12 (13.01%)	1 (0.9%)
Subanalysis of events			
VTE recurrence	0	0	0
Bleeding	11*	10 (6 major, 4 minor)	1 (minor)
VTE related death	2	2	0
*** Type of anticoagulant regimen during bleeding events**		3 Enoxaparin (3 major, 2 fatal) 3 Rivaroxaban (2 major) 2 Apixaban 2 Edoxaban (1 major) 1 Fondaparinux	

VTE: venous thromboembolism

collected. Results are expressed as mean ± standard deviation. Data were analyzed using chi-squared test or Fisher's exact test, when appropriate.

Results: Among 4052 suspected symptomatic PE, ISSPE were confirmed in 92 patients, with an overall incidence of 8.4%/years, that increase from 6.8% to 11.8%/years in the last period. 82 (89.2%) patients received an anticoagulant treatment, which was prolonged to at least 6 months in 47% of them. 10 patients (10.2%) were left untreated. After six months a VTE-related event occur in 13 (14.1%) patients. Among these, a VTE-related event occurred in 12 patients treated with anticoagulants (table 1) and in 1 patients left untreated. No recurrent VTE episode was observed in the cohort, while 6 six major bleeding (2 fatal) developed in the treated patients.

Conclusions: Symptomatic ISSPE is diagnosed in a not negligible proportion of patients. While the risk of VTE recurrence seems low even in ISSPE untreated patients, the incidence of VTE-related major adverse events is mainly due to bleeding during anticoagulation. Large prospective studies are needed to establish the optimal duration of anticoagulant therapy in symptomatic ISSPE patients.

PB2276 | Prospective Study of a Screening Protocol for Venous Thromboembolic Complications in Patients Operated on for Pelvic Ring Fractures

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Background: Pelvic fractures are complex fractures associated with a high-risk of venous thromboembolic (VTE) complications, with reported rates ranging from 10 to 50% and a risk of 0.5 to 10% of fatal pulmonary embolism.

Aims: To reduce the perioperative morbi-mortality related to these complications using a systematic screening protocol. The secondary objectives were the evaluation of this protocol and the identification of VTE risk factors.

Methods: This is a prospective monocentric study involving 100 consecutively enrolled patients with a pelvic fracture requiring surgery (ethics committee approval: 03/24/2017; number 66; NCT03535428). The screening protocol included a venous ultrasound examination of inferior vena cava and lower limbs and an abdominopelvic CT-venography within 48 hours prior to surgery. The main evaluation criteria were the mortality and the complication

TABLE 1 Comparison between patients with and without DVT

	NO DVT	DVT	Standard deviation no DVT	Standard deviation DVT	p
Pelvic fractures	19.0%	52.0%	39.5%	51.2%	0.002
Acetabular fractures	86.0%	67.0%	34.8%	48.3%	0.04
High displacement	49.0%	76.0%	50.3%	43.6%	0.03
Preoperative infection	5.1%	23.8%	22.1%	43.6%	0.007
Preoperative transfusion	6.3%	28.6%	24.5%	46.3%	0.003
Platelets	309.0	372.8	124.9	148.9	0.049

TABLE 2 Odds ratios (OR) for predictive factors

	OR	Confidence interval lower end	Confidence interval upper end	p
Pelvic fractures	4.6	1.7	13.08	0.004
Acetabular fractures	0.324	0.107	0.98	0.046
High displacement	3.3	1.1	9.83	0.047
Preopoerative infection	5.86	1.4	24.3	0.019
Preopoerative transfusion	5.9	1.6	22	0.01

rate. Secondary endpoints were the rate of diagnosed VTE events and the identification of risk factors.

Results: The mean age was 44. One patient died on D1 postoperatively of a massive pulmonary embolism. A protocol-related complication was reported (one migration of a vena cava filter on 10 filters insertions). No thrombotic recurrence or hemorrhagic complication was reported. The rate of diagnosed VTE events was 21%, and 90% of which were pre-operative asymptomatic events. CT venography did not show superiority over ultrasound. Patients with great fracture displacement, preoperative infection, higher platelets counts or having preoperative transfusion were at higher risk of developing deep vein thrombosis (DVT).

Conclusions: The rate of asymptomatic VTE events in pelvic fractures is extremely high and leads to the following recommendations: their preoperative investigation should be systematic by ultrasound. CT-venography should be performed in case of partial exploration of inferior vena cava or iliac veins. A vena cava filter must be placed in case of proximal DVT and the surgery must be postponed to allow anticoagulation treatment for at least 48 hours whatever the type of thrombosis.

PB2277 | D-Dimer Plasma Levels and the Anatomical Extension of Acute Symptomatic Deep Vein Thrombosis of the Lower Limbs

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Background: D-dimer is included in diagnostic algorithms for deep vein thrombosis (DVT) or pulmonary embolism for its high negative predictive value, thus reducing the need for imaging. However small thrombi may be associated with low D-dimer levels, thus influencing the yield of diagnostic algorithms, possibly increasing false negative results

Aims: To establish whether plasma D-dimer levels are associated with extent of acute deep vein thrombosis (DVT) of the lower limbs

Methods: We investigated consecutive outpatients with suspected DVT of the lower limbs referring to our vascular emergency department from 2013 to 2018. D-dimer testing (STA, Stago, cut-off: 500 ng/mL) was performed in all patients who then underwent pretest clinical probability (PTP) evaluation and compression ultrasonography.

Results: Among 3948 patients (M:1554 - 39%, mean age 65, median 69, range:18-103), 486 proximal (12.3%) and 348 distal (8.8%) DVTs were diagnosed. PTP was likely in 390 (80%) of patients with proximal DVT and in 180 (52%) of subjects with calf DVT. Patients with proximal DVT had higher D-dimer levels (mean: 7.13 microgr/mL, median: 3.96 microgr/ml; range:0.17-73 microgr/mL) than patients with calf DVT (mean: 2.38 microgr/mL; median:1.4 microgr/mL, range:0.03-60 microgr/mL) (p< 0.0001) and patients without DVT (mean 0.96 microgr/mL, median: 0.54 microgr/mL, range:0.02-23 microgr/mL) (p< 0.0001). D-dimer below the cut-off was detected in 10 (2.1%) of subjects with proximal DVT and in 51 (14.7%) of subjects with calf DVT.

Conclusions: D-dimer levels correlate with the extent of DVT and small calf thrombi are associated with low D-dimer levels.

PB2278 | Identification of Somatic Mutations Associated with Clonal Hematopoiesis in Unprovoked Venous Thromboembolism

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Background: Up to 50% of patients with venous thromboembolism (VTE) have no obvious risk factors, being classified as idiopathic or unprovoked. Recently, somatic mutations in clonal hematopoiesis of indeterminate potential (CHIP) are common, increase in prevalence with age and not only have been associated with a higher risk for hematologic malignancy but also with atherosclerotic cardiovascular disease. There are no data assessing the incidence of CHIP-mutations in VTE.

Aims: Analyze the presence of somatic CHIP-mutations in blood cells from patients with unprovoked VTE by Next-Generation Sequencing (NGS).

Methods: 486-patients with VTE from the Hospital of Salamanca are included in RIETE database. Sixty-six patients < 70 years with criteria for unprovoked VTE were sequenced searching CHIP mutations. Patients with major risk factors and epidemiological factors were excluded.

The mutational status of the most common mutated regions within 12-genes related to CHIP (*DNMT3A*,*TET2*,*TP53*,*ASXL1*,*SF3B1*,*IDH1*, *IDH2*,*PPM1D*,*JAK2*,*NRAS*,*KRAS* and *GNAS*) was analyzed in peripheral blood mononuclear cells (PBMCs) using a Custom Amplicon panel of NGS; the primers were designed by Paragon Genomics as a CleanPlex-Custom NGS panel. The mean of reads was 2721 [224-9302]; 95% of the regions were sequenced >1000reads. Mutations were validated by high-depth amplicon sequencing (NexteraXT).

Results: NGS analysis revealed the presence of 6 somatic mutations (9%) located in genes *DNMT3A*, *JAK2* and *TET2* (Table1). This incidence was higher than previously reported in population studies(< 3%). The most frequently mutated gene was *DNMT3A* (6%, 4/66). Three out of *DNMT3A* mutations were truncating leading to a *DNMT3A* loss-of-function. All CHIP mutations were detected in a low percentage of PBMCs (< 10%) except to one detected in 30% of cells.

Conclusions: This study identified somatic mutations related to CHIP in with unprovoked VTE.

TABLE 1 Somatic mutations detected by NGS

Age (years)	Gene	cDNA Mutation	Protein Change	Allele frequency
56	JAK2	c.G1849T	p.V617F	8.7
59	DNMT3A	c.2678G>A	p.W893X	29.8
56	DNMT3A	c.2638delA	p.M880fs	2.5
62	DNMT3A	c.2245delG	p.D748fs	2.0
63	DNMT3A	c.G890C	p.W297S	3.1
61	TET2	c.2454delT	p.P818fs	1.9

Studies with a larger number of patients are necessary to determine their association with VTE and if CHIP mutations emerge as a new risk factor for VTE.

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PB2279 | Venous Thromboembolism (VTE) Events in Elective and Emergency Orthopaedic Surgery in a Large Teaching Hospital 2011 to 2019

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Background: University hospitals Plymouth is a 900 bed teaching hospital and has been a national venous thromboembolism (VTE) exemplar site since 2009. VTE outcome data has been produced on all VTE events since 2011. In orthopaedic surgery, extended thromboprophylaxis (TP) with direct oral anticoagulants is used for elective hip and knee arthroplasty, with extended TP using low molecular weight heparin for emergency surgery.

Aims: All VTE events diagnosed following total hip replacement (THR), total knee replacement (TKR)and fractured neck of femur (#NOF) between 2011 and 2019, have been reviewed and compared to obtain trends over that time.

Methods: All VTE events from targeted investigations, comprising CT pulmonary angiograms, SPECT-CT scans and Doppler ultrasounds of upper and lower limbs were collected between January 1st 2010 and 31st December 2019. Positive scans reviewed to identifying whether VTE was a hospital acquired event (HAT) associated with orthopaedic surgery. HAT events following THR, TKR or # NOF were recorded and compared against annual numbers for surgery in these patients. Annual percentage rates of VTE for each operative type were calculated.

Results: The table shows all events over 9 years. VTE events in THR operations, range from 0.58% to 1.42% annual rates with overall 0.93% VTE events seen. For TKR the range was 0.21% to 1.53% with the overall rate of 0.67%. Finally for #NOF the range was 0.29% to 1.33% with an overall rate of 0.77%. Root cause analysis on all HAT events, identified 4 cases over nine years associated with inappropriate or inadequate TP.

Conclusions: VTE events were relatively stable in #NOF patients for 4 years, but have subsequently increased slightly. TKR showed initially high rates, followed by a significant reduction between 2014 and 2019 (p=0.02). Events associated with THR have remained consistent. The challenge is to facilitate further reductions in thrombosis in all, but particularly #NOF cohort.

TABLE 1 Venous Thromboembolism events in Orthopaedic surgery 2011 – 2019

YEAR	THR VTE Events	Total Operations	%	TKR VTE Events	Total Operations	%	#NOF VTE Events	Total Operations	%
2011	3	433	0.69	7	458	1.53	3	541	0.55
2012	3	420	0.71	6	416	1.44	3	657	0.46
2013	3	424	0.71	3	402	0.75	2	686	0.29
2014	5	374	1.34	1	383	0.26	3	645	0.47
2016	4	421	0.95	1	472	0.21	6	636	0.94
2017	2	345	0.58	1	312	0.32	7	665	1.05
2018	6	422	1.42	3	392	0.77	5	618	0.81
2019	7	819	0.86	3	810	0.37	9	675	1.33
Totals (Inc 2015)	37	3963	0.93	26	3896	0.67	44	5722	0.77

PB2281 | Safety of Using the Wells Rule and D-Dimer Test for Excluding Acute Recurrent Ipsilateral Deep Vein Thrombosis

L.F. van Dam[1]; G. Gautam[2]; C.E.A. Dronkers[1,3]; Å. Eckerbom[2]; W. Ghanima[4,5]; J. Gleditsch[6]; A. von Heijne[2]; H.M.A. Hofstee[3]; M.M.C. Hovens[7]; M.V. Huisman[1]; S. Kolman[8]; A.T.A. Mairuhu[9]; M. Nijkeuter[10]; M.A. van deRee[8]; C.J. vanRooden[11]; R.E. Westerbeek[12]; J. Westerink[10]; E. Westerlund[2]; L.J.M. Kroft[13]; F.A. Klok[1]

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Background: The diagnostic accuracy of clinical probability assessment and D-dimer testing for recurrent ipsilateral deep vein thrombosis (DVT) is largely unknown.

Aims: To evaluate the safety of ruling out acute recurrent ipsilateral DVT based on an unlikely Wells score for DVT in combination with a normal D-dimer test.

Figure 1. Study flowchart of 295 patients with suspected recurrent ipsilateral deep vein thrombosis. Note: DVT: deep vein thrombosis, n: number of patients, CDR: clinical decision rule

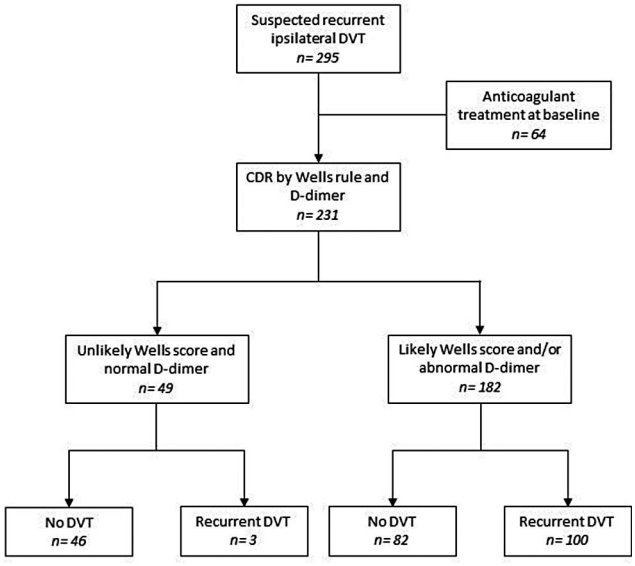

FIGURE 1

TABLE 1

Table 1. Baseline characteristics of 231 patients with suspected recurrent ipsilateral DVT of the leg and without anticoagulant treatment at inclusion.

Note: DVT: deep vein thrombosis, SD: standard deviation, no: number of patients, IQR: interquartile range, VTE: venous thromboembolism

Mean age (+/- SD) – years	56 (17)
Male – no (%)	109 (47)
Median duration of complaints (IQR) – days	4 (2-7)
More than 1 prior VTE episode – no (%)	50 (22)
Mean time since the last DVT episode (+/- SD) – years	6.9 (9.2)
Active malignancy – no (%)	10 (4.3)
Immobility for > 3 days or recent long travel >6 hours in the past 4 weeks – no (%)	9 (6.5)
Trauma/surgery during the past 4 weeks – no (%)	9 (3.9)
Hormone (replacement) therapy – no (%)	5 (2.2)
Known genetic thrombophilia – no (%)	18 (7.8)

Methods: This was a predefined endpoint of the Theia study (van Dam et al, Blood 2020) were the diagnostic accuracy of magnetic resonance direct thrombus imaging (MRDTI) for acute recurrent ipsilateral DVT was validated. The Wells rule and D-dimer test were performed as part of the study protocol, but not used for management decisions. The primary outcome of this analysis was the incidence of recurrent DVT at baseline (defined as a positive MRDTI for DVT) or during 3-month follow-up in patients with a negative MRDTI, in patients with an unlikely Wells score and a normal D-dimer test.

Results: Results of both Wells score and D-dimer tests were available in 231 patients, who were not on anticoagulant treatment at inclusion (Figure 1 and Table 1), with a DVT prevalence (at baseline and 3-month follow-up) of 45% (103/231). Three of 49 patients (6.1%, 95%CI 1.3-18%) who had an unlikely Wells score and normal D-dimer test had recurrent DVT at baseline or during follow-up, yielding a sensitivity of 97% (95%CI 92-99%) and a specificity of 36% (95%CI 28-45%). If clinical probability scoring and D-dimer testing had been applied, radiological imaging would have been avoided in 21% (49/231; 95%CI 16-28%) of patients.

Conclusions: Although the sensitivity of the Wells rule in combination with D-dimer testing was high, we observed a 6.1% incidence of recurrent DVT at baseline or during follow-up in those with normal results. Together with the limited specificity, our data do not support routine assessment of pre-test probability and D-dimer in the diagnostic workup of suspected recurrent ipsilateral DVT.

PB2282 | Immuno-Magnetic Separation (IMS) Allows Efficient and Selective Extraction of Microvesicles Bearing Tissue Factor Activity from Various Liquid Biopsies

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Background: Some microvesicle (MV) subtypes, including monocytic, endothelial and tumor-derived MV, often harbor TF-dependent procoagulant activity (TF-PCA) and are the most promising to predict thrombosis Therefore, measuring this MV-associated TF-PCA with high sensitivity using automated methods remains a crucial need. A new version of FXa generation assay (Vallier 2019) helps tackle sensitivity but the process of MV extraction from human fluids, actually based on high-speed centrifugation (HS-C), remains limiting.
Aims: First, to implement a reproducible method to isolate MV, compatible with TF-PCA assay and future automation. Second, to specifically purify individual MV sub-populations in order to identify the cellular origin of TF-PCA.
Methods: First, a Pan-MV Immuno-Magnetic Separation (IMS) tool was made by coating two widely reacting antibodies (CD29 and CD59) onto 1µm magnetic beads using streptavidin-biotin linkage. IMS-based extraction was compared to HS-C on different biofluids including untreated and LPS-activated plasma, plasma spiked with tumoral MV-TF, normal saliva and pleural fluids from cancer patients. Second, lineage-specific monoclonal antibodies were coated on IMS beads for selective extraction.
Results: In all tested models, CD29-59 IMS was more reproducible and efficient than HS-C to extract MV-TF, without losing specificity. Lineage-specific IMS clarified the origin of TF activity in plasma samples, locating TF-PCA on monocytic MV only (CD11b+/CD15-/HLA-DR+/CD45R-) in the LPS model. In all tested pleurisy samples, CD326 IMS systematically recovered TF-PCA, owing to overexpression of EpCAM-CD326 on carcinoma cells and their derived MV. In contrast, TF-PCA origin could not be identified in saliva due to frequent aggregation of MV subsets as seen with flow cytometry.
Conclusions: CD29-59 magnetic beads proved to be more efficient than HS-C to extract EVs. The new IMS-based MV-TF activity assay was both more reproducible and more sensitive than the HS-C-based assay without losing TF specificity and allowed selective extraction of MV subsets bearing TF-PCA.

PB2283 | A Combination of Age Adjusted Cut-Off and Preanalytical Conditions Monitoring for Increased DDIM Assay Specificity

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Background: D-dimer measurement is an important step in the diagnostic strategy of clinically suspected acute pulmonary embolism (PE) or deep venous thrombosis (DVT), but its clinical usefulness is limited in elderly patients.
Aims: The aim of our work is to verify the implementation of the age-dependent D-dimer cut-off value in conjunction with the evaluation of pre-analytical sample conditions to increase the diagnostic specifity of D-dimer in elderly patients suspected for PE and DVT.
Methods: All consecutive outpatients who were presented to the emergency department with clinically suspected PE or DVT were assessed by a sequential diagnostic strategy based on the clinical probability. They underwent highly sensitive D-dimer measurement with detection preanalytical conditions and the positive D-dimer finding was verified by imaging method (duplex sonography of the lower extremity veins or computed tomography angiography). D-dimer was be detected by HemosIL D-Dimer HS test with monitoring preanalytical condition by HIL modul on ACL TOP 750 CTS system.
Results: In 1952 patients with presumed PE or DVT included, the prevalence of DDIM positivity was 67.1% with fixed cut-off vs. 100%. 59.4% with age adjusted cut-off. The highest difference was in the age group of 70 years and over, where it formed 22.2% in the age group up to 75 years respectively. 18.6% up to 80 years. The measurement of preanalytic conditions led to only 1% improvement in negative prediction. However, this also represents in our group 20 patients who do not have to undergo time-consuming and more expensive imaging examinations.
Conclusions: Compared with a fixed D-dimer cut-off, the combination of pretest clinical probability assessment with age-adjusted D-dimer cut-off was associated with a larger number of patients in whom PE and DVT could be considered ruled out with a low likelihood of subsequent clinical venous thromboembolism.
Supported by: Grant LF-2020-002 and MH CZ - DRO (FNOl, 00098892).

PB2284 | Cost-Effectiveness of Magnetic Resonance Direct Thrombus Imaging for Diagnosing Recurrent Ipsilateral Deep Vein Thrombosis

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Background: The diagnosis of recurrent ipsilateral deep vein thrombosis (DVT) with compression ultrasonography (CUS) is hindered by persistent intravascular abnormalities after a previous DVT. We have shown that magnetic resonance direct thrombus imaging (MRDTI) can exclude recurrent ipsilateral DVT and prevent overdiagnosis by CUS. However, it is unknown whether the application of MRDTI in daily clinical practice is cost-effective.

Aims: To evaluate the cost-effectiveness of applying MRDTI in different diagnostic strategies for suspected recurrent ipsilateral DVT in the Dutch setting.

Methods: Patient-level data of the Theia study (van Dam et al, Blood 2020) were analyzed in 10 hypothetical diagnostic scenarios, including clinical probability scoring with the Wells rule in combination with D-dimer testing, and diagnostic imaging with CUS and/or MRDTI (Figure 1). CUS was judged as either positive, negative or inconclusive, which was accounted for in the diagnostic strategies. Both positive and inconclusive results were summarized as abnormal CUS result. The costs of diagnostic tests and anticoagulant treatment during one-year follow-up, including costs of false positive and false negative diagnoses, were compared.

Results: Compared to MRDTI alone, a combination of the Wells rule and D-dimer tests was associated with a higher failure rate (6.1%), and CUS alone with an excess of false positive diagnoses (19%) of recurrent ipsilateral DVT. Even though initial diagnostic costs for strategies with MRDTI were higher than strategies without MRDTI, the

Figure 1. Ten hypothetical scenarios for the diagnostic workup of suspected recurrent ipsilateral deep vein thrombosis (DVT), applying various combinations of a clinical decision rule and D-dimer (DD) testing and diagnostic imaging with compression ultrasonography (CUS) and/or magnetic resonance direct thrombus imaging (MRDTI).

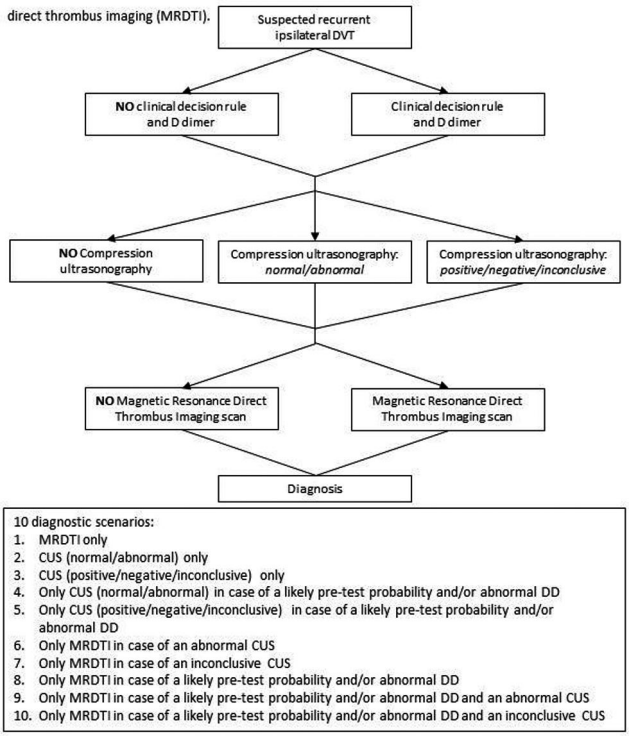

FIGURE 1

Figure 2. One-year healthcare costs per patient for the 10 diagnostic scenarios, and a scenario to treat all, treat none and treat those with a likely pre-test probability and/or abnormal D-dimer without diagnostic imaging. Note: CDR+DD: clinical decision rule + D-dimer testing, CUS: compression ultrasonography, CUSi: compression ultrasonography is positive, negative or inconclusive, MRDTI: magnetic resonance direct thrombus imaging, FN: false-negatives, FP: false-positives

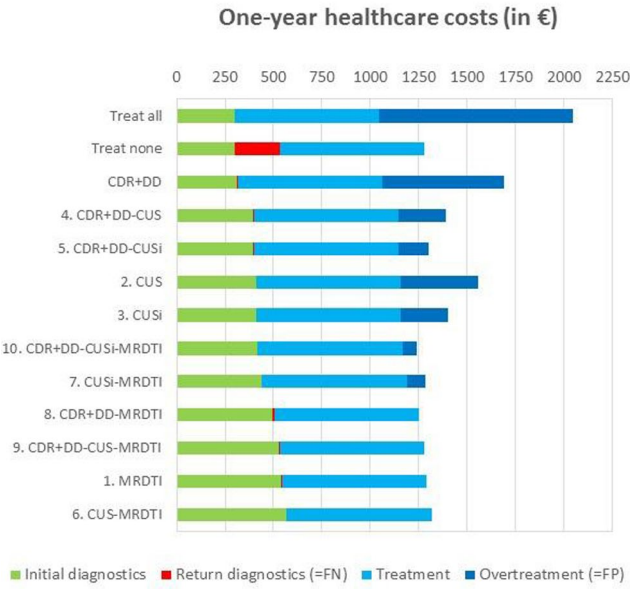

FIGURE 2

total 1-year costs with MRDTI were generally comparable or lower than without MRDTI due to superior diagnostic accuracy (Figure 2).

Conclusions: Our model shows that the diagnostic strategies for suspected recurrent ipsilateral DVT with MRDTI have higher diagnostic accuracy at generally lower total 1-year healthcare costs compared to diagnostic setups without MRDTI. Compared to CUS alone, applying MRDTI in clinical practice therefore will not increase healthcare costs.

PB2285 | A Simplified Management Strategy for Suspected Pulmonary Embolism: External Validation of the YEARS Diagnostic Algorithm

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Background: Validated diagnostic algorithms are used to manage patients with clinically suspected pulmonary embolism (PE). The recently published YEARS study proposed a simplified diagnostic strategy which appears to safely reduce the use of computed tomography pulmonary angiography.

Aims: To externally validate this diagnostic strategy in an independent cohort.

Methods: We analyzed data from three prospective cohort studies of outpatients with suspected PE collected between 2000 and 2006. We retrospectively applied the YEARS diagnostic algorithm to this prospectively collected data. The three YEARS clinical criteria are: clinical signs of deep vein thrombosis (DVT), hemoptysis, and PE as the most likely diagnosis. If zero YEARS criteria are met, a D-dimer < 1000 ng/mL will rule out a PE. If one or more YEARS criteria are met, a D-dimer < 500 ng/mL will rule out a PE.

Results: Of the 3314 analyzed patients, 731 (22.1%) had PE. Applying the YEARS diagnostic algorithm, 1423 (42.9%) patients could have had PE ruled out without the need for imaging. Of these patients, 17 (1.2%; 95% CI 0.8 - 1.9) were diagnosed with a PE at initial testing. All 17 had no YEARS item and a D-dimer < 1000 ng/mL. Of note, all 17 had a D-dimer level above their age-adjusted cutoff. Among the 272 patients with no YEARS criteria and a D-dimer < 1000 ng/mL but above their age-adjusted D-dimer cutoff, PE was diagnosed in 6.3% (17/272; CI 3.9 - 9.8%).

Conclusions: We provide external validation of the YEARS diagnostic algorithm in an independent cohort. Overall, the rule appears to safely exclude PE. However, caution might be required in patients with no YEARS item and a D-dimer < 1000 ng/mL but above their age-adjusted D-dimer cutoff, especially in centres with a higher prevalence of PE.

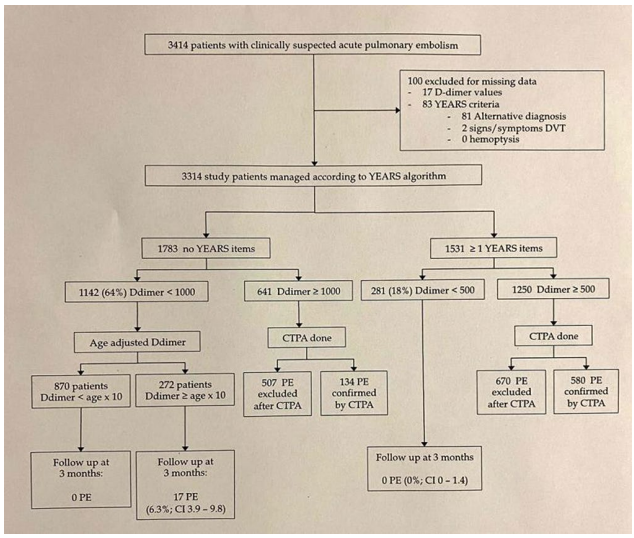

FIGURE 1 Study flowchart

PB2286 | Radiological Parameters on Computed Tomography Pulmonary Angiography Correlate Poorly with Early Mortality from Acute Pulmonary Embolism

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Background: Acute pulmonary embolism (PE) is associated with significant morbidity and mortality. Early risk-stratification of patients is crucial for therapeutic decision-making. The use of radiological data has been recommended to guide risk-stratification, but evidence for this is limited.

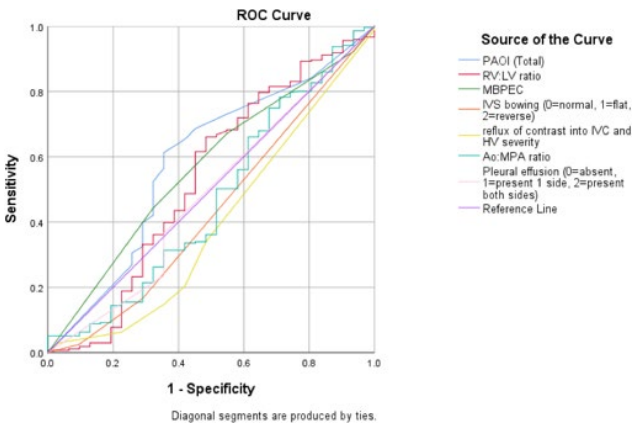

FIGURE 1 ROC Curve of radiological parameters for 30-day all-cause mortality

Test Result Variables	Area	Std Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
PAOI (Total)	.588	.057	.110	.476	.700
RV:LV Ratio	.532	.064	.559	.407	.657
MBPEC	.568	.053	.216	.464	.672
Interventricular Septum Bowing	.434	.058	.226	.320	.547
Reflux of contrast into feeding veins	.395	.059	.055	.280	.509
Ao:MPA Ratio	.470	.060	.586	.352	.588
Pleural Effusion	.482	.058	.747	.368	.596

Aims: To retrospectively evaluate the correlation between computed tomography pulmonary angiography (CTPA) radiological parameters in patients with acute PE with 30-day all-cause mortality.

Methods: Consecutive patients with acute PE diagnosed on CTPA from 2013 to 2016 in our institution were included. Clinical characteristics and outcomes were extracted from electronic patient records system. For each patient, mean bilateral proximal extension of clot (MBPEC) score, pulmonary artery obstruction index (PAOI), right ventricle to left ventricle ratio (RV:LV ratio) on transverse views, interventricular septum bowing, degree of contrast reflux into feeding veins and presence of pleural effusions were measured by experienced radiologists who were blinded to clinical outcome. Primary outcome was all-cause mortality at 30 days from diagnosis. The discriminatory power for 30-day all-cause mortality was assessed using the area under the receiver operating characteristic (ROC) curves (AUC).

This study received institutional ethics approval (National Healthcare Group Domain Specific Review Board, Singapore; approval number: 2016/00750).

Results: Mean age was 60.7 (17.5) years; 155 (48.3%) were male. Overall 30-day all-cause mortality was 10.3% (95% confidence interval [CI], 7.4%-14.1%). The AUC for 30-day all-cause mortality for all patients was 0.59 (95% CI, 0.47-0.70), 0.53 (95% CI, 0.41-0.66), 0.57 (95% CI, 0.46-0.67), 0.43 (95% CI, 0.32-0.54), 0.40 (0.28-0.51), 0.47 (95% CI, 0.35-0.59) and 0.48 (95% CI, 0.37-0.60) for respectively, MBPEC score, PAOI, RV:LV ratio, interventricular septum bowing, degree of contrast reflux into feeding veins and presence of pleural effusions.

Conclusions: In patients with acute PE, radiological parameters were not useful for prediction of 30-day all-cause mortality. Further studies are needed to evaluate more suitable markers of severity to guide therapeutic clinical decisions.

PB2287 | D-dimer Diagnostics: An International Assessment of the Quality of Laboratory Testing

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Background: D-dimer is used to rule out venous thromboembolism (VTE) in outpatients with a low pre-test probability of VTE and incorporated into clinical decision rules to determine recurrence risk in patients after an unprovoked VTE. To increase D-dimer specificity and reduce the number of patients requiring imaging, recent studies

TABLE 1 Commonest assays in use in the last sample survey of 2019 (Total - 801 samples analyzed and reported)

Assay	Unit	Proportion of samples assessed using this assay (%)
Siemens Innovance D-dimer	mg/L FEU	39.3
I.L. HemosIL D-dimer HS500	mg/L FEU	15.1
Stago Liatest D-dimer Plus	mg/L FEU	13.1
I.L. HemosIL D-dimer HS	mg/L DDU	4.9
Roche Tinaquant 2nd gen (cal heparin)	mg/L FEU	4.2
BioMerieux Vidas D-dimer	mg/L FEU	3.5
Stago/ Roche Liatest D-dimer	mg/L FEU	3.4
Other*	mg/L FEU OR mg/L DDU	16.5

FEU - Fibrinogen Equivalent Units DDU - D-dimer Units *Included in this group are assays with 25 or less samples tested per assay

TABLE 2 Between Participant variability in samples AT or CLEARLY ABOVE above the VTE exclusion cut-off

	Samples with D-dimer at or just above cut off of 0.5 mg/L FEU or 0.25 mg/L DDU			Samples with D-dimer clearly above cut off of 0.5 mg/L FEU or 0.25 mg/L DDU		
Assay	Samples assessed (2017-2019) n	Range of assigned value (mg/L)	Between participant variability CV Range (%)	Samples assessed (2017-2019) n	Range of assigned value (mg/L)	Between participant variability CV Range (%)
Siemens Innovance D-dimer (FEU mg/L)	1795	0.41-0.71	7.6-12.5	2918	0.75-1.85	5.4-8.4
I.L. HemosIL D-dimer HS500 (FEU mg/L)	578	0.37-0.5	9-17.3	922	0.70-1.7	5.7-10.2
Stago Liatest D-dimer Plus (FEU mg/L)	573	0.37-0.55	12.4-19	616	0.73-2.13	4.3-9.3
I.L. HemosIL D-dimer HS (DDU mg/L)	265	0.2-0.23	8.8-21.2	438	0.25-0.63	4.3-22.3
Roche Tinaquant 2nd gen (cal heparin) (FEU mg/L)	196	0.3-0.37	21.3-38.7	308	0.38-1.29	7.3-23.1
BioMerieux Vidas D-dimer (FEU mg/L)	191	0.36-0.53	5.3-6.8	318	0.69-2.1	3.7-7.9
Stago/ Roche Liatest D-dimer (FEU mg/L)	239	0.38-0.5	12.1-21.1	411	0.73-2.16	4.9-9.4

have suggested a clinically "adjusted" D-dimer cut-off based on patient age, or clinical probability of having VTE rather than a fixed assay-specific cut off. D-dimer assays are not standardized and expressed in different units. The use of universal VTE exclusion thresholds and differences in assay variability raises concerns about the generalizability of recent studies using specific D-dimer assays, and the effect on clinical decision rules.

Aims: To review the diagnostic performance of D-dimer assays based on external proficiency data in a large international cohort of laboratories.

Methods: From 2017 to 2019, the global External Quality Assessment programme of the ECAT Foundation distributed 24 D-dimer positive samples quarterly, to participating laboratories, mainly from Europe, North America and Australia who analyzed the samples and reported results to ECAT. Consensus values for 6 samples were at the D-dimer cut-off level of 0.5 mg/L FEU (or DDU equivalent), 8 were slightly elevated and 10 were clearly elevated above that cut-off.

Results: Between 578 and 640 of enrolled laboratories per year participated in the D-dimer surveys (response rates of 88-95%) using up to 35 unique assays. Over 65% of samples were assessed using three main assays as seen in the most recent survey of 2019 (Table 1). For D-dimer samples around the cut-off, the variability in individual assays for the same sample, was much higher than for samples clearly elevated above the cut-off (Table 2).

Conclusions: Increasing between participant variability for the same sample at the cut-off raises concerns about the diagnostic performance of D-dimer assays at VTE exclusion thresholds.

PB2288 | Vein Thrombosis after an Osteopathy Session

V. Kieffer
Orleans, France

Background: Present a rare case of a subclavian vein thrombosis after an osteopathy session of a young woman.

Aims: Discuss if there is a real relation between the osteopathy session and the thrombosis discovered just after.

Methods: A young woman 27 years old, consulting for pain and edema after her osteopathy session. She felt an acute pain during the osteopathy session and few hours after she started to see the edema of her left arm and subclavian area. The doppler echography shows a recent, hypo echogenic thrombus in the subclavian vein of her left arm. No jugular or humeral thrombosis. This thrombus is isolated. The patient has systemic lupus erythematosus, but any treatment, just a regular follow in internal medicine. She don't have the risk factors of thrombosis, no hormonal contraception, no smoking, no other pathology or treatment, no recent long trip, no recent surgery or infection, no recent hospitalization, no pregnancy.

Results: Her blood results are normal, no anti-phospholipid syndrome and no other thrombophilia.

She had three months Warfarine treatment and the subclavian vein was completely clear in the doppler control at the end of the treatment. The edema desapeared in the first 3 weeks of treatment, but she kept a residual local pain during few mouvements of the arm.

Conclusions: p.p1 {margin: 0.0px 0.0px 0.0px 0.0px; text-align: justify; font: 12.0px 'Times New Roman'; -webkit-text-stroke: #000000} span.s1 {font-kerning: none} We can put the question, if this is just a coincidence or the osteopathy session can be the origin of the thrombosis? Is her Lupus the origin? even if it is follow in internal medicine and it dose't need treatment. In the doubt, we consider that her Lupus is the risk persistent factor. But, should we let the warfarin treatment for life or she should stop the osteopathy?

PB2289 | Impact of Beta-Blockers on the Clinical Diagnosis of Patients with Pulmonary Embolism

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[1]*Chu Clermont-Ferrand, Clermont-Ferrand, France;* [2]*Universite Clermont Auvergne, Clermont-ferrand, France*

Background: The morbidity and mortality of patients with pulmonary embolism is high with 8 to 23 deaths per 100,000. In order to make the diagnosis, the emergency physician uses clinical scores that involve the presence or absence of a tachycardia.

Aims: The aim of our study was to assess the impact of beta-blockers on the heart rate of patient with a pulmonary embolism

Methods: We performed a retrospective, observational, monocentric study from June 2014 to May 2015. All consecutive patients admitted to our department with an objectived pulmonary embolism were included

Results: Among the 117 patients included, more than a quarter was under beta-blocker (n = 31). Patients with beta-blockers were older than those without (76+/-11 vs. 66+/-18 years, p = 0.03). The mean heart rate was lower in patient with beta-blockers than in those without (76.7 vs. 90.7 bpm, p < 0.001). Moreover, regarding the heart rate item in the revised Geneva score, those with beta-blockers were more likely to have a heart rate < 75 bpm (42% vs. 19%, p = 0.015) and less likely > 95 bpm (13% vs. 41%, p = 0.04) than those without. However, for the sPESI score, there was no difference between the mean score of those with and without beta-blockers (1.2+/-0.9 vs. 0.9+/-0.8, p = 0.104). Interestingly, regarding the heart rate item of sPESI score, none of the patient with beta-blocker had a heart rate > 110 bpm whereas 9 (10.5%) of those without beta-blocker had it (p = 0.11).

Conclusions: Our study showed that 26.5% of PE patient admitted in an emergency department were under beta-blockers. Those with beta-blockers had a lower mean heart rate than those without. This could have an impact on the different scores used by emergency physicians which could underestimated the PE score of some patient with beta-blocker.

PB2290 | Determining Predictive Factors of Venous Thromboembolism Using Design of Experiments

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Background: Statistical tools can be useful to determine the main factors of venous thromboembolism recurrence (VTR) since it extracts patterns among selected factors.

Aims: This work aimed to use techniques of fractional factorial design to obtain the main predictive factors of VTR, using the neural model for prediction of VTR, developed by Martins [Unicamp, Thesis (2018)].

Methods: Thirty-nine clinical and laboratory factors were considered. We considered 235 patients with a history of pulmonary, lower limbs and central nervous system thrombosis. Fractional factorial design techniques of five types were used. The statistical significance of the main factors was determined using t-student test. All calculations were performed using the neural model and the Statistica software.

Results: For all designs, the leukocyte level was indicated as statistical significant, with negative effect. The higher it's level, the lower the risk of a thrombotic recurrence episode. Also were founded as significant factors: tabagism and hormone therapy, with positive effects. A total of fifteen factors were found as statistical significant: time of use of anticoagulants, age, residual thrombus, proximal or distal thrombus, diabetes mellitus, renal, protein S, red blood cells, C-reactive protein, dyslipidemia, hemoglobin and platelets (and also the first three factors). In this study it was found that the longer the anticoagulation time, the greater the chance of recurrence of thrombosis. The literature shows that this effect is opposite. This result may be due to the fact that, the greater the chance of a recurrence, the longer the anticoagulation time to avoid it. This fact is inherent in the collected data.

Conclusions: The results of this work showed that it's possible to determine the main factors of VTR using a neural model, combined with a design of experiments technique. These factors can be used in the formulation of new (and more accurate) predictive models/scores.

PB2291 | High Thrombus Burden and its Correlation with In-hospital Mortality or Right Ventricular Dysfunction in Patients with Acute Pulmonary Embolism and Low-risk Simplified-PESI Score

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Background: Computed Tomography Pulmonary Angiography (CTPA) provides an assessment of thrombus burden (ThB) in acute pulmonary embolism (PE). The Simplified Pulmonary Embolism Severity Index (sPESI) is used to predict mortality in PE. It is unclear if the presence of high- ThB correlates with in-hospital mortality (IHM) or right ventricular (RV) dysfunction in patients with low-risk sPESI scores.

Aims: Determine the correlation between the presence of high-ThB and IHM or RV dysfunction in patients with PE and low-risk sPESI score.

Methods: Single-center observational cohort registry performed at a University Hospital. Patients hospitalized with a diagnosis of PE between 2008-2018 were eligible. Images from CTPA were reviewed and ThB was quantified using the modified Miller score (MMS). Two groups were formed (High-ThB ≥ 23 points vs Low-ThB < 23 points). RV dysfunction was determined by echocardiography and defined as RV dilatation and concomitant reduced tricuspid annular plane systolic excursion or RV wall motion abnormalities. Information was collected related to clinical presentation, troponin levels and electrocardiographic (ECG) pattern. All statistical analysis was carried out using the STATA version 13.1

Results: 230 consecutive patients were hospitalized with a diagnosis of PE. 53 patients with low sPESI score were included. Mean age 53±14 years; 49% female. High-ThB was present in16 patients (30.1%). IHM was 6.5%. Univariate analysis showed that High-TB correlated with chest pain (31.2 vs 5.4%; p:0.01), negative T waves in ECG V1-V4 leads (31.2 vs 5.4%; p:0.01), RV dysfunction (37.5 vs 8.1%; p:0.001) and RV wall motion abnormalities (18.7 vs 2.7%; p:0.04). Multivariate analysis showed that High-ThB correlated with RV dysfunction (OR 8.4; CI 95% 15-47.3; p:0.01). No deaths were observed in low sPESI score patients.

Conclusions: In this cohort of patients with low-risk sPESI score, High-ThB correlated with RV dysfunction but not IHM. Quantification of ThB may be useful for PE risk stratification.

PB2292 | Detection of MicroRNA-126 as a Plasma Biomarker for Diagnosis of DVT

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Background: DVT is the formation of thrombi in the deep veins, most commonly the large veins of the legs or pelvis. Numerous efforts have been made to identify reliable and predictive biomarkers to detect the early signs of deep vein thrombosis. Circulating microRNAs (miRNAs) are stably present in the plasma. Their diagnostic function has been investigated in many diseases.

Aims: The aims of present study were to assess the diagnostic ability of plasma miRNA-126 in DVT.

Methods: 47 DVT patients, 36 healthy controls were enrolled in this study. The TaqMan miRNA microarray was used to identify dysregulated miRNAs in the plasma of APE patients. The TaqMan-based miRNA quantitative real-time reverse transcription polymerase chain reactions were used to validate the dysregulated miRNAs. The receiver-operator characteristic (ROC) curve analysis was conducted to evaluate the diagnostic accuracy of the miRNA-126 identified as the candidate biomarker.

Results: Plasma miRNA-126 level was significantly higher in the DVT patients than in the normal controls. The ROC curve showed that plasma miR-126 was a specific diagnostic predictor of DVT with an area under the curve of 0.782 (95% confidence interval, P < 0.01).

TABLE 1 High vs Low thrombus burden. Univariate and Multivariate analysis

Variable	Low thrombus burden (n:37)	High thrombus burden (n:16)	p value
UNIVARIATE analysis			
Age (mean)	53	54	ns
Women	18 (48.6%)	8 (50%)	ns
EKG negative T waves (V1-V4)	2 (5.4%)	5 (31.2%)	0.01
Elevated troponin levels	6 (16.2%)	5 (31.2%)	ns
RV wall motion abnormalities	1 (2.7%)	3 (18.7%)	0.04
RV dilatation	3 (8.1%)	6 (37.5%)	0.001
MULTIVARIATE analysis	Odds ratio	CI 95%	p value
RV dilatation	8.4	1.5-47.3	0.01

Conclusions: Our findings indicated that plasma miR-126 is associated with DVT and it could be an important biomarker for the diagnosis of DVT.

PB2293 | The Significance of Haemostatic System's Changes in the Assessing of Unfavorable Prognosis in Patients with Burn Injury

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Background: Prognosis is important for treatment.
Aims: Comparison of haemostatic system's parameters in the patients with severe burn injury subject to their outcomes.
Methods: 54 patients with burns of II-III degree ranging from 22 to 75% of the TBSA were prospectively studied on day 1, 3 and 10 from the moment of injury. They were separated in 2 groups: 1 group- patients with favorable outcome, 2 group with unfavorable outcome.
Results: In the group 1 APTT and INR were within normal values on the day 1. In the group 2 there was an increase in APTT and INR, while the differences in APTT and INR between patients of groups 1 and 2 were not significant. In the group 1 the activity of protein C was within the normal range on the day 1, in the group 2 it was noticed significant decrease of its activity compared with the normal values and with the surviving patients ($p < 0.05$). The activity of ATIII was reduced in both groups. The activity of ATIII was significantly lower in the dead persons than in survivors ($p < 0.05$). In patients with the activity of ATIII below 75% on the day 1 after injury the lethal outcome was recorded in 63% of cases. If on the day 1 the activity of ATIII was more than 75% an adverse outcome occurred only in 18% of cases ($p < 0.05$). The impact of ATIII activity on the outcome of burn disease was assessed by constructing a Cox regression model. Low values of this parameter on the day 1 after burn injury were a significant predictor of an adverse outcome ($p=0.001$).
Conclusions: The anticoagulation system is more affected in patients with an adverse outcome. The determination of ATIII activity may have a prognostic significance for assessing of lethal outcome's risk.

PB2294 | The Prognostic Role of Neutrophil to Lymphocyte Ratio (NLR) in Venous Thromboembolism and Related Mortality

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Background: Previous studies reported that NLR is a simple yet useful biomarker for various clinical conditions. However, the role of NLR in predicting venous thromboembolism (VTE) remains controversial.
Aims: To investigate the association between NLR and VTE including deep vein thrombosis (DVT), pulmonary embolism (PE) and related mortality.
Methods: Major medical databases were systematically searched for observational study which assessed the association of NLR and VTE published until January 2020. The databases were searched with predefined protocol without language restriction based on PRISMA guideline and the selected studies were evaluated using GRADE approach. The analysis was performed in RevMan 5.3 (fixed-effects and random-effects model based on heterogeneity test) to provide pooled measures for Risk Ratio (RR), 95% Confidence Interval (95%CI), and weighted mean difference (WMD).
Results: Twenty-four studies were enrolled including 32,047 subjects. Higher NLR was indicated as independent predictors for VTE compared to lower NLR (RR=1.3, $p< 0.00001$), comprising DVT and PE (RR=1.57, $p< 0.00001$, and RR=1.08, $p=0.005$, respectively). There was also an association between higher NLR and risk of mortality after PE episode with RR=1.52 ($p< 0.0001$), including in-hospital-, short- (30-day), and long-term (>3-years) mortality (RR=1.5 [$p=0.02$], RR=1.41, $p=0.006$, and RR=1.65, $p=0.000$, respectively). VTE-group had a higher mean of NLR compared to control with WMD=1.64 ($p< 0.00001$), involving PE-group (WMD=2.71,$p=0.0006$) and DVT-group (WMD=1.08, $p=0.004$). Among PE patients, PE-related mortality-group had also higher mean of NLR in comparison to survivor-group with WMD=5.35 ($p< 0.00001$), comprising in-hospital (WMD=2.79, $p< 0.0001$) and 30-day mortality (WMD=5.88, $p< 0.00001$).
Conclusions: Higher NLR value was associated with the occurrence of VTE, including DVT, PE, and PE-related mortality. Therefore, the use of the potential value of NLR should be highlighted due to its affordability and accessibility particularly in limited-resource settings.

PB2295 | Acquired Type II (IIb) Protein S Deficiency, Case Report

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Background: We describe a patient with severe acquired functional deficiency of protein S (PS) and protein C (PC). The patient with a history of inflammatory bowel disease and primary biliary cirrhosis without long term specific treatment suffered from two spontaneous venous thromboembolic events in one year. He was treated with warfarin for twelve months when he developed skin necrosis. Subsequently, very low PS levels, low levels of PC and acquired APC (activated protein C) resistance were found.
Aims: The aim was to elucidate the underlying cause of PS deficiency in our patient.

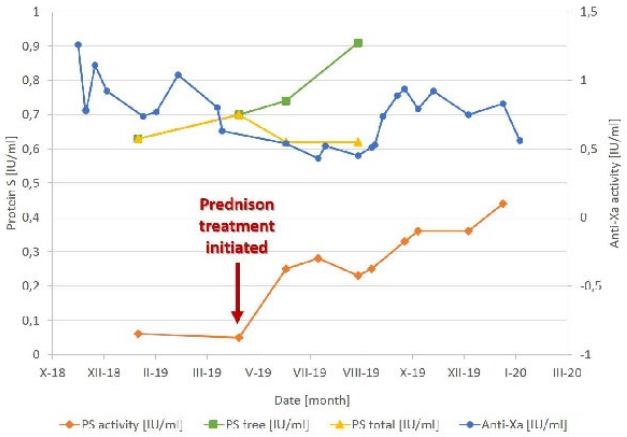

FIGURE 1 PS levels and anti-Xa activity during the follow-up. Initiation of prednison treatment is highlighted

Methods: We measured PS (activity, free PS, total PS) and PS inhibitor (Bethesda) and anti-PS IgG antibodies (ELISA Zymutest, Hyphen). We also measured PC (activity, antigen). The direct Sanger sequencing of all exons of *PROS1* was performed.

Results: The patient had to be treated with long term therapeutic doses of LMWH in order to prevent a new thromboembolic event. Attempts to switch him to DOACs resulted in severe side effects. A thorough laboratory testing and diagnostic imaging revealed no other underlying disease or cause of thrombophilia. Five months later, immunosuppressive treatment with prednisone and azathioprine was initiated by patient's hepatologist. Beside improving clinical condition, the treatment led to a fast increase of PS activity (Fig. 1). PC activity also increased (0.27 to 0.55 IU/ml). The LMWH dose could be lowered progressively. In light of these results we investigated possible presence of anti-PS antibodies, however it was not confirmed by Bethesda or anti-PS ELISA. We did not find any mutation in *PROS1* gene.

Conclusions: We ruled out inherited PS deficiency and the presence of specific antibodies. However, PS and PC levels increased after immunosuppressive treatment, which suggests the presence of inhibitory antibodies in the APC pathway, that might have a clinical effect on thrombosis in our patient.

PB2297 | Venous Involvement in Behcet Disease: Clinical Spectrum and Predictive Factors

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Background: Vascular involvement is frequent in Behcet disease (BD). Vascular manifestations are more frequently venous than arterial.

Aims: We aimed in this work to describe clinical characteristics, predictive factors and management of venous involvement during BD in the Tunisian context

Methods: We retrospectively studied 281 records of BD patients followed between January 2004 and December 2017 in the Internal Medicine Department and who fulfilled the International Study Group for BD (ISGBD) criteria. We described first clinical features of BD with venous involvement then predictive factors were studied in univariate then multivariate analysis.

Results: Among 281 patients, 61 have had venous involvement. They were 82% males and 18% females with a mean age of 32 years (12 to 55 years). Superficial venous thrombosis were diagnosed in 18 patients (29.5%) associated or not to deep venous thrombosis in 46 cases (75.4%) as follow: upper arm (16.4%), inferior limb (37.7%), inferior vena cava (13.1%), superior vena cava (1.6%) and mesenteric vein (1.6%). Venous involvement was associated to arterial manifestations in 9 patients (14.8%). Treatment consisted in high doses of steroids in 42.6%, cyclophosphamide in 21.3% and anticoagulation in 79.3%. Predictive factors independently associated with venous involvement in BD are male gender (OR=0.3, 95% CI= 0.14-0.67, p=0.004), erythema nodosum(OR= 4.2, 95% CI= 1.7- 10.6, p=0.002), ophthalmic involvement (OR= 0.46, 95% CI= 0.24- 0.9, p=0.024) and orchi epididymitis (OR= 4.8, 95% CI= 1.42 -16.38, p= 0.012).

Conclusions: Venous involvement in BD is frequent in the Tunisian context consisting in deep and superficial veins. Male gender, patients with erythema nodosum, ophthalmic manifestations or orchi epididymitis are more prone to develop venous complications needing therefore a close monitoring. In addition to anticoagulant, treatment should associate steroids and immunosuppressors.

PB2298 | Incidental Pulmonary Embolism in an Internal Medicine Department

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Background: Incidental pulmonary embolism (PE) defined as an unsuspected PE, is increasingly detected with the improved CT-imaging capable of thinner slices. However its significance remains unknown and its management is not codified.

Aims: To assess clinical characteristics, related factors and management of incidental PE.

Methods: A retrospective study over 16 years enrolling data of patients presenting an incidental PE in an internal medicine department.

Results: Seventeen patients were included. Sex ratio (H/F) was 0.7. The mean age was 61±12.4years. Five patients had an auto-immune or inflammatory systemic disease: lupus erythematosus(n=3), associated with dermatomysitis in one patient, Sjogren syndrome(n=1), rheumatoid arthritis(n=1) and Bechet disease(n=1). Malignancy was already diagnosed in four patients. CT scan was performed to

confirm or evaluate an interstitial lung disease(n=3), for staging or follow-up of a known neoplasm(n=3), for screening on an underlying neoplasia(n=3), for investigation of prolonged fever or worsening health status(n=3), for exploring abdominal pain(n=3) and for suspicion of thoracic extension of an hydatid cysts of the liver(n=1). In one case, PE was discovered when screening for Takayasu disease. Noted thromboembolism risk factors were: obesity(n= 5), sedentarity(n=5), recent surgery(n=3). Medical history of deep vein thrombosis was reported in 2 cases. In two cases, an underlying neoplasia was discovered. PE was segmental(n=16) and subsegmental in one case. The embolism was located in the lower lobe(n=11), middle lobe(n=3) and the upper lobe(n=3). It was bilateral in seven cases. The mean CT obstruction index was 42% ±6.6.

Patients were treated by Low-molecular-weight heparin followed by vitamin k antagonists(n=14) and by vitamin K antagonist only(n=2). One patient did not receive any anticoagulant treatment because of high risk of bleeding

Conclusions: Large outcome trials on the optimal management of incidental PE are lacking. However, the natural course of incidental PE seems to be comparable to that of symptomatic PE suggesting the application of similar management decisions.

PB2299 | Developing Fluidic Models that Replicate Cephalic Arch Geometry and Flow Conditions of Dialysis Patients to Understand and Predict Access Thrombosis

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Background: The current gold standard for End-Stage Renal Disease (ESRD) patients undergoing dialysis is creating an arteriovenous fistula (AVF) that allows a mature vein to withstand the vagaries of dialysis. Unfortunately, ESRD patients undergoing dialysis suffer AVF thrombosis, primarily in the cephalic vein arch.

Aims: We aim to understand why cephalic arch thrombosis occurs in ESRD patients through computational and experimental exploration of the complex interplay between abnormal hemodynamics, patient-specific vein geometry and biochemical factors that lead to endothelial cell activation.

Methods: We present personalized 3D millifluidic models of ESRD patients' cephalic arches at 3 and 12 months post AVF creation. These 3D models were created utilizing patient-specific Intravascular Ultrasound and venogram images that were coupled via SOLIDWORKS and AutoCAD. The patient-specific computational models were subjected to 1) Computational Fluid Dynamics simulations and 2) fabrication of fluidic devices in order to assess hemodynamics. These models were perfused with blood mimicking fluid containing fluorescent tracer beads at healthy (baseline) and patient-specific cephalic vein flow rates, measured 3 and 12 months after AVF creation (using Doppler measurements).

Results: Particle image velocity was used to calculate Reynolds number and local wall shear stress in the cephalic arch. We were able to visualize recirculation pools and calculate turbulent flow conditions across the cephalic arch.

Conclusions: Future patient-specific hemodynamic analyses using blood from patients and healthy donors, with and without the presence of clotting factors and anti-coagulants will permit identification of key parameters and regimes that correlate with thrombotic events. These fluidic devices allow isolating each contributing factor- geometry, flow dynamics, and biochemical factors- to understand their individual contribution in thrombosis. Elucidating the parameters of thrombogenesis in ESRD will enable more efficient and personalized treatments to maintain access and prevent complications during dialysis.

PB2300 | Analysis of Hemodynamic and Hemocoagulation Changes in Mortality Structure in Polytrauma Patients

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Background: Patients with polytrauma are the most difficult contingent in relation to venous thromboembolism.

Aims: The purpose of the study was to identify leading clinical and laboratory features in the mortality structure in polytrauma patients.

Methods: Materials and methods. The study included patients with multiple injuries who were in the ICU during the 2018-2019 follow-up period. The following cases: life-threatening injury, immediate death after an accident at the scene of the event and the first minutes of arrival at the hospital, patients with severe Brain Injury less than 10 points according GCS, APACHE II (19.8 ± 3.4)were exluded at the time of reception. The total number of patients was 241 patients according to the inclusion criteria, men-198 and women-43 (aged 42.03 ± 11.4 years). The hemodynamic parameters and the state of the coagulation system were studied, the influence of these factors on the mortality during the study period (28 days) were analyzed. Analysis of mortality was studied by disease histories of 24 (9.9%) death cases in the hospital during the control period.

Results: Results. The most severe category of patients after falling from a height. The total blood loss was 39% ± 0.19, mean AP-63.3 ± 16.1 mm Hg. This study evaluated the level of pH (7.21 – 7.31), mean AP(63.3 ± 16.1 mm Hg), and level of Lactate(3.94-0.22mmol / l), BE (-7.2±1.21) were significantly lower in patients who died. However, D-dimer levels were increased to 4205 ± 229.8 ng / ml, fibrinogen to 6.05 ± 0.82g / l. According to autopsy the causes of death were Shock-8, Pneumonia-6, Pulmonary Embolism-2, Cardiovascular insufficiency-7, Hemopericardium-2, Purulent-septic complications-3 cases.

Conclusions: Conclusions. Predictors of fatal complications were a low level of hemodynamics and increased levels of lactate, D-dimer and fibrinogen. At the same time the disorders of the

hemocoagulation system are not always directly related to fatal tromboembolism.

PB2301 | A Non-invasive Diagnostic Work-up Is Safe and Efficient to Exclude Pulmonary Embolism in Low Risk Pregnant Women with Suspected Pulmonary Embolism: A Systematic Review and Meta-analysis

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Background: Few studies evaluated the safety of a predefined diagnostic algorithm starting with pre-test probability assessment and D-dimer testing to rule out acute pulmonary embolism (PE) in pregnant women. Hence, the diagnostic accuracy of non-invasive diagnostic tests to rule out PE can be further established.

Aims: To establish the safety and efficiency of pre-test probability assessment, D-dimer testing and compression ultrasonography (CUS) in pregnant women with suspected acute PE in an individual patient data meta-analysis (IPDMA).

Methods: We performed a systematic review of the literature to identify all prospective diagnostic management studies in pregnant women with suspected acute PE, in which pre-test probability and D-dimer testing was used to rule out PE without imaging. Patient level data of the identified studies was collected and analyzed. The primary outcome was the 3-month incidence of venous thromboembolism (VTE) in patients with non-high pre-test probability and normal D-dimer test, using the Wells rule or YEARS algorithm. Efficiency was defined as the number of patients in whom CTPA was avoided. We additionally studied the diagnostic performance of CUS.

Results: We identified only two relevant studies, which ruled out the possibility of a meta-analysis. Because we excluded study-specific clustering of relevant patient characteristics in a multivariate model, data of the two studies were analysed as a whole. The combined studies totalled 893 pregnant women (Table 1). The diagnostic accuracy and efficiency of the individual diagnostic tests applied in the studies are summarized in Table 2. The YEARS algorithm as well as the Wells rule combined with D-dimer testing safely ruled out PE. The efficiency of bilateral CUS was limited, foremost in patients without symptoms of deep vein thrombosis (DVT).

Conclusions: This study underlines current guideline recommendations (ESC 2019) to apply pre-test probability assessment and D-dimer tests to rule out PE in low risk pregnant women, reducing potential harmful imaging tests.

TABLE 1 Baseline characteristics of 893 pregnant women with suspected pulmonary embolism

Baseline characteristics	*CTPE study (N=395)*	*Artemis study (N=498)*	*Merged (N=893)*
Mean age (+/- SD) – years	31 (6)	30 (5)	31 (6)
Median duration of pregnancy (IQR) - weeks	23 (9)	25 (9)	24 (9)
Trimester of pregnancy – no (%)			
First: 0 to 12 wk 6 days of gestation	73 (19)	74 (15)	147 (17)
Second: 13 wk 0 days to 26 wk 6 days of gestation	171 (43)	193 (39)	364 (41)
Third: 27 wk 0 days to 42 wk of gestation	151 (38)	231 (46)	382 (43)
YEARS criteria – no (%)			
Patients who met no criteria	91 (23)	252 (51)	343 (38)
Patients who met one to three criteria (total no.)	284 (72)	246 (50)	530 (59)
Clinical signs of deep-vein thrombosis	59 (15)	47 (9)	106 (12)
Hemoptysis	14 (4)	19 (4)	33 (4)
Pulmonary embolism as the most likely diagnosis	266 (67)	218 (44)	484 (54)
Travel in the previous 4 weeks – no (%)	15 (4)	12 (2)	27 (3)
Trauma/surgery in the previous 4 weeks – no (%)	6 (2)	5 (1)	11 (1)
Immobilization in the previous 4 weeks – no (%)	34 (9)	31 (6)	65 (7)
Current smoker – no (%)	71 (18)	37 (7)	108 (12)
Known heart failure – no (%)	0 (0)	5 (1)	5 (0.6)
Active malignancy – no (%)	0 (0)	1 (0.2)	1 (0.1)
Previous VTE – no (%)	29 (7)	28 (6)	57 (6)
Outpatient – no (%)	395 (100)	419 (84)	814 (91)
Mean heart rate (+/- SD) – bpm	91 (17)	92 (17)	92 (17)
Mean weight (+/- SD) - kg	73 (15)	74 (16)	74 (16)

Note: N: number of patients, SD: standard deviation, IQR: interquartile range, wk: weeks, VTE: venous thromboembolism, bpm: beats per minute, kg: kilograms

TABLE 2 Sensitivity, specificity, negative predictive value and efficiency of the non-invasive diagnostic work-up of suspected pulmonary embolism in

	Sensitivity (n/n, %, 95%CI)	Specificity (n/n, %, 95%CI)	Negative predictive value (n/n, %, 95%CI)	Efficiency (number of CTPA scans avoided; n/n, %, 95%CI)
YEARS	41/43 95.4% (84.5-98.7)	270/838 32.2% (29.1-35.5)	270/272 99.3% (97.4-99.8)	272/881 30.9% (27.9-34.0)
Wells rule/ D-dimer test	45/46 97.8% (88.7-99.6)	103/841 12.3% (10.2-14.6)	103/104 99.0% (94.8-99.8)	104/887 11.7% (9.8-14.0)
Bilateral CUS (overall)	11/34 32.4% (19.1-49.2)	450/450 100.0% (99.2-100.0)	450/473 95.1% (92.8-96.7)	11/484 2.3% (1.3-4.0)
Bilateral CUS for suspected DVT	8/16 50.0% (28.0-72.0)	85/85 100.0% (95.7-100.0)	85/93 91.4% (83.9-95.6)	8/101 7.9% (4.1-14.9)
Bilateral CUS without signs of DVT	3/18 16.7% (58.4-39.2)	365/365 100.0% (99.0-100.0)	365/380 96.1% (93.6-97.6)	3/383 0.78% (0.27-2.3)

Note: CTPA: computed tomographic pulmonary angiography, CI: confidence interval, CUS: compression ultrasonography, DVT: deep vein thrombosis, NA: not applicable

PB2302 | Right Ventricular to Left Ventricular Diameter Ratio Assessment with Non-ECG Gated Computed Tomography Scans and its Correlation with Right Ventricular Dysfunction in Acute Pulmonary Embolism

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Background: The presence of right ventricular (RV) dysfunction in acute pulmonary embolism (PE) is associated with poor prognosis. RV dilatation based on right-to-left ventricular end diastolic diameter ratio (RV/LV) on computed tomographic pulmonary angiography (CTPA) has a good correlation with echocardiographic (Echo) parameters. RV/LV assessment with non-ECG gated CTPA and its correlation with RV dysfunction is not well established.

Aims: Establish the correlation between RV/LV diameter ratio assessed by non-ECG gated CTPA and RV dysfunction determined by Echo in acute PE.

Methods: Single-center observational cohort registry performed at a University Hospital. Patients hospitalized with a diagnosis of PE and RVD between 2008-2018 were eligible. RV dysfunction was defined as the presence of RV dilatation and concomitant reduced tricuspid annular plane systolic excursion or RV wall motion abnormalities. CTPA considered the maximal distance between the ventricular endocardium and the interventricular septum, perpendicular to the long axis for both ventricles to calculate the RV/LV. All statistical analysis was carried out using the STATA version 13.1. 1

Results: 230 consecutive patients were hospitalized with a diagnosis of PE. 45 patients with RVD were included. Mean age 64±16 years; 54.6% female. Global IHM in patients with RVD was 10.7%. Multivariate analysis showed that patients with RV dilatation on Echo had a RV/LV > 2 (Std.Err. 0.7, IC 95% 0.4-3.5; p:ns) while those without RV dilation had a RV/LV > 1.3 (Std.Err. 0.2, IC 95% 0.8-1.9; p: ns). No patient with RV/LV > 2 presented normal RV function.

Conclusions: In our setting a RV/LV >2 assessed by non-ECG gated CTPA correlated with RV dysfunction in patients with acute PE. Determination of RV/LV with non-ECG gated CTPA may thus be useful in clinical practice.

PB2303 | Particularities of Venous Thromboembolism in Systemic Vasculitides

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Background: Venous thromboembolic disease (VTED) can reveal systemic vasculitides (S V) or appear during their evolution.

Aims: The aim of our study was to determine the particularities of venous thromboembolic events in patients with SV.

Methods: We conducted a retrospective study of in patients hospitalized for VTED records in the internal medicine department over a 23-year period (1995-2018).

Results: Among 1553 cases of venous thromboembolism, 107 patients (6.8%) have SV including 75%men and 25% women (sex ratio F / H at 0.3).The average age at the time the diagnosis of thrombosis was 44 years. VTED was indicative of the underlying vascularity in 71% of the cases, it occurred during monitoring of the vascularity involved in the rest of cases. Thrombophlebitis was localized : in the lower limbs in 62% cases, pulmonary embolism was found in 11% of cases, portal thrombosis, of the superior vena cava and jugular thrombosis were found in 6% each, cerebral thrombosis in 4%, and in the upper limb in 2%. the vasculitides found were : Behcet's disease(n= 102), granulomatosis with Polyangiitis (n=1), microscopic polyangiitis (n=1), Cryoglobulinemic Vasculitis (n=1) and indeterminate vasculitis(n=2). Anticoagulant therapy was indicated in all patients for an average duration of 18 months. The corticosteroid therapy was indicated in 81% of cases and treatment immunosuppressant in 56% cases. Thrombophlebitis was recurrent in 9 cases under anticoagulant treatment.

TABLE 1 Clinical characteristics and in-hospital mortality. Group 1 (RV/LV >1.3 and <2) vs.Group 2 (RV/LV≥ 2)

VARIABLE	GROUP 1 (RV/LV >1.3 and < 2)	GROUP 2 (RV/LV < gequal > 2)	p value
Age (mean)	68 (SD 13)	62 (SD 19)	ns
Female	11 (47.8%)	8 (50%)	ns
Syncope	2 (8.6%)	2 (12.5%)	ns
sPESI >0	20 (86.9%)	10 (62.5%)	ns
Chest pain	3 (13%)	3 (18.7%)	ns
Heart rate > 100 bpm	14 (60.8%)	8 (50%)	ns
Elevated troponin levels	8 (34.7%)	6 (37.5%)	ns
RV wall motion abnormalities	15 (65.2%)	11 (68.7%)	ns
in-hospital mortality	2 (8.6%)	2 (12.5%)	ns

Conclusions: There is more and more evidence of links between the presence of a systemic vascularity and the occurrence thromboembolic events. A rigorous etiological investigation before a first episode of venous thrombosis can allow an early positive diagnosis of the vasculitis in question. In our series, behcet's disease was the most common cause which joins literature data.

PB2304 | Deep Venous Thrombosis with Atypical Location: About 22 Cases

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Background: Venous thromboembolism (VTE) of atypical location involves organ-related venous territories such as splanchnic, renal, gonadal, and cerebral venous segments.
Aims: To characterize the spectrum of atypical deep venous thrombosis (DVT) in our department of Internal Medicine.
Methods: We reviewed the records of 22 patients with documented DVT of atypical location.
Results: In our study, 22cases of DVT with atypical location, 11men (50%) and 11women (50%) were analyzed, with a mean age of 52±20 years. In 9cases (41%) no risk factors were detected. Other risk factors were noticed : bed rest in 6cases (27%), tobacco in 5cases (23%) and obesity in 2cases (9%). When making the diagnosis, only 1patient (4.5%) were asymptomatic.Atypical locations of DVT were: digestive tract veins in 16cases (72.73%), upper extremity vein in 5cases (23%), jugular vein in 2cases (9%), ovarian vein in 2cases (9%), vena cava in 2cases (9%), renal vein in 1case (4.5%) and cerebral vein in 1 case (4.5%). Idiopathic thrombosis was observed in 8cases(36.7%). The other underlying conditions were surgery in 4cases (18%), neoplasm in 2cases (9%), postpartum in 2cases (9%), essential thrombocythemia in 1case (4.5%), presence of a central venous catheter in 1case (4.5%), anti-thrombin deficiency in 1case (4.5%), Behçet disease in 1case (4.5%), tuberculosis in 1case (4.5%) and nutcracker syndrome in 1case (4.5%).The median duration of treatment 9±5 months. No patient died during this period. Nevertheless, hemorrhage was observed in 2cases and recurrence of DVT was noticed in 1case.
Conclusions: The most common forms of VTE are deep vein thrombosis in the lower limbs and pulmonary thromboembolism. There are few revisions of unusual cases of VTE. In most cases, VTE occurs in the upper limbs, unlike our series. Due to their uncommon expression, we suggest a thorrow approach to reveal underlying transient factors and local or hypercoagulable states mainly neoplasms.

PB2305 | Photoshop-GSM *versus* Customized-GSM Software to Analysis of Echogenicity of Residual Venous Obstruction

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Background: Studies has been shown that patients with DVT managed within an established clinical care incorporating the presence of Residual Venous Obstruction (RVO) had relatively low incidences of DVT recurrence. Analysis of echogenicity of RVO by gray scale median (GSM) appear improve this kind of analysis. However, the GSM analyses has poor availability and is operator-dependent.
Aims: The aim of this study was to compare echogenicity of RVO by GSM using Photoshop vs customized GSM software.
Methods: RVO from 76 DVT patients, follow from 2017 to 2019 in Blood Center UNICAMP-Campinas were analysed for both methodology. Only common femoral vein was considered for a homogeneous analysis. Photoshop 2019 (Adobe Systems) and local customized GSM (CIMT) softwares were used. The row data collected were median, number of Pixels and weight median of each thrombus. The median time between DVT and RVO detection was 46 months (from 4 to 401months).
Results: The difference between CIMT-GSM and Photoshop-GSM results were high among the low to high values that classify the RVO as hypoechoic (figuere1). The median were 42 (1-216) vs 23 (7-119) to GSM and Photoshop, respectively. The patients that presented post thrombotic syndrome (60%) showed better sensitivity and specificity with a Photoshop evaluated by Area under the ROC curve, 0.6273 (95% confidence interval: 0.3710-0.8837 p=0.2801) against 0.5810 (95% confidence interval: 0.3692-0.7928 p=0.4918). When the correlation between the time of clinical event (DVT) were evaluated with the echogenicity, patients that had DVT until 12 months of RVO evaluation showed better results with Photoshop-GSM (Spearman correlation r = 0.6669 for photoshop-GSM vs 0.5000 for CIMT-GSM).
Conclusions: Photoshop is an easily method, not operator-dependent, which can be included in clinical practice for RVO analysis. If results confirm the importance of RVO echogenicity in DVT recurrence, the Photoshop can be a practical tool on this setting.

VTE EPIDEMIOLOGY

PB2306 | Risk Factors and Hemostatic Aspects of Aseptic Cerebral Venous Sinus Thrombosis

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Background: Although aseptic cerebral venous sinus thrombosis (ACVST) has been clinically recognized for more than a century, physicians often overlook the diagnosis. In recent years, the septic forms have become quite rare with the use of antibiotics. No precise prevalence and predictors of ACVST have been established due to rarity of this condition.

Aims: To study risk factors and hemostasis in patients with ACVST.

Methods: Two groups: group 1 - 47 patients (age 62.7±7.7 years) with ACVST; group 2 (control) - 40 patients (59.7±10.2 years) without ACVST and VTE were formed in non-randomized retrospective-prospective study. Brain MRI and CT-/MR-venography were performed. Fibrinogen level, fibrinolysis index, fibrinolytic activity, INR, APTT, Lee-White coagulation time, D-dimers, B-fibrinogen, antithrombin III (ATIII), hematocrit platelet count, protein C and S levels, von Willebrand factor (VWF), blood coagulation factors (FV, FVII, FVIII, FXII), homocysteine, antibodies to cardiolipin and lupus anticoagulant - were determined.

Results: Isolated ACVST was detected in 23.4%, and two or more venous sinuses thrombosis - in 76.6% of ACVST cases. The main risk factors for the development of ACVST in women were pregnancy, coagulopathy, contraceptives usage and hyperhomocysteinemia; in men - hyperhomocysteinemia and coagulopathy. ACVST was cryptogenic in 23.4% of cases among women and 34.0% of cases among men.

We revealed in the group 1, compared with the group 2, an increase in the level of FVIII and VWF antigen, a decrease in the level of ATIII, protein C, protein S, and hyperhomocysteinemia (table 1).

Conclusions: Identified risk factors and markers of thrombosis can be used for routine and screening examinations of patients with suspected ACVST to allocate the "highest risk" group with VWF antigen (above 161%), ATIII (below 80%), FVIII (above 128%), protein C (below 92%), protein S (below 88%) and homocysteine (above 14 μmol/l).

PB2307 | Epidemiologic Study of Patients with Thrombotic Events Referred to Tertiary Hospital in Southern Iran

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Background: Epidemiologic study of patients with thrombotic events referred to tertiary hospital in southern Iran.

Aims: We aimed to evaluate the occurrence of thrombotic events and related risk factors in a group of Iranian patients.

Methods: In this cross-sectional study, all patients (n=99) who were complicated by thrombotic events referred to the Hematology Research Center of Shiraz University of Medical Sciences were investigated from 2015 to 2017, in Shiraz, Southern Iran. Data were collected from their medical records by a designed data gathering form.

Results: The mean age of the occurrence of thrombosis was 50 ± 20 years (range: 4-89 years). From all thrombotic events 52.5% occurred in females. Venous thrombosis was more prevalent (61.6%) than arterial. Hypertension, diabetes mellitus and ischemic heart disease were the most associated disease with thrombosis. Most of the patients (79.8%) had no episodes of relapse and the occurrence

TABLE 1 Hemostatic indicators (statistically significant) in an aseptic cerebral venous sinus thrombosis

Indicators, mean value	1st group with ACVST (n=47)	2nd group without ACVST and VTE (n=40)	ROC-analysis, hemostatic predictors for the development of ACVST
Von Willebrand factor (VWF), % (norm 61.3-117.5%)	195.5 ± 22.1*	157.1 ± 15.9	above 161
F VIII,% (normal 50-150%)	142.3 ± 15.1*	94.2 ± 11.8	above 128
Antithrombin III, (ATIII) % (norm 71-115%)	74.6±11.2*	87.4 ± 8.3	below 80
Protein C, % (norm 70-150%)	83.6 ± 13.9*	105.7 ± 10.2	below 92
Protein S, % (norm 60-130%)	79.4 ± 12.5*	99.6 ± 11.4	below 88
Homocysteine μmol/l (norm 0-12 μmol/l)	16.14 ± 6.8**	11.4 ± 2.5	above 14

P value: * - < 0.05; ** - < 0.01, Mann-Whitney U-test.

of relapse had no significant relationship with thrombophilia and underlying disease. Acceptable response rate for warfarin therapy was achieved in 46.5% with 5 mg and 43.4% with 5-7.5 mg. Frequency of the associated diseases with thrombotic events is shown in figure1.

Conclusions: The most common risk factors for thrombotic disease in our study were atrial fibrillation and rheumatic heart disease. It is reasonable to recognize high risk patients for recurrence of venous thromboembolic and arterial events. Moreover, prophylaxis is necessary in high risk patients to reduce the complications and healthcare cost.

PB2308 | Management Strategies and Clinical Outcomes in Patients with Inferior Vena Cava Thrombosis: Data from the GARFIELD-VTE Registry

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Background: Thrombosis of the inferior vena cava (IVC) is a rare form of deep venous thrombosis (DVT). Optimal treatment strategies and clinical outcomes are not well established.

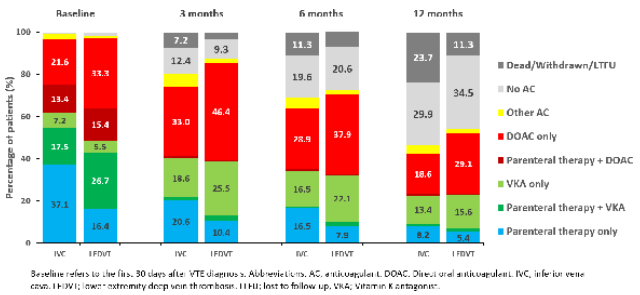

FIGURE 1 Anticoagulation treatment patterns over 12 months' follow-up

TABLE 1 12-month clinical outcomes

Event	IVC (n=100)		LEDVT (n=7646)		HR [95% CI]	P-value
	n	Rate [95% CI]	n	Rate [95% CI]		
All cause mortality	14	16.6 (9.8-28.0)	478	6.8 (6.2-7.4)	2.39 [1.40-4.06]	0.0013
Recurrent VTE	4	4.9 (1.8-13.0)	172	5.4 (4.8-5.9)	0.88 [0.33-2.36]	0.7994
Any bleeding	6	7.4 (3.3-16.4)	680	10.3 (9.5-11.1)	0.69 [0.31-1.55]	0.3708
Major bleed	1	1.2 (0.2-8.5)	356	2.5 (2.1-2.9)	*	

A hazard ratio >1.0 indicates patients with IVC thrombosis are at higher risk. Event rates are shown per 100 person-years and hazard ratios were unadjusted. Abbreviations: VTE; venous thromboembolism, HR; hazard ratio, CI; confidence interval, IVC; inferior vena cava, LEDVT; lower extremity deep vein thrombosis. *Event rates too small to calculate hazard ratio.

Aims: Compare the baseline characteristics, treatment strategies, and 12-month clinical outcomes of patients with lower extremity DVT (LEDVT) with and without IVC thrombosis enrolled in the Global Anticoagulant Registry in the FIELD - Venous Thromboembolism (GARFIELD-VTE).

Methods: GARFIELD-VTE (ClinicalTrials.gov: NCT02155491) is a global, prospective, non-interventional study of real-world treatment practices. Of the 10,870 patients with objectively confirmed VTE enrolled between May 2014 and January 2017, 7,746 were eligible for analysis in this study.

Results: One-hundred IVC thrombosis patients (all with concomitant LEDVT) and 7,646 LEDVT patients with no IVC involvement were compared. IVC thrombosis patients were younger (51.9 vs. 59.8 years), more frequently had active cancer (23.0% vs. 7.5%) or a history of cancer (21.0% vs. 10.2%), and less frequently had recent trauma or surgery.

At baseline, a higher proportion of patients with IVC thrombosis received thrombolysis (11.3% vs 3.8%) or surgical mechanical interventions (4.1% vs. 1.4%) than those without. Patients with IVC thrombosis more frequently received parenteral anticoagulants alone (37.1% vs. 16.4%), and less frequently received vitamin K antagonists (24.7% vs. 32.2%) or direct oral anticoagulants (± parenteral therapy) (35.0% vs. 48.7%). Treatment duration over 12 months of follow-up was similar (Figure 1). The rate of all-cause mortality was higher in patients with IVC thrombosis (16.6 vs. 6.8 per 100 person-years), however the proportion of cancer-associated deaths was comparable (51.9% vs. 57.1%). Rates of VTE recurrence (4.9 vs. 5.4 per 100 person-years) and bleeding (7.4 vs. 10.3 per 100 person-years) were similar (Table 1).

Conclusions: Patients with IVC thrombosis are younger, and have higher all-cause mortality rates than LEDVT patients, only partly attributable to malignancy.

PB2309 | The Influence of Anemia on Clinical Outcomes in Venous Thromboembolism: Results from GARFIELD-VTE

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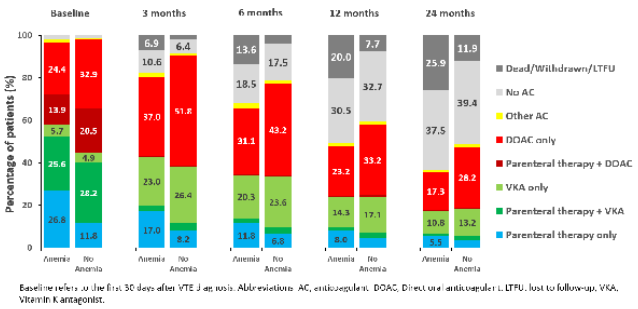
Background: Venous thromboembolism (VTE) patients with concomitant anemia are at increased risk for bleeding.

Aims: Compare the baseline characteristics, treatment patterns, and 24-month outcomes in patients with and without anemia (Hb < 12.0 g/dL for women, < 13.0 g/dL for men) enrolled in the Global Anticoagulant Registry in the FIELD (GARFIELD)-VTE.

Methods: GARFIELD-VTE (ClinicalTrials.gov: NCT02155491) is a global, prospective, non-interventional study of real-world treatment practices. Of the 10,870 patients with objectively confirmed VTE enrolled between May 2014 and January 2017, 7,703 (71%) were eligible for analysis in this study.

Results: The distribution of VTE events was comparable between patients with (n=2,779) and without anemia (n=4,924) (DVT alone: 61.1% vs. 56.0%, PE ± DVT: 38.9% vs. 44.0%, respectively). Anemic patients (vs. no anemia) were more likely to be Asian (29.4% vs. 13.0%), older (59.9 years vs. 57.5 years) and have a lower BMI (27.4 kg/m^2 vs. 29.1 kg/m^2). Platelet counts were higher in anemic patients (267.8 10^9/L vs. 237.3 10^9/L). Several VTE risk factors were more common in anemic patients, including hospitalization (22.0% vs. 6.9%), surgery (19.2% vs. 8.2%), cancer (17.4% vs. 4.2%) and acute medical illness (8.2% vs. 4.2%). Anemic patients were more likely to be initiated on parenteral therapy alone compared with patients without anemia (26.8% vs. 11.8%), and less likely to receive a direct oral anticoagulant (38.3% vs. 53.4%). The same trends were observed at 3, 6, 12 and 24 months after VTE diagnosis (Figure 1). After 24 months of follow-up, anemic patients were at an increased risk (hazard ratio [95% confidence interval] of all-cause mortality (2.66 [2.17-3.26]) and major bleeding (3.25 [2.45-4.31). The risk of recurrent VTE was comparable between groups (Table 1).

Conclusions: VTE patients with concomitant anemia are at an increased risk of major adverse clinical outcomes.



Baseline refers to the first 30 days after VTE diagnosis. Abbreviations: AC, anticoagulant; DOAC, Direct oral anticoagulant; LTFU, lost to follow-up; VKA, Vitamin K antagonist.

FIGURE 1 Anticoagulation treatment patterns over 24 months' follow-up

TABLE 1 24-month clinical outcomes

Event	Anemia (n=2,779)		No anemia (n=4,924)		HR [95% CI]	P-value
	n	Rate [95% CI]	n	Rate [95% CI]		
All-cause mortality	477	11.23 [10.26-12.28]	244	2.80 [2.47-3.17]	2.66 [2.17-3.26]	<0.0001
Major bleed	161	3.92 [3.36-4.58]	79	0.91 [0.73-1.14]	3.25 [2.45-4.31]	<0.0001
Recurrent VTE	179	4.42 [3.82-5.11]	345	4.13 [3.72-4.59]	0.96 [0.79-1.16]	0.6486
Any bleeding	410	10.79 [9.80-11.89]	515	6.40 [5.87-6.98]	1.45 [1.27-1.67]	<0.0001
Stroke/TIA	37	0.87 [0.63-1.21]	44	0.51 [0.38-0.68]	1.37 [0.87-2.15]	0.1758
MI/ACS	31	0.74 [0.52-1.05]	33	0.38 [0.27-0.53]	1.83 [1.10-3.03]	0.0199

A hazard ratio >1.0 indicates patients with anemia are at higher risk. Event rates are shown per 100 person-years. Hazard ratios were adjusted for cancer status, age, ethnicity and body mass index. Abbreviations: VTE; venous thromboembolism, TIA; transient ischemic attack, MI; myocardial infarction, ACS; acute coronary syndrome, HR; hazard ratio, CI; confidence interval.

PB2310 | Pregnancy-Associated Venous Thromboembolism: Insights from GARFIELD-VTE

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Background: The risk of venous thromboembolism (VTE) is increased in pregnancy and postpartum such that VTE is a leading cause of maternal mortality.

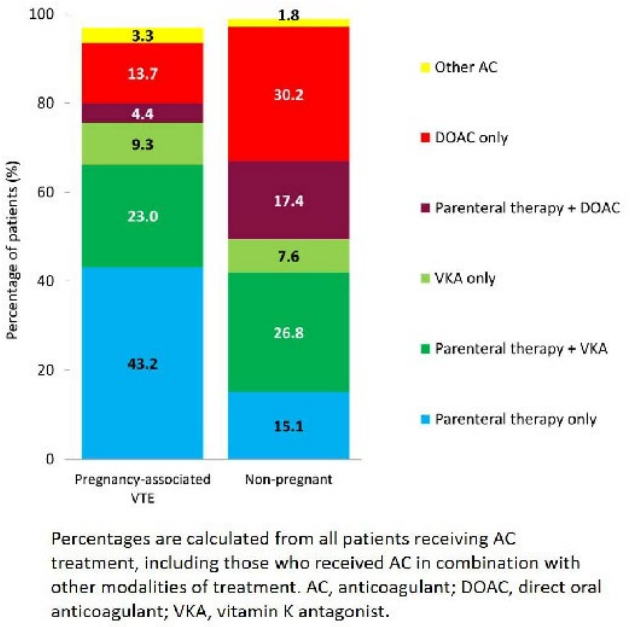

FIGURE 1 Anticoagulant treatment at baseline (up to 30 days after VTE diagnosis)

TABLE 1 12-month clinical outcomes

Event	Pregnancy-associated VTE (n=183)		Non-pregnant (n=1,187)		HR [95% CI]	P-value
	n	Rate [95% CI]	n	Rate [95% CI]		
All-cause mortality	3	1.71 [0.55-5.29]	43	3.86 [2.87-5.21]	0.59 [0.18-1.98]	0.3959
Major bleed	3	1.73 [0.56-5.37]	29	2.65 [1.84-3.81]	1.13 [0.33-3.90]	0.8499
Recurrent VTE	3	1.71 [0.55 5.29]	56	5.19 [4.00 6.75]	0.82 [0.34 1.94]	0.6458
Any bleeding	16	9.78 [5.99-15.96]	140	13.85 [11.74-16.35]	0.81 [0.47-1.41]	0.4648

A hazard ratio >1.0 indicates pregnancy-associated VTE patients are at higher risk. Event rates are shown per 100 person-years. Hazard ratios were adjusted for age, ethnicity and body mass Index. Abbreviations: VTE; venous thromboembolism, HR; hazard ratio, CI; confidence interval.

Aims: Investigate the clinical characteristics, diagnostic strategies, treatment patterns and 12-month outcomes of women with pregnancy-associated VTE enrolled in the Global Anticoagulant Registry in the FIELD (GARFIELD)-VTE.

Methods: GARFIELD-VTE (ClinicalTrials.gov: NCT02155491) is a non-interventional study observing real-world treatment practices. Of the 10,870 patients with objectively confirmed VTE enrolled between May 2014 and January 2017, women of childbearing age (< 45 years) were stratified into those with (n=183) or without (n=1187) pregnancy-associated VTE.

Results: Women with pregnancy-associated VTE were younger (30.5 years vs. 34.8 years) and less likely to have had a pulmonary embolism (PE) than those without (19.7% vs. 32.3%). Deep vein thrombosis (DVT) in women with pregnancy-associated VTE occurred more frequently in the left leg (73.9%) than in women without pregnancy-associated VTE (54.8%). The most common VTE risk factors in pregnancy-associated VTE patients were hospitalisation (10.4%), previous surgery (10.4%), and family history of VTE (9.3%). Compression ultrasonography was used to diagnose DVT in 98.7% of pregnancy-associated VTE patients, whilst spiral/chest computed tomography scan was frequently used to diagnose PE (75.0%). 43.2% of pregnancy-associated VTE patients received parenteral therapy alone, 50.4% received a VKA or a DOAC (Figure 1). After adjustment for baseline characteristics, the risk of all-cause mortality (HR 0.59 [95% CI: 0.18-1.98]), recurrent VTE (HR 0.82 [95% CI 0.34-1.94]) and major bleeding
(HR 1.13 [95% CI 0.33-3.90]) were comparable between patients with or without pregnancy-associated VTE (Table 1).

Conclusions: Half of all patients with pregnancy-associated VTE received either a VKA or a DOAC, despite limited evidence for their use in this population. The rate of clinical outcomes was comparable between patient groups.

PB2311 | Influence of Body Mass Index on Outcomes in Patients with Venous Thromboembolism: Insights from GARFIELD-VTE

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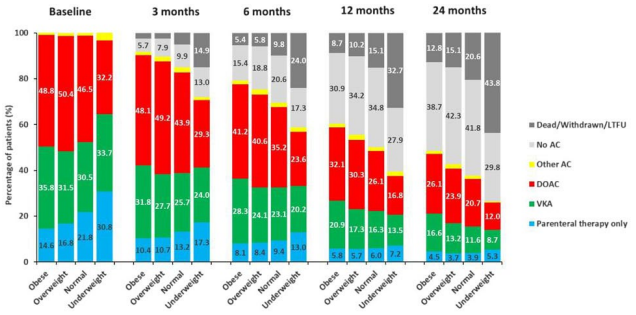
Background: There is limited information on the influence of obesity on outcomes in venous thromboembolism (VTE) patients.

Aims: Investigate the influence of body mass index (BMI) on baseline characteristics, treatment patterns and 2-year outcomes in VTE patients.

Methods: GARFIELD-VTE is a prospective, non-interventional registry of 10,870 patients with objectively confirmed VTE, enrolled between May 2014 and January 2017. Patients were grouped according to BMI as follows: < 18.5 (underweight; n=215); 18.5-24.9 (normal; n=2865); 25.0-29.9 (overweight; n=3332); ≥ 30 (obese; n=3076).

Results: The mean age and distribution of VTE events were comparable across groups. Compared with patients with a normal BMI, obese patients were more frequently Caucasian (77.3% vs. 57.9%), treated in the outpatient setting (30.4% vs. 23.2%); less likely to have active cancer (5.9% vs. 11.6%) but more likely to have had a previous VTE (17.6% vs. 11.6%). At baseline, obese patients were less likely than those with a normal BMI to receive parenteral therapy alone (14.6% vs. 21.8%), and a greater proportion of obese patients remained on anticoagulation treatment over 2 years (48.5% vs. 37.6%) (Figure 1). After 24 months of follow-up, the risk of all-cause mortality was lower in obese patients than in those with normal BMI (adjusted hazard ratio: 0.66; 95% confidence interval: 0.55-0.79). The risks of recurrent VTE and major bleeding were comparable (Table 1). Among obese patients, the unadjusted rate of all-cause mortality was higher in those receiving parenteral therapy (12.30 per 100 person-years) than in those receiving a DOAC (1.66 per 100 person-years) or VKAs (2.88 per 100 person-years). Patients with a BMI≥40 had an increased risk of any bleeding compared with those with BMI of 35-39.9 or 30-34.9 (11.01, 8.56 and 6.56 per 100 person-years, respectively).

Conclusions: Obese VTE patients have a reduced risk of mortality compared with patients with normal BMI, even after adjustment for baseline characteristics.



Baseline refers to the first 30 days after VTE diagnosis. DOAC and VKA groups may include parenteral bridging.
Abbreviations: AC; anticoagulant. DOAC; Direct oral anticoagulant. LTFU; lost to follow-up, VKA; Vitamin K antagonist.

FIGURE 1 Anticoagulation treatment patterns over 24 months' follow-up

TABLE 1 24-month clinical outcomes in VTE patients according to BMI

Event	Underweight		Normal		Overweight		Obese		HR [95% CI], *p*
	n	Rate [95% CI]	n	Rate [95% CI]	n	Rate [95% CI]	n	Rate [95% CI]	
All-cause mortality	69	24.76 (19.56-31.35)	336	7.31 (6.56-8.13)	272	4.81 (4.27-5.42)	191	3.56 (3.09-4.10)	0.66 [0.55-0.79], *0.0001*
Major bleed	17	6.29 (3.91-10.12)	88	1.95 (1.58-2.40)	91	1.63 (1.33-2.01)	86	1.63 (1.32-2.02)	0.92 [0.68-1.25], *0.5894*
Recurrent VTE	10	3.76 (2.02-6.98)	158	3.57 (3.06-4.17)	223	4.12 (3.61-4.70)	194	3.76 (3.27-4.33)	1.10 [0.88-1.36], *0.4048*
Any bleeding	28	10.77 (7.44-15.60)	302	7.11 (6.36-7.96)	361	6.94 (6.26-7.69)	377	7.73 (6.99-8.55)	1.09 [0.93-1.27], *0.3007*
MAST	2	0.72 (0.18-2.88)	54	0.72 (0.18-2.88)	55	(0.98 (0.75-1.28)	59	1.11 (0.86-1.43)	1.01 [0.69-1.48], *0.9602*

A hazard ratio >1.0 indicates patients with obesity are at higher risk (reference group: normal BMI). Unadjusted event rates are shown per 100 person-years. Hazard ratios were adjusted for age, ethnicity and body mass index and cancer status. Abbreviations: VTE; venous thromboembolism, MAST; myocardial infarction, acute coronary syndrome, stroke or transient ischemic attack, HR; hazard ratio, CI; confidence interval.

PB2312 | Risk Factors of Thrombotic Complications in Thai Thalassemia Patients

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Background: Thalassemia is an inherited red blood cell disorder characterized by reduced or absent production of the globin chain. Thromboembolic events are one of complications that may lead to morbidity and mortality of thalassemia patients. The prevalence of thromboembolic events in Thai patients with thalassemia has not been well described.

Aims: This study aimed to determine the prevalence and risk factors of thromboembolic events in patients with thalassemia.

Methods: This was a single center, cross-sectional study. Data were retrieved from Chiang Mai Thalassemia Registry between 2014 to

Figure 1. Sites of thrombosis in patients with thalassemia

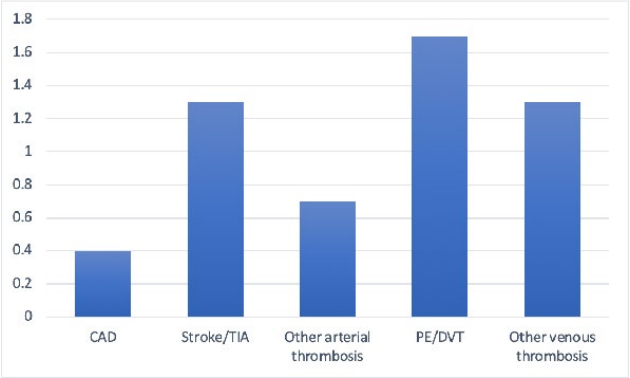

FIGURE 1 Sites of thrombosis in patients with thalassemia

2019. We included all adult patients (age 15 years old or older) who were diagnosed with thalassemia, using a high-performance liquid chromatography (HPLC) methods. We collected data regarding to age, sex, type of thalassemia, transfusion requirement, cirrhosis, cardiomyopathy and basic laboratories. The primary outcome was the prevalence of objectively confirmed arterial or venous thrombosis.

Results: There were 543 thalassemia patients included in the study. The median age was 33 years (interquartile range 24-49 years) and 42.1% were males. Of these, 200 (36.9%) patients were transfusion dependent and 343 (63.2%) patients were transfusion independent. Splenectomy was performed in 38.9% of patients in this cohort. All of splenectomized patients had received aspirin. Thromboembolic events were observed in 29 patients, which translated into the prevalence of 5.3% (95% confidence interval [CI] 3.6% to 7.6%). Of these, 44.8% were arterial thrombosis and 55.2% were venous thrombosis. The sites of thrombosis are demonstrated in figure 1. The univariable analysis revealed that, older age (odds ratio [OR] 1.02, 95% CI 1.01-1.04, p = 0.049) and cirrhosis (OR 6.96, 95% CI 2.40-20.17, p< 0.001) were associated with thrombosis.

Conclusions: Thromboembolic complication was not uncommon in Thai thalassemia patients. Older age and liver were associated with higher risk of thrombosis.

PB2313 | The Association of BMI and Waist Circumference Improves the Prediction of VTE Risk as Compared to BMI Alone

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Background: Obesity, as defined by the body mass index (BMI), is independently associated with the risk of venous thromboembolism (VTE), while abdominal obesity, defined by the measurement of waist circumference, is a predictor of cardiovascular events.

Aims: We assessed the role of BMI or waist circumferences and their combination to predict unprovoked VTE in the population of the longitudinal Moli-sani study.

Methods: Moli-sani is a prospective cohort study carried out in the general population of the Molise region in Italy. A total of 23,758 individuals (48% men, age ≥35 years) enrolled between 2005- 2010 were eligible for this analysis. Patients on life-long anticoagulant treatment for any reason were excluded. Obesity was defined by a BMI >30 Kg/m^2 while abdominal obesity was defined by waist circumference ≥102 cm for men or 88 cm for women. The long-term incidence of unprovoked VTE in subjects with and without obesity or abdominal obesity, both measured at baseline, was assessed. Hazard Ratio (HR) and 95% confidence intervals (CI), adjusted by age, sex, income, history of VTE, cardiovascular disease, chronic kidney disease and alcohol drinking habits, were estimated by a Cox regression.

Results: Overall, 29.7% of individuals were obese and 44.3% had abdominal obesity. A total of 68 unprovoked VTE events were diagnosed during a median follow-up of 8.2 years. VTE occurred more frequently in obese (n: 33, 0.47%) than in non-obese subjects (n: 35, 0.21%), HR 1.63, 95%CI 0.98-2.69, and in subjects with abdominal obesity (n:45, 0.43%) than in those without (n:23, 0.17%), HR 2.14, 95%CI 1.21-3.81. In a combined model, neither obesity alone (1.34, 0.31-5.80) nor abdominal obesity alone (2.03, 0.97-4.27) were independently associated with VTE, whereas their concomitant presence was significantly associated (2.27, 1.22-4.24).

Conclusions: The concomitant assessment of BMI and waist circumference should be used to improve the prediction of VTE risk.

PB2314 | Multidisciplinary Approach of Deep Venous Thrombosis (DVT) in Vall d'Hebron University Hospital: Two Years' Experience

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Background: DVT is a frequent cause or morbidity and mortality, and multiple factors are involved in its etiopathogenesis. In Vall d'Hebron University Hospital, we have stablished an ordered multidisciplinary system to follow our DVT patients and to try to ensure an appropriate correct approach of the disease.

Aims: To identify our DVT population demographics and characteristics of the disease, as well as mid-term complications within our DVT unit.

Methods: Observational and prospective study between January 2017-December 2018. Two years of follow up.

Results: We included 295 patients with DVT, 55.9% were men, the average age was 66.8 years old, 84.4% were in lower limbs and 17% associated pulmonary embolism (PE). We tested 34.6% for thrombophilia and only 13.2% were positive. 42.2% were idiopathic DVTs, most of them without personal or familiar history of DVT, and only 20% of positive thrombophilia test. All patients started LMWH treatment for 7-10 days but 50.2% switched to VKA and 18.3% to DOACs, 0.7% changed to Fondaparinux. After 3-6 months of treatment, 33.22% resolved and 41.4% had residual/chronic DVT. After 2 years of follow up, we found 16.6% of post thrombotic syndrome (PS) and 1.35% of bleeding complications. We had 4 PEs, 1 splenic thrombosis, and 1 recurrent DVT after stopping treatment. One patient presented extension of DVT during LMWH treatment. We identified 9 patients with cancer, 6 of them diagnosed during the extended study of the idiopathic DVT.

Conclusions: We found an important percentage of idiopathic DVT with most negative thrombophilia tests, which suggests a lack of biological/genetic scores that reach a good predictive role in recurrent DVT. Hidden neoplasms should be investigated, as DVT can be the first sign. After 2 years of follow up, we found a low rate of PS (40% in the literature), 2% of recurrent thrombosis and only 1.35% of severe hemorrhage as complications.

PB2315 | A Retrospective Study of Incidence of Venous Thromboembolism (VTE) and Prevalence of Risk Factors Associated with VTE in Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplantation: A Single-Center Experience

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Background: Allogeneic hematopoietic stem cell transplantation (aHSCT) involves multiple complex risk factors that can place the patient under an increased risk of venous thromboembolism (VTE). Limited studies in aHSCT recipients have reported a wide range in the incidence of VTE, from 0.5% to 13%.

Aims: To study the incidence of VTE following aHSCT and to study the prevalence of risk factors commonly associated with VTE in this setting.

Methods: We studied a retrospective cohort of adult patients ≥18 years of age who underwent aHSCT between January 1, 2016, and December 31, 2019, at the Mazumdar Shaw Medical Center, Bangalore. Patient, transplant, and outcome-related factors were extracted from the transplantation database. A pharmacy and radiology record review was done to identify patients with VTE who received systemic anticoagulation.

TABLE 1 Baseline characteristics of patients

Baseline characterstics	N (Total =158)
Median age (range)	34.5 years (18.69)
Males: females	101: 57
Diagnosis:	
Acute leukemias, myelodysplastic syndromes & other malignancies	135
Aplastic anemia, hemoglobinopathies & others	23
Donor type:	
Matched sibling: matched unrelated: haplo-identical	112: 9: 37
Disease status:	
First remission: second remission	98: 37

TABLE 2 Prevalence of risk factors for VTE

Risk factors	N (Total=158)
Central venous catheter (tunelled or non-tunelled) & PICC	158
Family history of VTE	0
Previous history of VTE	4
Prolonged hospitalizations (>30 days)	9
Myeloablative conditioning	73
Acute GVHD: Chronic GVHD	49: 19

Results: A cohort of 158 patients who underwent aHSCT in the time period mentioned were studied. The Baseline characteristics of the cohort are as shown in *Table 1*. The incidence of VTE was 2.5% with a median follow-up period of 5 months (Range 1 - 42 months). Two patients had upper limb deep vein thrombosis (DVT), 1 patient had thrombosis of medullary veins of the brain and, 1 patient had splanchnic vein thrombosis with a median time to development of VTE of 36 days, 18 days, and, 20 days respectively. Prevalence of risk factors such as central line, prolonged hospitalizations, myeloablative conditioning, acute or chronic graft versus host disease (GVHD), prior VTE, and family history of VTE was studied (*Table 2.)*. The patients had a median of 3 risk factors in both the group of patients (VTE *versus* no VTE).

Conclusions: VTE is a rare complication following aHSCT (2.5% incidence in this study and the patients had a median of 3 risk factors) and further studies are warranted in this special population subset.

PB2316 | Risk Factors and Patterns of Lower Limb Deep Venous Thrombosis in Gombe, North-Eastern Nigeria

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Background: Deep venous thrombosis (DVT) is one of most common causes of morbidity and mortality among hospitalized patients. Many risk factors ranging hereditary to acquired are associated with increased risk of DVT. There is paucity of data on the pattern and risk factors of DVT in this part of the world.

Aims: The aim of the study was to review the pattern and risk factors of DVTs in Gombe.

Methods: We retrospectively reviewed the cases of Doppler ultrasound confirmed lower limb DVT that were managed in the Department of Haematology, Federal Teaching Hospital Gombe, North-eastern Nigeria over a 2 year period (January 2018 to December 2019). Data obtained were analyzed using SPSS v.20.

Results: Forty-two (42) patients were seen during the study period. Females were the majority, 64% (n=27). The age range was 18 to 92 years with a mean of 47.7 (±18.7) years. Young adults (18-45 years) were the majority (50%), followed by middle age (46-60 years) 29% and then elderly (>60 years) 21%. The DVT affects the left lower limb in 69% (n=29), right in (29%) (n=12) while only one (2.3%) patient had bilateral DVT. Eighteen (43%) had proximal DVT, 16 (38%) distal DVT while 8 (19%) had extensive DVT. Three patients (7%) had no identifiable risk factor, 20 (46.5%) had one while the remaining 20 (46.5%) had 2 or more risk factors. Majority of the patients had provoked DVT (67%/28). The leading provoking factors were immobilization 45%; recent surgery 21% and pregnancy/puerperium 10%. Among those with provoked DVT, young adults were the majority (66.7%), followed by middle aged group (28.6%) and then elderly (10.7) (p-value 0.04).

Conclusions: Our study showed the preponderance of left sided DVT and the majority of the cases were provoked affecting young adults.

PB2317 | Clinical Spectrum and Prothrombotic Risk Factors of Cerebral Venous Sinus Thrombosis in Children

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Background: Cerebral venous sinus thrombosis (CVST) is a rare and life-threatening cerebrovascular disease in children.

Aims: In this study, clinical presentations, prothrombotic risk factors, thrombosis localization, and anticoagulant treatment results of children with CVST were evaluated.

Methods: Between January 2011 and September 2018, 29 patients (0-18 years) diagnosed with CSVT in Eskişehir Osmangazi University Faculty of Medicine, Department of Child Health and Diseases were retrospectively examined.

Results: Twentty nine children were included in this study, 15 (51.72%) were male and 14 (48.28%) were female. Median age was 11 years (3 days-17 years). The most common neurological symptoms and signs were headache, vertigo, seizure, hemiparesis. At least one clinical and / or hereditary thrombophilic risk factor was detected in 89.66% of patients. The most common clinical risk factors were infections, venous catheter and dehydration. The most common inherited thrombophilic risk factors were Heterozygous Factor V Leiden Mutation and high lipoprotein (a). The most common thrombosis localization was the transverse sinus. All patients received anticoagulant therapy. No child with CVST died from his or her thrombotic episodes. Epilepsy developed in five patients, hydrocephalus in one patient, and intracranial hypertension in one patient.

Conclusions: Clinical and hereditary thrombophilic risk factors should be well known to clinicians in CVST. It should be remembered that the risk of acute complications and long-term sequela can be significantly reduced with the appropriate treatment approach initiated early in the early diagnosis.

PB2318 | Saddle Pulmonary Embolism: Demographics, Clinical Presentation, and Outcomes

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Background: Saddle pulmonary embolism (PE) is an uncommon type of venous thromboembolism (VTE) that can lead to sudden hemodynamic collapse and death. Saddle PE can be difficult to recognize, and data on its presentation, clinical features and associated complications are sparse.

TABLE 1 Presenting patient characteristics

Age, median (range)	61 (28-94)	Oxygen saturation, (range)	94.8 (78-100)	DVT Laterality	
Race		Systolic BP, median (range)	126 (78 - 188)	Left	29 (23.3)
White, n (%)	28 (23.3)	Diastolic BP, median (range)	77 (26 - 122)	Right	32 (27.5)
Black, n (%)	54 (45.0)	Heart rate, median (range)	104 (60-150)	Bilateral	17 (15.0)
Other, n (%)	38 (31.7)	Respiratory rate, median (range)	22 (14-50)	None/Not Performed	42 (34.2)
Hispanic, n (%)	28 (23.3)	Hemoglobin g/dl, median (range)	12.8 (5.1 - 16.7)	DVT Location	
Female, n (%)	69 (57.5)	Creatinine mg/dl, median (range)	1 (0.5 - 6.5)	Proximal	38 (32)
Prior history of VTE, n (%)	22 (18.3)	D-Dimer mcg/ml, mean, (range) n=27	14.8 (1.43-55)	Distal	4 (3)
Provoked Transient Risk, n (%)	53 (44.2)	BMI kg/m2, median (range) n=104	33 (18.6 - 64.6)	Both	36 (29)
Active Malignancy	23 (19.2)	Right heart strain (n=104), n (%)	70 (67.3)	N/A	42 (36)

TABLE 2 Patient outcomes

Cardiac Arrest, n (%)	13 (10.8)
In-hospital Mortality, n (%)	11 (9.2)
Alive at 6 months, n=105, n (%)	85 (81.0)
ICU Admission, n (%)	71 (59.2)
LOS days, median (range)	9 (0 - 106)
IVC Filter placement, n (%)	41 (34.2)
Systemic tPA, n (%)	23 (19.2)
Recurrent VTE n (%)	13 (11.9)
On Anticoagulation	9 (69.2)
Off Anticoagulation	4 (30.8)

Aims: To describe the demographics, presentation and outcomes of saddle PE.

Methods: Montage software (Nuance®, Burlington MA, US) was used to retrospectively identify patients ≥18 years of age that had been diagnosed with saddle PE from 1/1/2012 to 12/31/2018. Charts were reviewed for demographics, diagnostics, laboratory data, presenting vital signs, presence of DVT or malignancy, choice of anticoagulation and thrombolysis, in-hospital mortality, 6-month survival, and incidence of recurrent VTE.

Results: 120 patients with saddle PE were identified. Median age was 61 years and 57.5% were women. Events were provoked by a transient risk factor in 44.2%. We found a 9.2% in-hospital mortality and an 81% 6-month survival. In-hospital mortality was higher in women (11.6%), compared to men (3.9%) but this was not significant (p=0.28). Both Ventilation/Perfusion (VP) scans and CTPA were performed in 10 patients. None of the VP scans diagnosed the saddle PE; 6/10 diagnosed peripheral PEs and 4 were indeterminate. On presentation, mean systolic/diastolic blood pressures were 128.6/75.9 mmHg and mean heart rate was 103.8 bpm. Only 5% of patients presented with vitals concerning for massive PE. Right heart strain was present in 70/104 (67.3%). Thrombus was visualized in the right heart in 8/104 (7.7%) and this group had a 37.5% in-hospital mortality. Systemic tPA was administered in 19.2% of cases. Recurrent VTE occurred in 13/109 (11.9%) of survivors.

Conclusions: Despite presenting without accepted criteria for massive PE, saddle PE has a very high in-hospital mortality. VP scan is unable to diagnose saddle PE. Visualized right heart thrombi portend an even higher in-hospital mortality.

PB2320 | Risk of Receiving a Permanent Work-Related Disability Pension After Incident Venous Thromboembolism

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Background: Although long-term complications of venous thromboembolism (VTE) (e.g.post-thrombotic syndrome) are known to hamper functional activities and impair quality of life, it remains unclear whether VTE is associated with subsequent risk of permanent work-related disability.

Aims: To investigate whether incident VTE is associated with subsequent work-related disability in the general population.

Methods: Using medical and administrative databases, a Danish nationwide cohort of 41,928 patients < 65 years with incident VTE during 1995-2016 was established. An age,-sex,-and calendar-year-matched comparison cohort was identified from the general Danish population

< 65 years (n=209,640). Incident VTE was ascertained at study inclusion with permanent work-related disability as study outcome. Cox-regression models were used to compute hazard ratios (HRs) with 95% confidence intervals (CIs) for disability in analyses stratified by sex and age-group (25-39, 40-54, 55-64 years) and adjusted for comorbidities (history of cancer, CHD, diabetes, stroke, obesity and COPD).

Results: 41,928 subjects with incident were included. Of these 3,893 were granted a permanent work-related disability pension (incidence rate=13.0 per 1,000 person-years). Subjects with VTE had a 3-fold (HR 2.90, 95%CI: 2.79-3.02) higher risk of subsequent disability pension than those without VTE. Adjustment for comorbidities had a modest impact on the risk estimate (HR 2.47, 95% CI: 2.37-2.58). The risk of disability pension decreased slightly by age groups (HR 2.96, 95% CI: 2.68-3.26 in the youngest to HR 2.19, 95% CI: 2.02-2.38 in the oldest). The risk of disability was somewhat higher in men versus women (HR 2.67, 95% CI: 2.50-2.84, vs. HR 2.32, 95% CI: 2.18-2.46), especially in younger men (HR 3.66, 95% CI: 3.12-4.30).

Conclusions: Our findings clearly showed that incident VTE is associated with subsequent permanent work-related disability pension, independent of comorbidities such as cancer and other cardiovascular diseases. The indirect costs and social consequences associated with lost work-ability may add to the economic burden of VTE.

PB2321 | A Single-Centre Case Series of Venous Thromboembolic Disease

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Background: Venous thromboembolic disease (VTE) is a relatively common condition with significant morbidity. In its clinical spectrum includes both deep vein thrombosis (DVT) and pulmonary thromboembolism (PE). The treatment for this pathology is anticoagulation. They are numerous risk factors for venous thromboembolism, we should know them and suspect its presence when there is a compatible clinical picture. Clinical diagnosis of VTE can be difficult and unreliable as presenting symptoms can be caused by a multitude of other etiologies. We shouldn't forget the impact of thromboembolic disease on health-related quality of life.

Aims: To analyze the common characteristics of Venous thromboembolic disease in a specific medical consultation.

Methods: A descriptive study was performed. It included first VTE cases, admitted at specific internal medicine consultation. Seventeen patients met the inclusion criteria. Data and variables collected were: age, sex, diagnosis, treatment, comorbidity, toxic consumption and quality of life related to health. The health questionnaire SF-36V2 was used as a measuring instrument.

Results: About the 64.7% of the sample were males. Mean age was 58.6 ±11.2 years (range: 40-74 years). Ten (58.8%) patients had deep venous thrombosis (DVT), five (29.4%) had pulmonary embolism (PE). Two patients had a concomitant DVT and PE. All patients received low molecular weight heparin and 76.2%, four patients were with oral vitamin K antagonist while one was with direct oral anticoagulants. The patients presented as main comorbidities: arterial hypertension, diabetes, osteoarticular pathology, hematooncological pathology. Five (29.4%) were smokers. Only four patients valued their health as good, while thirteen (76.7%) valued their general health as bad.

Conclusions: n this registry the most frequent clinical picture presented is DVT and the main comorbidity referred to is osteoarticular pathology. In relation to treatment there is a predominance of the therapeutic use of vitamin K antagonists against new anticoagulants. There is a predominant perception in negative health terms.

PB2322 | Impact of Socioeconomic Status on the Risk of Receiving a Permanent Work-Related Disability Pension After Incident Venous Thromboembolism

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Background: Socioeconomic status (SES) affects the risk of incident venous thromboembolism (VTE), and VTE has long-term complications that influence functional activity and life quality. No previous study has investigated the effect of SES on the risk of work-related disability pension after VTE.

Aims: To investigate the combined effects of SES and incident VTE on work-related disability pension in the general population.

Methods: Using medical and administrative databases, a Danish nationwide cohort of 41,928 patients < 65 years with an incident VTE during 1995-2016 was established. An age,- sex,-and calendar-year-matched comparison cohort was identified from the general population < 65 years (n=209,640). Incident VTE and SES (categorized low, medium, high based on similar categories for education, employment, and income) were exposures and work-related disability pension outcome. Cox-regression models were used to compute hazard ratios (HRs) with 95% confidence intervals (CIs) in analyses stratified by sex, age-groups (25-39, 40-54, 55-64 years) and adjusted for comorbidities (cancer, CHD,diabetes, stroke, obesity and COPD). The reference group consisted of subjects with medium SES and no VTE.

Results: 41,928 subjects with incident were included whereof 3,893 were granted permanent work-related disability pension (incidence rate=13.0 per 1,000 person-years). Subjects 25-39 years with low SES without VTE had a 4.6-fold higher risk (HR 4.58, 95% CI: 4.08-5.14) of disability pension than the reference group. Low SES combined with incident VTE yielded a 13-fold increased risk of disability

pension (HR 13.16, 95% CI: 11.65-14.86). Adjustment for comorbidities had modest effect on the risk estimate (HR 12.50, 95% CI: 11.07-14.13). This risk estimate decreased gradually with increasing age and displayed similar trends for both sexes.

Conclusions: Our results showed that SES affected the risk of work-related disability in patients with VTE, independent of comorbidities. This implies that awareness of SES is important when designing programs to reduce the social consequences and economic burden of VTE.

PB2323 | High Levels of Uric Acid Are Associated with Increased Risk of Recurrent Venous Thromboembolism

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Background: The link between increased level of uric acid (UA) and the risk of cardiovascular disorders is well established. However, the impact of UA levels on the risk of VTE recurrence is currently unknown.

Aims: The aim of this study was to understand the role of uric acid levels on the risk of VTE recurrence.

Methods: We performed a prospective study on 280 patients with a previous episode of VTE, without history of recurrence and that completed the oral anticoagulant period. UA levels were measured at the enrollment and correlated with the risk of VTE recurrence.

Results: Fifty episodes of VTE recurrence occurred during a mean follow-up time of 71.1±29.2 months. Comparison of baseline characteristics showed that patients with future VTE recurrence had significantly higher levels serum UA (5.57 ±1.27 vs 4.95 ±1.32 mg/dl, p=0.003) and lower levels of eGFR (78.4 ±20.5 vs 88.8 ±17.3 ml/min/1.73m2, p=0.007) compared to patients without recurrent VTE. When patients were stratified according to UA terziles, Kaplan-Meyer survival analysis showed that subjects belonging to the lower terzile of UA (< 4.38 mg/dl) had significant lower risk of future VTE recurrence as compared to patients with higher UA levels (p=0.003). No difference were seen among patients belonging to the second and the third terzile of UA distribution. A multivariate Cox regression analysis also showed that level of UA > 4.38 mg/dl was associated with a significant increase in the risk of VTE recurrence independently from age, gender, BMI, eGFR, hypertensive status and the type of VTE (HR 3.07, IC95% 1.22 - 7.74, p=0.017).

Conclusions: Higher levels of UA are associated with increased risk of future VTE recurrence independently from traditional risk factors.

PB2324 | Impact of Clostridium Difficile Infections on In-Hospital Outcomes of Venous Thromboembolism (DVT or PE) Hospitalizations

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Background: Clostridium difficile infection (CDI) is one of the leading causes of hospital acquired infections. It causes a significant health burden and is associated with high morbidity and mortality. It creates a pro-inflammatory state and past studies have shown increased rate of VTE in patients with CDI. However, literature regarding impact of CDI on outcomes of hospitalized VTE patients is scarce.

Aims: Our study aimed to assess the impact of CDI on in-hospital outcomes amongst VTE hospitalizations.

Methods: The 2016 National Inpatient Sample (NIS) was used to identify all adult hospitalizations with a primary discharge diagnosis of acute VTE. Hospitalizations with Deep Venous Thrombosis (DVT) or Pulmonary Embolism (PE) were included under VTE. The sample was stratified based on the presence or absence of active CDI. Rao-Scott modified chi-square test and weighted Student's t-test were

TABLE 1 In-hospital outcomes of VTE hospitalizations with and without Clostridium Difficile Infection

Outcomes	Incidence in CDI vs no CDI	Adjusted Odd's Ratio with 95% CI	P-value
In-hospital mortality	6% vs. 3%	1.54 (1.09-2.17)	0.014
Acute Kidney Injury	24.2% vs 9.9%	2.32 (1.9-2.84)	<0.0001
Shock state	7.8% vs 1.9%	3.21 (2.35-4.39)	<0.0001
Bleeding	16.9% vs 6.4%	2.55 (2.05-3.17)	<0.0001
Acute respiratory failure	18% vs 7.4%	2.22 (1.8-2.76)	<0.0001
Mechanical ventilation > 96 hours	6% vs 1.2%	4.33 (3.05-6.16)	<0.0001
Length of stay	5.4±0.03 days vs 13.7±0.6 days	2.10 (1.98-2.24)	<0.0001
Average hospital costs	$55193±$764 vs $147356.5±$12734.1	2.01 (1.87-2.17)	<0.0001

used to analyze categorical and continuous variables, respectively. The adjusted odds ratio for various clinical outcomes was calculated using multivariate logistic regression analysis. Subgroup analysis comparing DVT and PE hospitalizations was also performed. The analysis was done using SAS. Statistical significance was defined by p-value < 0.05.

Results: We identified n=382,585 weighted hospitalizations for VTE. Of which, 0.8% (n=3080) had concomitant CDI. The presence of CDI was associated with increased in-hospital mortality. (6% vs 3%, AOR=1.54, 95% CI 1.09-2.17, p< 0.01). The odds of bleeding and acute respiratory failure requiring prolonged ventilation were also higher in patients with CDI. Overall hospitalization cost and length of stay increased with the presence of CDI(Refer to Table 1). On subgroup analysis, the odds of stroke were higher in patients with CDI and DVT (1.3% vs. 0.1%, AOR=6.21, 95% CI=1.5-25.71, p< 0.01).

Conclusions: In patients with VTE, CDI independently increases in-hospital mortality. Prevention of CDI in the VTE population may prevent unforeseen complications, improve in-hospital outcomes, and lower health care costs.

PB2325 | Plasma Levels of Complement Factor D and Future Risk of Incident Venous Thromboembolism

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Background: Complement factor D (CFD) is a serine protease mainly synthesized by adipose tissue, which has a key role in activation of the alternative pathway of the complement system. Recently, we have reported that complement activation was associated with future risk of venous thromboembolism (VTE). As obesity is a strong and consistent risk factor for VTE, we hypothesized that CFD could mediate the risk of VTE in obese subjects. However, whether CFD is associated with VTE is unknown.

Aims: To investigate the association between plasma CFD levels and future risk of incident VTE.

Methods: A nested case-control study consisting of 410 subjects with incident VTE and 838 age- and sex-matched controls was derived from a population-based cohort (the fourth survey of the Tromsø study). Baseline information was collected by physical examination and blood samples in 1994-95. Logistic regression was used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE across quartiles of CFD levels determined in the control group. Ethical approval and informed consent were obtained.

Results: Body mass index (BMI) showed a positive correlation with plasma CFD levels (Spearman's rho 0.29, *P*< 0.001). Plasma CFD levels were not associated with VTE risk across quartiles. In the age- and sex-adjusted model, participants with CFD values in the highest quartile (≥5.14 μg/mL) had an OR for VTE of 1.00 (95% CI 0.71-1.42) compared to those with CFD in the lowest quartile (< 3.23 μg/mL). Further adjustment for BMI and C-reactive protein did not substantially change risk estimates. Similar results were obtained in subgroup analyses of deep vein thrombosis, pulmonary embolism, and provoked and unprovoked events.

Conclusions: Our results indicate that plasma levels of CFD are not associated with future risk of VTE. Our findings suggest that CFD does not serve as a mediator for the VTE risk in obese subjects.

PB2326 | Correlation between Intrinsic D-Dimer Levels and D-Dimer Measured at First Venous Thromboembolism (VTE)

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Background: Plasma D-dimer levels in healthy subjects have been associated with increased risk of incident VTE and coronary heart disease. Intrinsic D-dimer has therefore been proposed as a potential marker of inherent hypercoagulability. However, whether high intrinsic D-dimer levels correlate with D-dimer levels in response to a venous thrombotic event has not been investigated.

Aims: To explore the correlation between intrinsic D-dimer and D-dimer at the time of VTE diagnosis.

Methods: Participants were recruited from the fourth survey of the Tromsø study, conducted in 1994-1995, and followed until 1 September 2007. During the follow-up, 198 cancer-free patients with a first life-time VTE were identified and included in the present study. Intrinsic D-dimer was analyzed from stored plasma samples collected at inclusion (1994/95), and D-dimer levels at the time of VTE diagnosis were assessed as a part of the diagnostic work-up of suspected VTE. The Pearson's correlation coefficient was used to test for correlation between intrinsic D-dimer and D-dimer at the time of VTE diagnosis.

Results: Intrinsic D-dimer and D-dimer measured at time of VTE diagnosis were positively correlated (r = 0.18, p = 0.001). Further stratification by sex revealed that the correlation was stronger in women (r = 0.28, p = 0.004) than in men (r = 0.09, p = 0.381). The mean time between the measurements of intrinsic and diagnostic D-dimer was 7.4 years in women and 7.8 years in men. The estimated correlation was strongest during the first year after the intrinsic measurement and decreased thereafter.

Conclusions: Our findings suggest a positive correlation between intrinsic D-dimer and D-dimer at the time of VTE diagnosis in women

but not in men, suggesting that inherent hypercoagulability partly explain plasma d-dimer levels at VTE diagnosis.

PB2327 | Impact of Competing Risk of Death on the Cumulative Incidence of Venous Thromboembolism in a Nationwide Danish Cohort of Men and Women

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Background: Studies investigating risk of venous thromboembolism (VTE) in men and women have had diverging results. In younger age groups, incidence is higher in women, while in older age groups, incidence is higher in men. Since men have higher mortality rates than women in all age groups, VTE incidence may be overestimated across all age groups of men. No study has investigated the gender-specific cumulative incidence of deep vein thrombosis (DVT) and pulmonary embolism (PE) in a nationwide cohort, while taking into account the competing risk of death.

Aims: To assess the impact of the competing risk of death on cumulative incidence of DVT and PE in men and women within different age groups in a nationwide cohort.

Methods: The population of Denmark served as the source cohort. All persons with a first-time VTE in the period 1995-2016 were identified from the Danish National Registry of Patients. Age was used as the time scale, and the cumulative incidence function of DVT and PE was estimated in men and women after taking into account the competing risk of death.

Results: During follow-up, 124,890 incident VTEs (74,389 DVTs and 50,501 PEs) occurred. The cumulative incidence of DVT was higher in women than in men among persons aged < 60 years, but higher in men than in women among those aged >60 years (Figure 1A). The cumulative incidence of PE was higher in women than in men among persons aged < 60 years, while no difference was observed among those aged >60 years (Figure 1B).

Conclusions: When the competing risk of death was taken into account, the cumulative incidence of both DVT and PE was higher in women than in men in the younger age groups. In the elderly, the cumulative incidence of DVT was higher in men than in women. No sex difference was observed for PE.

Figure 1. Cumulative incidence function of deep vein thrombosis (panel A) and pulmonary embolism (panel B) by increasing age after accounting for competing risk by death.

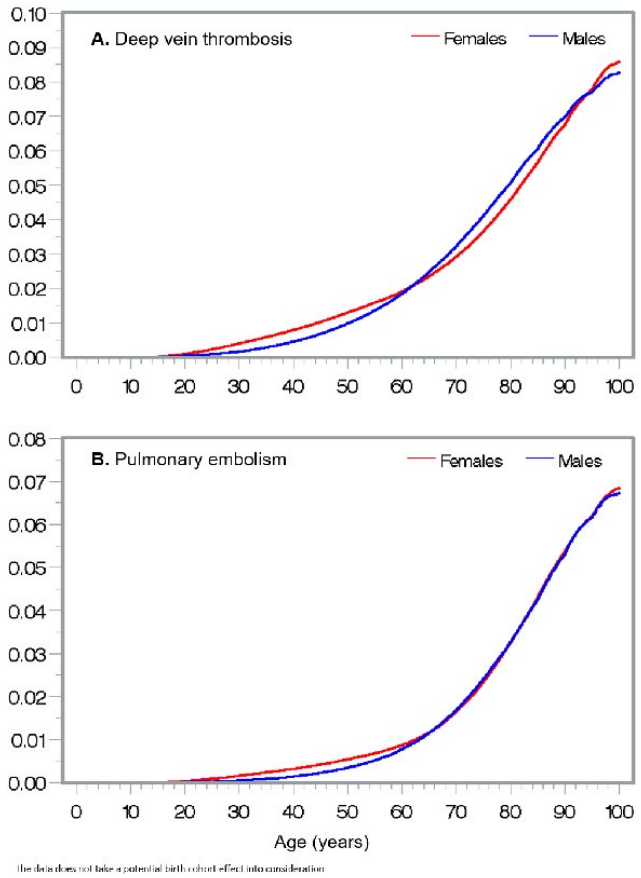

FIGURE 1 Cumulative incidence function of deep vein thrombosis (panel A) and pulmonary embolism (panel B) after accounting for competing risk by death

PB2328 | Recovery from Acute Kidney Injury in Patients with Pulmonary Embolism: A Monocenter Study

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Background: Acute kidney injury (AKI) is a complication of acute medico-surgical diseases, increasing the risk for chronic kidney insufficiency. We recently found that AKI was found in one in three patients with pulmonary embolism (PE), and associated with an increased risk of death and bleeding. Evolution of renal function in patients with AKI and PE is unknown.

Aims: To report the proportion of PE patients with AKI who recover a normal renal function, after PE.

Methods: We performed a post-hoc analysis of patients included in one prospective monocentric registry, from September 2013 to October 2018. Population study corresponded to all adult patients with symptomatic PE, in whom AKI (according to KDIGO guidelines) was present at the time of PE and at least one Serum Creatinine value assessment during the follow-up. The primary outcome was the proportion of patients who recover from AKI. We determined

TABLE 1 Evolution of renal dysfunction according to baseline characteristics

	AKI baseline (n=41)	Recovery (n=30)	Non Recovery (n=11)	p value
Female gender (%)	23 (56.1)	19 (63.3)	4 (36.36)	0.16
Age (mean ± Sd)	78.05 ± 8.38	77.7 ± 9.07	79 ± 6.41	>0.2
Stage of AKI at admission : 1/2/3	35/4/2	26/3/1	9/1/1	>0.2
Chronic heart failure	4	3	1	>0.2
Chronic lung disease	4	4	0	>0.2
sPESI : 0 / ≥ 1	8/33	5/25	3/8	>0.2
CT scan diagnosis	22	19	3	0.056
Cancer	9	4	5	0.04192
Time of recovery: < 2 days / [2 - 7[days / [7 - 90[days /> 90 days		12 / 12 / 5 / 1		

final recovery status as disappearance of AKI criteria during follow-up (any creatinine assessment during the follow-up).

Secondary outcomes were timing of recovery, and characteristics associated with AKI recovery during follow-up, in univariable and multivariable analysis.

Results: Among the 270 patients with acute pulmonary embolism, 41 (15.19%, 95%CI [11.12-20.03]) had AKI at baseline. Recovery occurred in 30 patients (73%, 95%CI [57-86]), during the first week in 24 patients. Baseline characteristics and AKI evolution are presented in table 1. In univariate analysis, presence of cancer was associated with absence of recovery. No item was found to be associated with recovery in multivariate analysis.

Conclusions: In PE patients with AKI, renal recovery is frequent (73% (95%CI [57-86]) and rapid (7 first days). However, about a quarter of patients did not fully recover, at least 90 days after PE. Further research are needed to develop prophylactic strategies improving the recovery rate.

PB2329 | Tissue Factor Pathway Inhibitor, Activated Protein C Resistance and Risk of Venous Thrombosis Associated with Menopausal Hormone Therapy

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Background: Menopausal hormone therapy (MHT) increases venous thrombosis (VT) risk.

Aims: We aimed to further unravel the mechanism by which MHT leads to an increased risk of VT.

Methods: A case-control study of VT risk was conducted nested in two Women's Health Initiative (WHI) trials where women were randomized to conjugated equine estrogen (CEE) alone and to CEE plus medroxyprogesterone (MPA) versus placebo. Activated Protein C resistance (APCsr, ratio) and tissue factor pathway inhibitor (TFPI, free:ng/ml, total:ng/ml, and activity:%) were measured at baseline and 1 year after follow-up.

We assessed whether MHT led to a more prothrombotic profile by measuring the change in biomarker levels from baseline to 1 year. We also assessed the VT risk by baseline biomarkers and MHT and

TABLE 1 Odds ratio (95% CI) of VT by baseline biomarkers and MHT

APCsr, ratio (>75th pctl)/ MHT Use	OR (95% CI)	Free TFPI, ng/ml (<5th pctl)/MHT use	OR (95% CI)	Total TFPI, ng/ml (<5th pctl)/ MHT use	OR (95% CI)	TFPI Activity, % (<5th pctl)/ MHT use	OR (95% CI)
No/No	Ref	No/No	Ref	No/No	Ref	No/No	Ref
Yes/No	1.05(0.53,2.06)	Yes/No	2.27(0.82,6.24)	Yes/No	1.58(0.54,4.64)	Yes/No	1.60(0.59,4.35)
No/Yes	2.13(1.40,3.25)	No/Yes	2.42(1.70,3.45)	No/Yes	2.43(1.71,3.46)	No/Yes	2.40(1.69,3.42)
Yes/Yes	2.89(1.69,4.95)	Yes/Yes	3.19(1.21,8.43)	Yes/Yes	2.22(0.85,5.77)	Yes/Yes	2.60(0.89,7.58)

Adjusted for Age at baseline, BMI at baseline, Race/Ethnicity, History of VTE, Trial Randomization date, Hysterectomy status.

TABLE 2 Odds ratio (95% CI) of VT after Year 1 by 1-year change in biomarkers

Biomarker change	OR (95% CI)	Biomarker change	OR (95% CI)	Biomarker change	OR (95% CI)	Biomarker change	OR (95% CI)
APC Sensitivity Ratio Change Tertiles		Free TFPI Change Tertiles		Total TFPI Change		TFPI Activity (%) Change Tertiles	
Low (-0.46)	1.36(0.72,2.58)	Low (-0.59)	1.06(0.61,1.85)	Increase (0.11)	Reference	Low (-0.38)	1.30(0.73,2.29)
Medium (0.15)	Reference	Medium (-0.11)	Reference	Decrease (-0.21)	0.69(0.41,1.13)	Medium (-0.11)	Reference
High (0.81)	1.01(0.54,1.89)	High (0.34)	1.46(0.86,2.49)			High (0.09)	1.67(0.94,2.98)

Adjusted for Hysterectomy Status, Age at baseline, BMI at baseline, Race/Ethnicity, Previous History of VTE, Trial Randomization date.

whether a prothrombotic change in biomarker values (increase in APC resistance or a decrease in TFPI) after 1 year due to MHT was associated with VT risk.

Results: There were 217 VT cases and 817 controls in both trials. Those assigned to MHT showed more prothrombotic changes in biomarkers over time compared with placebo; +0.39 (SD=0.54) for APC resistance, -0.24 (SD=0.22) for TFPI activity, -0.21(SD=0.50) for free TFPI and -0.22(SD=0.20) for total TFPI.

Table 1 shows the VT risk by baseline biomarkers and MHT. The risk of VT due to MHT was not further increased in women with a prothrombotic profile at baseline. Women with a prothrombotic response to MHT did not have higher VT risk than women who did not show a prothrombotic response (Table 2).

Conclusions: Though MHT leads to a more prothrombotic profile, i.e. an increase in APC resistance and a decrease in TFPI levels over one year, these prothrombotic changes were not associated with an increased risk of VT.

PB2330 | Incidence of Chronic Thromboembolic Pulmonary Hypertension: Results from a Longitudinal Prospective Cohort (Osiris Study)

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Background: Chronic thromboembolic pulmonary hypertension (CTEPH) is one of the leading causes of severe pulmonary hypertension (PH) and the only form of PH with a potentially curative treatment. The true incidence is unknown and varies from 0.4% to 6.2% depending on the nature of screened populations, and diagnosis criteria.

Aims: To determine the incidence for CTEPH at 2 years of an acute PE and to prospectively assess the barriers and limitations to obtain CTEPH incident-cases.

Methods: A prospective, long-term, follow-up study in consecutive patients diagnosed of pulmonary embolism (PE) in 18 Spanish hospitals was carried out. A questionnaire for CTEPH suspicion was the first step of the diagnosis algorithm and additionally, a transthoracic echocardiography (TTE) at follow-up 24months was performed.

Results: 1191 patients were recruited.The incidence of CTEPH, at 2 years after a PE, was of 2.27 [CI95%:1.56-3.28]. The Osiris questionnaire to assess the suspicion of CTEPH had a sensitivity of 85% [95%CI: 67.5-94], specificity of 91%[95%CI: 89-93] and negative predictive value of 99.4% [95%CI: 98.4-99.8].

Patients with PE but without symptoms of deep vein thrombosis, with varicose veins, in those surgery was absent as PE factor risk were significantly more frequent in CTEPH group. Moreover, CTEPH patients had lower PO2 level or SaO2< 90%, sPAP>50 mmHg on TTE and increased NT-proBNP at the time of PE diagnosis. In 354 patients the patient-centered algorithm for CTEPH detection was not fully completed. There were two kind of incompleted diagnostic algorithm causes, related to the doctors and patients decisions.

Conclusions: The incidence in our study was low but expected. Unknown risk factors have been identified. The Osiris questionnaire could be a useful clinical tool to implement in the PE follow-up.

Limitations to improve CTEPH diagnosis have been detected from doctors and patients.

PB2331 | The Characteristics of Patients with Isolated Superficial Vein Thrombosis of the Legs, their Follow-up and Outcomes

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Background: Superficial vein thrombosis (SVT) shares many common features with deep vein thrombosis (DVT) but epidemiological data about longer follow-up of SVT patients are scarce.
Aims: The aim of our study was to evaluate the characteristics of SVT patients, complications during follow-up and their potential predictors.
Methods: We performed a retrospective analysis of prospectively collected data from consecutive patients with isolated SVT of the legs who had been followed in thrombosis clinic. All provided an informed consent. We evaluated demographic, clinical and laboratory characteristics; thrombotic events or death during follow-up. For statistics we used T-test, Fisher's exact test, Wilcoxon test and a logistic regression.
Results: We included 127 patients (mean age 57.6±14.5 years; 65.4% females), most (89%) with varicose vein SVT. The personal/family history of SVT was positive in 44.9%/33.9%; that of DVT/pulmonary embolism (PE) in 35.4%/29.9%. Thrombophilia was present in 36.2%. All the index events had been treated with anticoagulation, in very variable schemes. Follow-up duration was in a wide range (3-174 months, median 39 months). SVT recurred in 49 patients, in 22 repeatedly (up to 6 times). The interval to the first recurrence was 1-110, median 16 months. Only the history of previous SVT was significantly associated with recurrence during follow-up in a multivariate analysis (hazard ratio 1.69; P=0.027). DVT/PE occurred in 21 patients (3-124 months after the index SVT, median 18 months), in two patients repeatedly, none was fatal. No significant predictor was found for DVT/PE during follow-up. Six patients died (6-105 months after SVT).
Conclusions: In 127 patients with an isolated SVT of the legs, there was a high rate of recurrent thrombotic events during follow-up - mainly SVTs and, to a lesser extent, also DVT/PE. The history of previous SVT was the only significant predictor of further SVT recurrence. Our study underlines the need for well-designed epidemiologic research of SVT.

PB2332 | Retinal Vein Thrombosis and the Association with Inherited Thrombophilia, Cardiovascular Risk Factors and the Risk of Occult Cancer. a Prospective Study

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Background: Retinal vein occlusion (RVO) is the second most common retinal vascular disease after diabetic retinopaty. For this type of thrombosis the correlation with thrombophilic conditions, cardiovascular risk factors and the risk of the future development of cancer is still debated.
Aims: To evaluate the association between RVO and inherited thrombophilia or the common cardiovascular risk factors. To investigate the relationship between RVO and the risk of future development of cancer.
Methods: In this prospective study we enrolled a total of 145 subjects at their first event of RVO. Sixty-six healthy subjects who did not present with a history of thromboembolic events acted as the controls. A total of 1376 and 384 patient observation-years were considered in cases and controls, respectively.
Results: Sixteen subjects in the group of cases and six in the controls were identified as carriers of thrombophilia with a prevalence of 12.4% (95% CI 7 to 18) and 10% (96% CI 3.7 to 20.5) respectively[RR 1.2 (95%CI 0.5 to 3)].
One hundred and seven subjects in the group of cases and 38 in the controls presented with cardiovascular risk factor with a prevalence of 73% (95% CI 66 to 81) and 58% (96% CI 44 to 70) respectively [RR 1.2 (95%CI 0.9 to 1.5)].
During the observation nineteen tumors developed in the group of cases (13%) as compared to four in the controls (6%). Overall, annual incidences of cancer were 1.4% (95% CI, 0.8 to 2.2) in cases and 1.05% (95% CI 0.3 to 2.7) in controls, respectively (RR, 1.3 [95% CI 0.5 to 4]).
Conclusions: Inherited thrombophilia is not associated with RVO while cardiovascular risk factor are weakly associated. RVO does not seem to be a marker for occult cancer.

PB2333 | Association of Surgical and Medical Hospitalizations with Venous Thromboembolism (VTE) in a Primary Care Population

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Background: Yearly one million U.S. individuals will be diagnosed with VTE, ~30% occurring during or within 3 months of hospitalization. The absolute and relative rates of VTE during and post hospitalization are not well studied.

Aims: Identify the relative and absolute risks of VTE for during and after medical and surgical hospitalizations.

Methods: Structured electronic health record data identified hospitalizations and VTE events for patients aged ≥18 years receiving their care within an academic health network between 2010 and 2016. We defined VTE using 1 inpatient or 2 outpatient VTE codes 7-180 days apart in addition to anticoagulant use within 30 days. We censored at the first VTE event. Cox models compared risk of a VTE during and following hospitalization relative to the rate among those without a current hospitalization or hospitalization in the prior 3 months, adjusted for age and sex and stratified by medical versus surgical hospitalization.

Results: From 2010-16, 87,821 patients contributed to 371,429 person-years of follow-up and 749 VTE. Of these, 21,963 patients were hospitalized 57,837 times (13,250 surgical admissions). Relative outpatients with no hospitalization in prior 3 months, the age and sex adjusted hazard ratio for VTE was 37.9 and 46.1 during medical and surgical hospitalizations respectively (Table). This comparison was 16.8 and 22.8 for the first month post discharge, 6.0 for both in the second month, and 5.0 and 4.9 in the third month post-discharge.

Conclusions: Medical and surgical hospitalizations were associated with a similarly increased risk of hospital acquired and post-discharge VTE. While we did not assess for procedures done during hospitalization, these data challenge the dogma that surgical hospitalizations are higher risk than medical hospitalizations for VTE and require further study as to who (if anyone) may benefit VTE prophylaxis inpatient or after discharge.

TABLE 1

Timeframe	Person-years (PY) follow-up	N of VTE	VTE events per 1000 PY	Age- and Sex-adjusted HR
No current or recent (<3 mo) hospitalizations	361,789	527	1.5	1(ref. Group)
Related to non-surgical hospitalization				
During hospitalization	482	37	76.8	37.9 (27.0, 53.2)
<1 month after discharge	2,746	97	35.3	16.8 (13.4, 21.2)
≥1 & <2 months after discharge	2,009	24	11.9	6.0 (3.9, 9.1)
≥2 & <3 months after discharge	1,586	8	5.0	2.6 (1.3, 5.3)
Related to surgical hospitalization				
During hospitalization	203	15	73.9	46.1 (27.5, 77.1)
<1 month after discharge	945	30	31.7	22.8 (15.8, 32.9)
≥1 & <2 months after discharge	858	7	8.2	6.0 (2.8, 12.6)
≥2 & <3 months after discharge	810	4	4.9	3.6 (1.4, 9.7)

PB2334 | Prevalence of Bilateral DVT in Ghanaian Patients

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Background: Every year, there are approximately 10 million cases of venous thromboembolism (VTE) worldwide.Deep vein thrombosis(DVT) prevalence rate in Africa varies between 2.4%-9.6% in patients after surgery and between 380 and 448 per 100,000births per year in pregnant and postpartum women. DVT is most commonly unilateral, but in few cases, it can be bilateral. A few retrospective studies have found an association between bilateral DVT and cancer, age >60 years, lower extremity trauma, and recent hospitalization

Aims: This study was done to determine the prevalence and risk factors associated with bilateral DVT in patients attending the Korle Bu Teaching Hospital Anticoagulation clinic.

Methods: Korle Bu Teaching Hospital established a dedicated Anticoagulation clinic in 2011 to manage patients on anticoagulation for various health conditions including VTE. A retrospective study (January 2017 to December 2019) was conducted. Data were analysed using Chi square test and Mann- Whitney U test, p< .05 was considered significant.

Results: A total of 104 patients who had their DVT confirmed by Doppler Ultrasonography with available clinical records were included in this study.

The prevalence for bilateral DVT was 12.5% (13/104). Median age of patients with bilateral DVT was 60y (13-100y) vs 55y (25-78y) for those with unilateral DVT (p=0.56) There was no significant difference in gender distribution between patients with unilateral and bilateral DVT (p=0.52). The most common risk factor for bilateral DVT was malignancy (Table1)

None of the patients with bilateral DVT had evidence of clot resolution after an average of 6 months on therapy.46.2% (6/13) were lost to follow up.

Conclusions: Prospective studies will be needed to better determine risk factors and outcome of bilateral DVT in Ghanaian patients.

TABLE 1 Risk factors for Bilateral DVT

Risk Factors	Male	Female	Percentage(%)
Malignancy	1	4	38.5
Post	3	0	23.1
Post partum	0	2	15.3
Unprovoked	2	1	23.1

PB2335 | A Decade of Experience in Peripherally Inserted Central Catheter (PICC) Related Thrombosis in Hematological Malignancies of a Single Spanish Center

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Background: Peripherally inserted catheters (PICCs) are becoming widely used in hematological patients. However, PICC-related thrombosis (PRT) is still one of the most important complications. We present our experience with PRT in the last decade.

Aims: To analyze the incidence and identify risk factors of symptomatic PRT in hematological patients.

Methods: Data of 317 adult patients in a single center with PICC insertion and diagnosed of hematological malignancies from the last decade (May 2010 to June 2019) were retrospectively collected. Experimented nurses' tunneled PICCs with the blinding Seldinger technique from 2010 to 2014 and guided by ultrasonography (US) technique from 2015 to 2019. Symptomatic PRT was confirmed by Doppler US.

Results: The overall incidence of PRT was 6.9% (n=22). The baseline characteristics are shown in (Table 1).

The main diagnoses related to PTR were acute lymphoblastic leukemia (ALL=9) and Non-Hodgkin Lymphoma (NHL=5). Seventeen patients had active disease when PICC was tunneled (17/22=77%). Nine patients were treated with L-asparaginase. The median onset of PRT was 26 days, (range: 0-230); and eleven of them (50%) in the first 30 days after insertion. Ten patients (45%) who developed PRT were being treated with granulocyte colony-stimulating factors (G-CSF). In fact, 8 out of 10 PRTs (80%) occurred during the first 30 days vs 20% (2/10) that were later (p< 0, 01). All PICCs were removed within 72 hours of PRT and treatedwith Low Molecular Weight Heparin (LMWH)to a median of 3 months (range: 1-10). Finally, for patients with PRT, L-ASA was the only risk factor of PRT (OR 7.25; CI 2.73-19.29;*p*< 0.001) in the multivariate analysis(Table 2).

Conclusions: Patients with L-ASA may be predisposed to developing PRT compared with those with other types of therapies.

TABLE 1 Baseline patients' characteristics

Median age (range)	57 years (14-86)
Sex: male / female	174 (55%) / 143 (45%)
Hematological malignancies: n (%)	- NHL: 130 (41.3%) -Acute myeloid leukemia and myelodysplastic syndrome: 89 (28.25%) - ALL: 37 (11.75%) -Multiple myeloma / Amyloidosis: 25 (7.9%) - HL: 23 (7.3%) - Others: 11 (3.4%)
Status disease when PICC was tunneled	Active: 245 (77.3%) / Remission: 72 (22.7%)
Drugs related thrombosis	Immunomodulatory drugs (IMIDS): 30 (9.5%) L-Asparaginase (L-Asa): 31 (9.8%) Erythropoietin: 24 (7.6%)
Thromboprophylaxis	LMWH: 29 (9.1%) ASA: 22 (6.9%) Oral anticoagulants: 3 (0.9%) Doubled antiagregated: 1 (0.3%)
PICCs tunneled technique	Guided by US: 213 (67.2%) / Blinding: 104 (32.8%)
Catheter tip location	Right atrium (RA): 112 (35.3%) / Cava-RA: 185 (54.8%)

TABLE 2 Univariate (U) and multivariate (M) analysis to identify risk factors of PRT

	U: OR [IC 95%]; p-value	M: OR [IC 95%] p-value
Male sex	0.81[0.34-1.93] 0.634	—
Age (>50 years)	0.4 [0.166-0.99] 0.042	p>0.05
-ALL -HL -NHL	5.896[2.331-14.91] <0.001 2.171[0.59-7.96] 0.233 0.417 [0.16-1.1.16] 0.085	p>0.05 — —
-IMIDs -L-Asparaginase -Stimulant growth factors	0.43 [0.06-3.37] 0.416 6.76 [2.57-17.8] <0.001 0.52 [0.22-1.24] 0.134	— 7.25 [2.73-19.29]; <0.001 —
Blinding Seldinger technique Right atrium location tip	0,58[0.2-1.63] 0.298 1.72 [0.62-4.79]. 0.295	— —

PB2337 | Seasonality in Hospitalizations for Pulmonary Embolism between 2006 - 2016

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Background: Seasonality in pulmonary embolism (PE) has been described and debated for a long time as we try to understand the risk factors leading towards its presentation.

Aims: We aim to replicate the findings of other works regarding PE seasonality in hospital admissions of the United States of America during the years of 2006 to 2014.

Methods: The NIS database was queried from 2006 to 2014 for adult patients with a discharge diagnosis of PE by ICD-9 CM codes. A time series analysis was performed with the monthly admission frequency for every year. All data was weighted and analyzed with a

Winter's Additive model as well as Auto-correlation Function (ACF) and Partial Auto-correlation Function (PACF) to identify a seasonality pattern. The population was then stratified per age group between patients ≤64 years of age and ≥ 65 years old. Finally a parallel analysis was performed in the described population involving a discharge diagnosis of both PE and deep vein thrombosis interpreted as a total of VTE patients.

Results: A total of 4'239.406 patients were analyzed for VTE and 2'545.320 (60% of total VTE) for PE. Of them 52.4% were females and 51.3% were ≥65 years old. The analysis demonstrated a seasonal variability with a statistically significant Ljung-Box Q test of < 0.001. 7% more admissions were observed during the month of January and 5% less admissions in June compared to the central tendency. Results obtained from the VTE analysis showed a similar trend. During the subgroup analysis a seasonality could not be demonstrated on the ≥ 65 years old population. There was an exponentially rising trend from 2006 to 20014 demonstrating a positive correlation (R^2=0.974) over the years.

Conclusions: Our data contributes to the building literature in favor of seasonality as an underlying factor driving PE and VTE incidence over the years.

PB2338 | Relevance of Immobility as Deep Vein Thrombosis Risk Factor for Medically Ill Patients

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Background: In surgical patients, the risk of deep vein thrombosis (DVT) associated with immobilization is well recognized, whereas the level of risk conferred by immobility in patients hospitalized in medical wards is less clear.

Aims: The aim of this study was to establish the relevance and how the length of immobilization relates to the risk of developing DVT in acutely ill medical patients.

Methods: A single centre longitudinal study was performed. Patients from medical wards of our academic hospital with suspected lower-extremity DVT were evaluated with whole leg ultrasound to confirm the presence of DVT. Immobility was defined as total bed rest or sedentary without bathroom privileges as defined in the EXCLAIM study.

Results: Among 500 inpatients (age 78.1±13.2 y, males 40.6%), frequency of immobilization was 50.4%. Thromboprophylaxis was used in 63.9% of patients with immobilization and in 47.6% of patients without immobilization (p< 0.001). Whole leg ultrasound showed 112 (22.4%) DVTs, of which 47 (42.0%) were proximal and 65 (58.0%) were isolated distal DVT. Patients with immobilization had a two-fold risk for DVT (2.4 CI 95% 1.6-3.8, p< 0.001) vs. patients without immobilization. After three day of immobilization, the frequency of DVT was similar in patients with and without immobilization, whereas after 7 and 14 days the frequency was 0.29±0.04 vs. 0.20±0.03 and 0.57±0.06 vs. 0.31±0.07, respectively. At multivariable analysis, predictors of DVT were immobilization (HR 1.6, 95%CI: 1.1-2.2, p=0.03) and thromboprophylaxis (HR 0.57, 95%CI: 0.38-0.85, p=0.005), whereas age, sex, cancer, heart failure, recent stroke were not.

Conclusions: In medical hospitalized patients, immobility is an important DVT risk factor and DVT risk is correlated with the duration of immobilization.

PB2339 | Risk of Venous Thrombosis in the Elderly: Lifestyle and its Joint Effect with Factor V Leiden and Prothrombin 20210A

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Background: Several lifestyle risk factors have been associated with the risk of VT in young and middle-aged population.

Aims: To investigate whether lifestyle factors (BMI, body height, body weight, smoking, and alcohol use) are associated with the risk of VT in elderly and to assess the joint effect with genetic risk factors for VT (i.e, the factor V Leiden (FVL) or prothrombin 20210A (PT20210) mutation).

TABLE 1 The risk of venous thrombosis(all VT, DVT only and PE±DVT) associated with lifestyle factors

Risk factor	All VT OR*(95%CI)	DVT only OR*(95%CI)	PE±DVT OR*(95%CI)
BMI(kg/m2)			
normal	1(ref)	1(ref)	1(ref)
obese	1.3(0.9-1.9)	1.5(0.9-2.4)	1.2(0.7-1.9)
Smoking status			
former+never	1(ref)	1(ref)	1(ref)
current	0.8(0.5-1.2)	1.0(0.5-1.7)	0.7(0.4-1.1)
Alcohol intake status			
not current	1(ref)	1(ref)	1(ref)
current	1.0(0.7-1.4)	1.1(0.8-1.7)	1.0(0.7-1.4)

TABLE 2 Combined effect of some exposures with the factor V Leiden(FVL) or prothrombin(FII)20210A mutations the risk of venous thrombosis

Risk factor	FVL/FII20210A	Patients	Controls	All VT OR*
BMI(kg/m2)				
normal	no	114	145	1(ref)
overweight	yes	24	12	2.9(1.3-6.3)
Height				
<P50	no	150	189	1(ref)
≥P50	yes	23	13	3.1(1.4-6.7)
Weight				
<P50	no	150	196	1(ref)
≥P50	yes	23	11	3.1(1.4-6.8)

Methods: Analyses were performed in the AT-AGE study, a multicenter case-control study performed in Vermont, USA and Leiden, NL, comprising of 401 cases with a first VT and 431 control subjects, all aged >70 years. To assess the risk of VT, odds ratios (OR) with 95% confidence intervals were calculated, after adjustment for potential confounders (age, sex, and study center). Weight and height were divided into quartiles based on the distribution in the controls.

Results: BMI as well as height and weight separately were positively associated with the risk of VT albeit risk estimated were modest. ORs were 1.3 (0.9-1.9) for BMI>30 compared with BMI< 25 kg/m^2 and 1.5 (1.0-2.4) and 2.2 (1.2-3.9) for weight and height in the highest compared with the lowest quartile, respectively. No association with VT was observed for smoking or alcohol use (table 1). Risks were similar for DVT and PE separately. The risk of VT was most pronounced in the presence of multiple risk factors, i.e, the OR was ~3 in individuals who were overweight or had a height or weight in the highest quartile in the presence of FVL or PT20210A (table 2).

Conclusions: In the elderly, BMI as well as body weight and height separately were associated with the risk of VT, while smoking and alcohol use were not. The risk of VT was highest in the presence of FVL or PT20210A.

PB2340 | Characterising the Burden of Hospital-acquired Venous Thromboembolism Arising during Admission and Following Discharge in an Irish Hospital Group Serving a Population of over One Million Individuals

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Background: Hospital-acquired venous thromboembolism (HA-VTE) is the leading cause of preventable hospital-associated mortality. Over 50% of HA-VTE occurs following discharge.

Aims: We aimed to characterise HA-VTE within the population served by the Ireland East hospital group (IEHG).

Methods: A 22-month (Jan 2018 to Oct 2019) retrospective study was conducted using validated hospital discharge datasets which record VTE diagnosed during admission as a 'secondary diagnosis'. In this study, 'secondary VTE' together with 'primary' VTE associated with a preceding hospital admission within 30 days were considered to represent a surrogate (but likely underestimation) of HA-VTE (not possible to capture VTE associated with a more remote preceding admission). Datasets were imported to SPSSv24 for analysis.

Results: 3055 VTE events occurred (annual incidence of 1.61 (95% CI 1.53-1.69) per 1000; IEHG total population, 1 036 279), 1657 (54.2%) of which were HA-VTE.

The annual incidence of HA-VTE was highest among individuals aged >85 years (11.5 per 1000) and the majority (83.6%) occurred during an emergency admission. Patients with HA-VTE were most commonly under the care of a medical specialty (76.2%). 69.2% (1147) of HA-VTE occurred in the two tertiary care hospitals within the group. All-cause mortality among HA-VTE patients was 9.1%.

The subgroup diagnosed with HA-VTE following discharge ('readmitted group') represented a distinct population, with a higher proportion aged ≤45 years in contrast to HA-VTE diagnosed during admission (14.7% vs 9.6%, p=0.008). Patients aged ≤45 had an odds ratio of 1.63 (95% CI 1.14-2.33) for being in the 'readmitted group' vs those >45 years. No deaths were recorded in this group.

Conclusions: HA-VTE accounted for at least 54.2% of VTE events. Quantifying HA-VTE events occurring post-discharge and determining the characteristics of this patient sub-group remains a challenge. Prioritising VTE prevention protocols and further research exploring optimum thromboprophylaxis strategies is vital in order to address this major patient safety concern.

PB2341 | The Season Role in the Occurrence of Thromboembolic Disease

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Background: There are numerous researches dealing with correlation between the seasons and climatic factors and pathogenesis of thromboembolic disease (VTE).
Aims: To investigate the influence of the season in the occurrence of VTE.
Methods: Retrospective, monocentric comparative study, having collected patients admitted for VTE between 2000 and 2018. We divided patients in 4 groups: group 1 included patients in whom VTE was diagnosed in autumn, group 2 in summer, group 3 in winter and group 4 in spring. We compared frequencies of VTE in four groups and their epidemiological, clinical, etiological and evolutive characteristics.
Results: The total population consisted of 1553 subjects (826 men, 727 women). The mean age of our population was 55.03 years old.
Overall, the number of VTE diagnoses was higher in winter compared to other seasons with the largest difference between winter and spring (17.1% higher).
There were no significant differences between 4 groups in gender ratio. No significant seasonal differences were observed in elderly patients (more than 65 years old). Risk factors are summarized in table 1. Non provoked VTE were significantly more frequent in spring and autumn (p=0.004). Frequencies of unsual location of VTE were significantly higher in group 4 than other groups (p=0.001).
Among patients with pulmonary embolism, no significant difference between groups. Thus, constitutional thrombophilia was the only etiology with a significant higher frequency in autumn (p=0.003). Recurrence were more frequent in group 1 and 4 (12.8% and 17%) versus 9.4 and 8.8% in groups 2 and 3 (p=0.019). Post phlebitis syndrome was significantly less frequent in spring (p=0.002). Seasonal variation was significantly contributive in VTE incidence.
Conclusions: In our series, VTE were more frequent in winter with significant differences between seasons.Further prospective researches could aim on testing the correlation between both climatic and thrombotics factors and the pathogenesis of VTE.

PB2342 | Catheter-associated Thrombosis at the Hematology Center: Analysis of 2212 Medical Records from a Developing Country

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Background: Catheter-associated thrombosis (CAT) is a serious complication observed both in children and adults, and can vary in different populations. The reported rate of CAT is between 1 to 5% in the published literature. The data on catheter-related thrombosis in Armenian population is unknown.
Aims: The aim of our study was to investigate CAT in Armenian patients admitted to the hospital with different hematological and oncological disorders.
Methods: A retrospective review of all medical records from 2019 admissions from both outpatient and inpatient clinics at the Hematology Center after Prof. R.H.Yeolyan, were collected and analyzed.
Results: In 2019 there were 2212 total admissions for different hematological and oncological disorders, who received treatment at our Center and all of them had catheter insertion. 1573 admissions were in the adult clinic (both hematologic malignant and benign

TABLE 1 Comparaison of frequencies of thrombotic factors between groups

Risk factors	Group 1	Group 2	Group 3	Group 4	p
Bed rest	15.7%	23.8%	21.5%	22.4%	NS
Varicose veins	15.7%	15.6%	14.7%	10.2%	NS
obesity	19.9%	24.7%	24.5%	18.1%	0.055
tobacco	36.2%	38.1%	29.5%	36.2%	0.001
post-partum	13.5%	14.7%	9.2%	3.8%	0.038
Surgery	9.6%	11.3%	12%	11.4%	NS
Neoplasia	13.8%	15.8%	13.3%	10.2%	NS
Constitutional thrombophilia	9.3%	6.3%	4%	5.3%	0.003
Antiphospholipid syndrome	2.6%	0.7%	1.6%	2.4%	NS

disorders, as well as solid tumors) and 639 at the pediatric clinic (all pediatric cancer and blood disorders in Armenia).

In the whole cohort of pediatric and adult admissions there we reported 19 (0.86%) cases of CAT. 9 patients were female. From these 19 patients 2 had multiple myeloma, 2 Hodgkin disease, 3 acute lymphoblastic leukemia, 1 aplastic anemia, 2 acute myeloid leukemia, and 9 Non-Hodgkin lymphomas. Out of 19 patients, only one was a child (below 18 years), a 7 years old female.

All the cases of CAT were confirmed by the duplex ultrasound. Interestingly, all recorded cases were among the inpatient admissions.

Conclusions: Our data shows that the incidence of CAT at our institution is much lower than in the published literature, which is most probably due to lower detectability and registration, especially for patients receiving outpatient therapy. Wider awareness campaigns and patient education programs may improve the situation.

PB2343 | Prevalence of Pulmonary Embolism in Patients Accessing the Emergency Department, Reported by Country of Study: A Systematic Review and Meta-analysis

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Background: There is an impression that North American ED patients tested for PE differ from those who are tested in Europe, based on the low prevalence of PE diagnosis.

Aims: Our aim was to compare the prevalence of PE, the rates of pulmonary imaging and the positive yield of imaging among ED patients tested for PE in Europe and North America.

Methods: We searched MEDLINE and EMBASE for studies reporting consecutive ED patients tested for PE. Two authors screened full texts, performed risk of bias assessment and data extraction. We conducted a meta-analysis of proportions specifying a random-effects model for each outcome. We used multiple meta-regression to evaluate whether the effect of the geographical region was independent of study year.

Results: From 3109 unique publications, 44 were included in the systematic review (23 with low risk of bias). The prevalence of PE in Europe was 23% (95% CI 21%-26%) and North America 8% (95% CI 6%-9%) (Figure1). The odds ratio (OR) for diagnosing PE in the study population in Europe as compared to North America, adjusted for year of study, was 1.16 (95% CI 1.11 - 1.22). Computed tomography pulmonary angiography (CTPA) was used in 59% (95% CI 51%-68%) of European and 38% (95% CI 24%-51%) of North American patients tested for PE (adjusted for year, OR 1.25 (95% CI 0.88 - 1.52)). The CTPA diagnostic yield was 29% (95% CI 26%, 32%) in Europe and 13% (95% CI 9%, 17%) in North America, [adjusted for year, OR 1.16 (95% CI 1.09 - 1.24)] (Figure2).

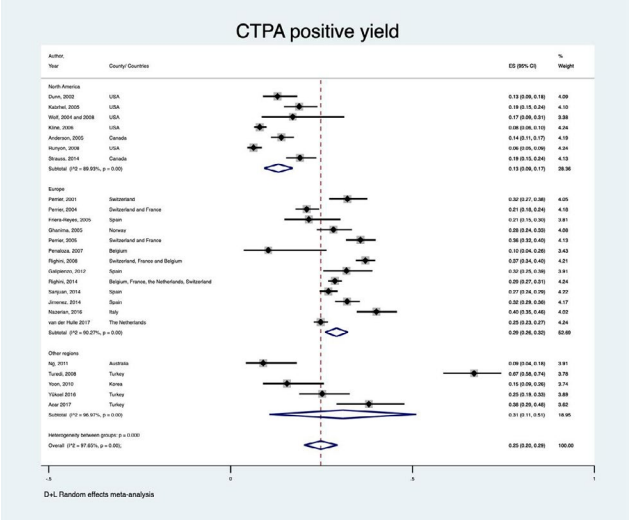

FIGURE 1 Forest plot for prevalence of pulmonary embolism

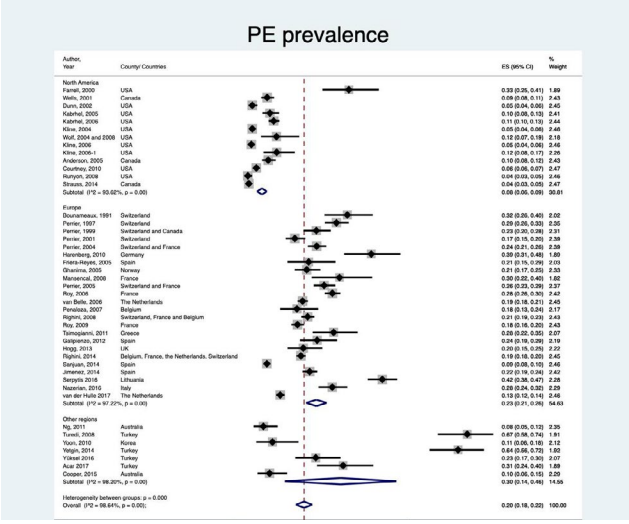

FIGURE 2 Forest plot for yield of computed tomography pulmonary angiography

Conclusions: Compared to North America, European ED studies have a higher prevalence of PE, higher rate of CTPA use and a higher diagnostic yield from CTPA compared to North America. This supports the hypothesis that those tested in North American EDs have a lower risk of PE compared to Europe.

PB2344 | The Impact of Body Mass Index (BMI) Dynamics on the Risk of Venous Thromboembolism Recurrence (VTE)

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Background: While elevated BMI is a risk factor for recurrent VTE, it is unknown whether a change in BMI influences this risk.

Aims: Evaluate the effect of baseline and changing BMI on risk of VTE recurrence.

Methods: Utilizing resources of the Rochester Epidemiology Project, all residents of Olmsted County, MN, diagnosed with incident VTE between 1996-2010 were identified. Baseline demographics, exposure to known VTE risk factors within 92 days of incident VTE, and VTE recurrence in these individuals were abstracted via medical record review. BMI at incident VTE diagnosis and change in BMI during follow-up were extracted. Cox proportional hazards model was utilized to evaluate the association between recurrent VTE and baseline BMI as well as change in BMI during follow-up, conditional on surviving free of recurrent VTE for 1 year after incident VTE.

Results: 1571 Olmsted County residents developed incident VTE between 1996-2010 and were free of VTE recurrence for 1 year following diagnosis (baseline demographics: Table 1). 325 of these individuals eventually experienced a recurrent VTE event. Individuals with VTE recurrence were slightly older and were more likely male (Table 1). A slightly larger percentage of patients with recurrence had a baseline BMI ≥ 30 (52% vs. 40%). Each increment in the baseline weight category (Table 1) was associated with increased hazard for VTE recurrence (hazard ratio: 1.14; p=0.02). Decreased BMI, conditional on surviving 1 year, was associated with increased risk of VTE recurrence (p< 0.01).

Conclusions: Our data suggest that baseline BMI and change in BMI following incident VTE impact risk of VTE recurrence. Further research is necessary to understand if and how, VTE risk and outcomes can be altered by lifestyle modification.

PB2345 | The Review of the Data from the National Registry of Thrombophilic States in the Slovak Republic

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Background: Thrombophilia is an inherited or acquired state predisposing to the formation of thrombosis in the blood flow. National Centre of Haemostasis and Thrombosis with the seat at the Department of Haematology and Transfusiology in Martin is in charge of the function of the National Registry of Thrombophilic States in the Slovak Republic. This database collects the data about the individuals with thrombophilia provided by the specialists from the regional haematological outpatient departments with the informed consent of the patient in the simple form.

Aims: The authors would like to point out to the significance of the diagnosis of thrombophilia and use of the data from this registry to increase the quality of life of the patients.

Methods: Data in the presented analysis were obtained during the yearly collection and processing the information about the patients with diagnosed thrombophilia.

Results: Up to date, the authors have registered 7025 patients with thrombophilia from more than 50 outpatient departments in the Slovak Republic. In the presented study, women were more prevalent than men (65.48% vs. 34.52%). In these patients, more than 30 prothrombotic states were detected.

Conclusions: National Registry of Thrombophilic States provides the collection of the data usable for the assessment of the prevalence, clinical manifestation, their frequency and possibilities of the diagnosis applicable in the clinical practice.

Keywords: Thrombophilia, risk factor, registry.

Acknowledgements: The authors would like to thank the support of projects of the Agency for the Support of Research and

TABLE 1 Baseline demographics of subjects with incident VTE based on VTE recurrence status

Baseline demographics by VTE recurrence status	No VTE recurrence N=1246	VTE recurrence N=325	Total N=1571
Median age in yrs. (IQR)	59 (44, 75)	61 (47, 74)	59 (45, 74)
Male sex (%)	534 (42.9%)	171 (52.6%)	705 (44.9%)
Baseline weight category			
Underweight (BMI<20; %)	90 (7.3%)	9 (2.9%)	99 (6.4%)
Normal weight (BMI 21-25; %)	296 (24.2%)	80 (25.4%)	376 (24.4%)
Overweight (BMI 26-29; %)	344 (28.1%)	63 (20.0%)	407 (26.4%)
Class 1 obesity (BMI 30-34; %)	243 (19.8%)	89 (28.3%)	332 (21.6%)
Class 2 and 3 obesity (BMI >35;%)	252 (20.6%)	74 (23.5%)	326 (21.2%)
Median BMI (IQR)	28.5 (24.9, 33.8)	30.2 (25.4, 34.8)	28.8 (25.0, 34.0)

Development (APVV) APVV-16-0020 and Scientific Grant Agency (Vega) 1/0549/19.

Informed consent of the patients was obtained and the study was approved by the Ethical Committee of the Jessenius Faculty of Medicine in Martin.

PB2346 | Apo(a) Polymorphisms rs3798220 and rs10455872 in a Brazilian Population of Deep Venous Thrombosis Pacients

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Background: Apo(a) is the main constituent of lipoprotein(a) (Lp(a)) and its homology with plasminogen Kringles IV and V is thought to underlie the artherogenic and thrombogenic characteristics of the protein. Polymorphisms in Kringle IV-2 repeat are highly heritable traits, directly associated with genetic variants, such rs3798220 and rs10455872, which influences the levels of the protein. Despite of substantial evidence of impact of Lp(a) in cardiovascular disease and its association with fibrinolysis, the role in deep venous thrombosis (DVT) is still inconsistent.

Aims: Assess the relationship between Apo(a) variants rs3798220 and rs10455872 and DVT.

Methods: We genotyped 329 DVT outpatients and 121 healthy subjects from Hematology and Hemotherapy Center of Campinas, Brazil, using TaqMan Allelic Discrimination Assay (Applied Biosystems). We also assessed plasma Lp(a) concentration of 154 of these subjects, using Tina-quant Lipoprotein(a) Gen.2 (Roche). Chi-square test was performed for comparative analysis of general characteristics.

Results: The total allelic frequencies (rs10455872: T=97% and C=3%; rs3798220: A=94% and G=6%) followed the observed in Global population studies (i.e.TopMed). Of DVT patients, 7.6% were caries of the rs10455872 variant and 7.7% of the rs10455872 variant (respectively, 25 and 33 subjects), while the corresponding frequencies were 2.5% and 3.4% in controls (respectively, 3 and 15 subjects). There were no significant differences of the genotypic proportions between groups. The profile of DVT patients and the correspondent genotypes are presented in Table 1. The rs10455872 variant was significant associated with the Lp(a) levels of the subjects and no association was observed with rs3798220 variation (Figure 1).

Conclusions: The present study showed no association of the variants to the occurrence of an DVT, but differences between subgroups might be concealed by the sample size. The fibrinolitic system will still be evaluated in samples of the 73 variant carriers, as well as non-carriers with high Lp(a) levels through Simultaneous Thrombin and Plasmin generation tests.

PB2347 | Epidemiology and Risk Factors for Thrombosis in Pregnancy and Puerperium. Experience in One Reference Center in Mexico

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Background: Embolism is the fifth leading causes of death in the world (3.2%, 78 000 cases annual cases). It is estimated that VTE complicates between 1 and 1.5 per 1,000 pregnancies. A pregnant woman is at a 5 times greater risk of developing DVT than non-pregnant woman. The major VTE risk is during puerperium. The Royal College of Obstetricians and Gynecologists (RCOG) has published a risk TVE assessment tool that is underutilized in our country.

Aims: Determinate the number of thromboembolic events in pregnant, describe risks factors for VTE and compare the score in pregnancy and puerperium.

Methods: Retrospective and longitudinal cohort. It was included patients in pregnancy and puerperium between April 2019 to January 2020. We describe by medians and frequencies baseline characteristics, risks factors and VTE risk score antenatal and postnatal.

Results: We analyzed 145 women. Their median age of pregnancy was 26 (12-40) and the parity 2 (1-16). Three patients (2.3%) had thrombosis during pregnancy. We found as pre-existing risk factors: BMI 30-40 in 28; 2 patients had previous personal history of thromboembolism; 3.4% had history of cancer, 1% heart failure, 1% active systemic lupus, 2% nephrotic syndrome and 1% type I diabetes mellitus with nephropathy; one patient had a known high-risk thrombophilia and two a family history of TVE; smoke in 10% and 2 patients had gross varicose veins. The obstetric and transient risk factors were described in table 1 and 2 respectively. The median of the VTE risk score antenatal was 1 (0-7) founding only 17% at high risk and at puerperium the median was 4 (0-14) founding more than a half (64%) at high risk.

TABLE 1 Obstetric risk factors

Pre-eclampsia in current pregnancy	21 (15%)
Assisted reproductive technology	1 (<1%)
Multiple pregnancy	8 (6%)
Cesarean section in labor	40 (30%)
Elective cesarean section	46 (34%)
Mid-cavity or rotational operative delivery	1 (<1%)
Prolonged labor (>24 hours)	13 (10%)
Postpartum haemorrhage	10 (8%)
Preterm birth <37 weeks	33 (25%)
Stillbirth in current pregnancy	11 (8%)

TABLE 2 Transient risk factors

Any surgical procedure in pregnancy or puerperium	54 (40%)
Hyperemesis	0 (0%)
Ovarian hyperstimulation syndrome	0 (0%)
Current systemic infection	9 (7%)
Immobility, dehydration	40 (28%)

Conclusions: Although the percentage of thrombosis during pregnancy in our patients does not seem to be high, if there is a marked increase in the percentage of risk during pregnancy and the puerperium.

VTE PROPHYLAXIS

PB2348 | Factors Associated with Major Bleeding Risk in Medical and Surgical Patients: Findings from a Multi-Center, Cross-Sectional Study (DissolVE-2)

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Background: Despite the practice guidelines, venous thromboprophylaxis remains to be improved in China where the utilization of anticoagulants is suboptimal, partially due to the perceived bleeding risks.

Aims: To assess the in-hospital major bleeding incidences and to identify the risk factors associated with major bleeding in medical and surgical inpatients.

Methods: DissolVE-2 was a multicentre, observational, cross-sectional study (ChiCTR-OOC-16010187) that screened patients for eligibility between March and September 2016 in 44 major cities at 60 teaching hospitals with >500 beds. We used data from DissolVE-2 study to assess in-hospital major bleeding incidence and to identify risk factors at admission associated with in-hospital major bleeding risk. Logistic regression analysis was used to calculate odds ratio to assess association between risk and covariates.

Results: Of the 13,609 patients (6,986 surgical and 6,623 medical) analysed, mean age was 63.2±15.65 years, and 57.5% were males. The cumulative incidence of in-hospital major bleeding in medical and surgical patients was 3.5% and 2.8%, respectively (Figure 1). The

TABLE 1 Significant risk factors associated with major bleeding based on multivariate logistic regression analysis in medical and surgical patients

Risk factors in medical patients	Odds Ratio (95% CI)	P-Value	Risk factors in surgical patients	Odds Ratio(95% CI)	P-Value
Active gastroduodenal ulcer	6.04 (1.68, 21.73)	0.006	Age≥85 y (vs<40 y)	5.86 (1.42, 24.27)	0.015
Bleeding in 3 months before admission	6.12 (2.61, 14.38)	<0.001	Coagulation dysfunction	7.22 (1.81, 28.79)	0.005
Platelet count <50×10 9/L	5.71 (2.77, 11.76)	<0.001	Concomitant use of antiplatelet therapy	4.11 (1.57, 10.75)	0.004
Hepatic failure (INR>1.5)	9.56 (2.33, 39.28)	<0.001	Severe renal or hepatic failure	2.68 (1.08, 6.68)	0.034
Severe renal failure(GFR<30 mL/min/m2)	4.51 (2.21, 9.18)	<0.001	History of diabetes	2.719 (1.2, 6.06)	0.014
ICU or CCU admission	2.54 (1.18, 5.48)	0.017	Abdominal surgery	6.62 (3.81, 11.52)	<0.001
Central venous catheter	2.96 (1.43. 6.13)	0.004	Cardiac surgery	10.24 (2.56, 40.96)	0.001
			Craniotomy	9.28 (4.79, 17.98)	<0.001
			Spinal trauma	8.37 (2.83, 24.72)	<0.001

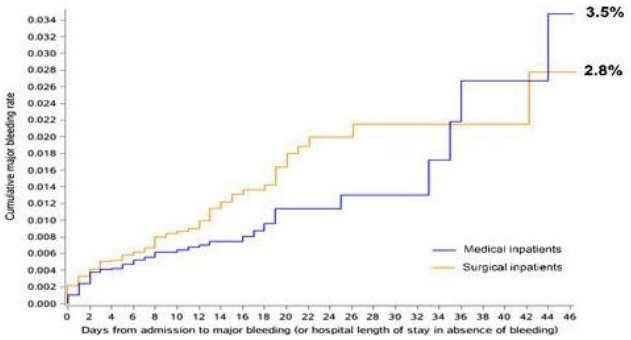

FIGURE 1 K-M curve on cumulative incidence of major bleeding

significant risk factors associated with major bleeding in medical patients were active gastroduodenal cancer, bleeding within 3-months before hospitalization, platelet count< $50X10^9$/L, hepatic failure, severe renal failure, ICU or CCU admission and central venous catheter. In surgical patients aged ≥ 85 years, coagulation dysfunction, antiplatelet therapy, diabetes, severe renal/ hepatic failure, abdominal surgery, cardiac surgery, craniotomy, spinal trauma were the major risk factors (Table 1). The bleeding risk scoring system developed showed higher incidence of major bleeding in medical patients with ≥3 than < 3 (11.3% vs. 0.5%) and surgical patients with score ≥ 4 than < 4 (12.4 vs. 0.9%).

Conclusions: We developed the major bleeding risk scoring system based on the independent risk factors associated with major bleeding. This information would assist physicians in prescribing an appropriate prophylaxis to reduce the risk of VTE in hospitalized patients at risk of major bleeding.

PB2349 | Post-Discharge Hospital-Associated Venous Thromboembolism

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Background: Hospital-associated venous thromboembolism (HA-VTE) morbidity and mortality has potentially been underestimated to date. Our centre is a tertiary, multi-campus health service which manages 265,000 admissions a year. Our VTE committee, comprising haematologists and other physicians, quality service representatives and pharmacists, routinely audits inpatient VTE (IP-VTE) for potential preventability against VTE prophylaxis guidelines. Over the study period, 81 IP-VTE were reviewed with 23% deemed potentially preventable however post-discharge VTE (PD-VTE) is not routinely captured. This is where VTE, presumably because of the index admission, is diagnosed after discharge, leading to re-admission.

Aims: To compare the incidence of PD-VTE against IP-VTE and review PD-VTE characteristics, adherence to VTE prophylaxis guidelines and potential preventability.

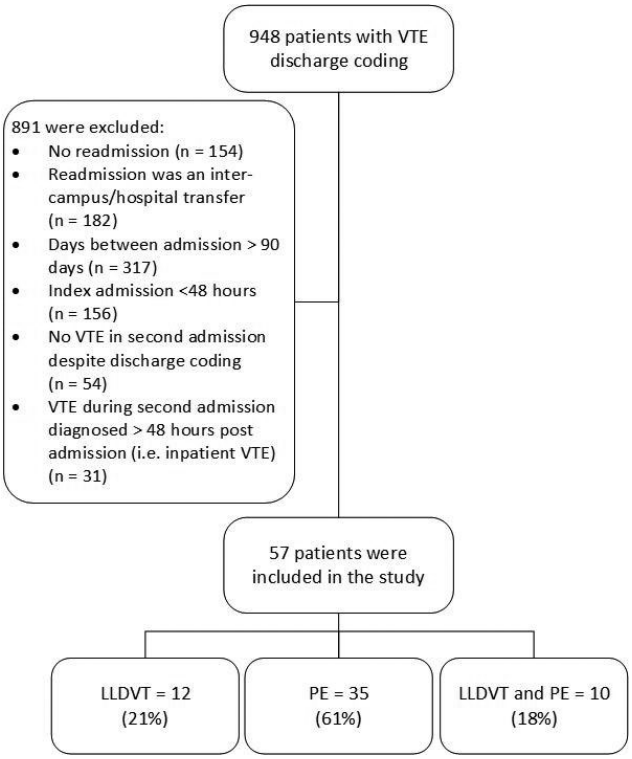

FIGURE 1 Flow chart on the identification of eligible cases

TABLE 1 Characteristics of the study population

Characteristics	Patients N = 57
Age (median, range, years)	71 (26 to 92)
Male (%)	54
Initial emergency admission (%)	26
Initial medical admission (%)	61
Initial admission duration (median, range, days)	7 (2-67)
Initial modified Padua score (median, range)	8 (0-12)
Days between initial admission and VTE admission (median, range, days)	19 (2-86)
History of VTE (%)	18
BMI (median, range, kg/m^2)	27.1 (18.1-48.9)

Methods: A retrospective audit of ICD-10 Australian Modification discharge coding for lower limb deep vein thrombosis (LLDVT) and pulmonary embolism (PE) in adults between January 2018 and May 2019 at Monash Health. Cases were filtered for criteria such as presence of new, objectively confirmed, symptomatic VTE within 90 days of an index admission greater than 48 hours (Figure 1). Our VTE committee reviewed all eligible cases.

Results: 948 encounters had VTE discharge coding. Of these, 891 were excluded with 57 eligible patients (Figure 1 and Table 1). Median age was 71 years (range, 26 to 92) and 32% with active cancer. Most index admissions were medical (61%) with a median of 19 days between admissions (range 2-86). VTE prophylaxis during

the index admission was concordant with guidelines in 51%. Non-adherence included delayed prescription (5%), interruptions (23%), none (14%) and inadequate prescriptions (e.g. inappropriately dose-reduced enoxaparin 7%). Overall, VTE prophylaxis adherence was 68% with 35% VTE events deemed potentially preventable. There were no deaths from PD-VTE.

Conclusions: Our data show that PD-VTE incidence is substantial. Adherence to VTE prophylaxis guidelines was poor resulting in potentially preventable VTE.

PB2350 | Risk Factors and Frequency of Recurrent Long-Term Venous Thromboembolism After Superficial Vein Thrombosis of The Lower Limbs

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Background: Superficial vein thrombosis (SVT) of the lower limbs is associated with an increased risk of short-term and also long-term risk of recurrent venous thromboembolism (VTE), but risk factors and frequency of late VTE up to five years are unknown.

Aims: To identify risk factors and frequency of recurrent VTE in patients with lower limb SVT beyond the initial three-month period of high risk.

Methods: Consecutive patients with SVT were treated with subcutaneous tinzaparin (Innohep™, LEOPharma, Denmark) for up to three months. Patients with thrombi measuring less than 5 cm on Duplex or reaching the saphenofemoral junction (last 3 cm of the great saphenous vein) were excluded. The composite primary endpoint of this prospective cohort study was recurrent VTE, defined as occurrence of clinically evident SVT recurrence, deep-vein thrombosis or pulmonary embolism, developing after three months. Demographics, clinical and ultrasonic variables were recorded.

Results: A total of 147 patients with a median age of 58.2 years were treated. Two patients died of VTE during the first three months. The remaining 145 patients were followed-up for a maximum of five years, with 19 episodes of recurrent VTE occurring in 13/145 patients (9%), including 15 events of recurrent SVT, three events of deep-vein thrombosis and one event of pulmonary embolism. On univariable Cox regression, several factors showed association with recurrent VTE during the five-year follow-up period (Table, p≤0.20, selected for multivariable Cox regression analysis). Only erythema over the thrombosis area at two weeks and development of VTE during the initial three-months (Figure) were independent predictors of recurrent VTE during long-term follow-up (hazard ratio 3.6 and 4.1, respectively, and p=0.030 and p=0.035, respectively).

Conclusions: The risk of recurrent VTE in patients with SVT continues for several years after the initial event. Erythema and early VTE seem to represent proinflammatory and prothrombotic conditions, respectively.

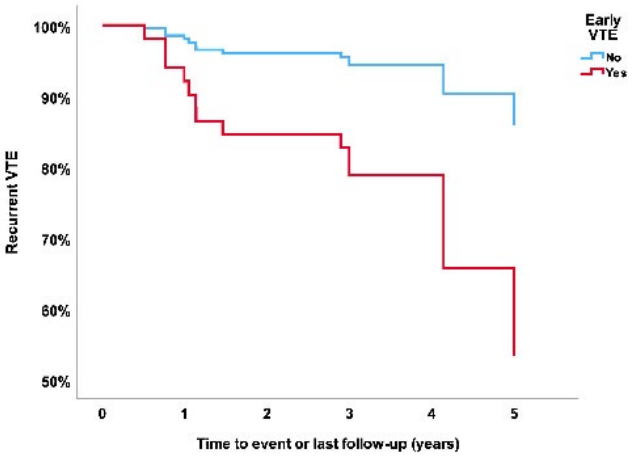

FIGURE 1 Erythema over the thrombosis area at two weeks and development of VTE during the initial three-months were independent predictors of reccurent VTE

TABLE 1 On univariable Cox regression, several factors showed association with recurrent VTE during the five-year follow-up period

Variable	Hazard ratio	p value
SVT of the left leg	1.7	0.077
Presence of contralateral varicose veins	3.8	0.200
Dyskinesia at baseline	1.8	0.130
Dyskinesia at two weeks	2.1	0.151
Erythema over the thrombosis area at two weeks	1.8	0.053
Swelling of the leg at three months	3.2	0.028
Development of VTE events, including symptomatic recurrent SVT, during the run-in phase of three months	3.3	0.068

PB2352 | Clinical Indications and Efficacy of Low Molecular Weight Heparin (LMWH) in Pregnancy

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Background: The use of LMWH in pregnancy not only includes management and prophylaxis of venous thromboembolism, but it is also frequently prescribed in unexplained recurrent miscarriages and the optimization of IVF pregnancies despite the lack of discrete scientific evidence.
Aims: The aim of this study was to evaluate the clinical indications and efficacy of Low Molecular Weight Heparin in pregnancy.
Methods: We analyzed data regarding 107 pregnant women receiving LMWH during 2018-2019.Our cohort had a median age of 27.4 years and a BMI of 31.2.They were divided into 4 groups: those with a history of VTE [Group-A: 03], those with unexplained recurrent miscarriages[Group-B: 94], those undergoing IVF [Group-C: 06] and those carrying prothrombotic tendency (thrombophilia, family history of VTE,) [Group-D: 02]. Mean duration of LMWH administration was 34 weeks. Out of 107 patients, 102 patients received prophylactic dose of LMWH while 05 patients received therapeutic dose of LMWH. Monitoring was generally not required, but whenever needed, it was done through activated partial thromboplastin time (APTT), due to non availability of Anti Xa assay at our hospital.
Results: Anticoagulation with LMWH was efficacious and well tolerated. No major bleeding events were observed while 04 clinically relevant non-major bleeding events were recorded. There were 03 cases of pre eclampsia/eclampsia along with 01 case of pre term labour. A total of 02 miscarriages and 04 cases of intrauterine growth retardation (IUGR) were noted, while there was 01 fetal death.
Conclusions: LMWHs are used extensively during pregnancy and postpartum in Pakistan for VTE treatment and prophylaxis and for a variety of other indications. Clear scientific data and effective guidelines are required on appropriate dosage, duration of treatment and indications for the use of LMWHs in pregnancy, beyond thromboprophylaxis.

PB2353 | VTE Prophylaxis Performance in a Tertiary and Secondary Level Hospital in Ireland

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Background: Venous thromboembolism (VTE) is a common complication of hospital admission. The VTE mortality rate in Ireland is approximately 4,000 per year, 48% of which are deemed preventable [1][2]. In 2008, University Hospital Limerick (UHL) conducted an audit showing inadequate thromboprophylaxis among medical patients. A medical proforma was subsequently introduced with a dedicated thromboprophylaxis prompt and follow up audits in 2012, 2015, 2018. This increased prophylaxis rates by 39% in high-risk patients by 2015.
Aims: This study aims to assess current practise of thromboprophylaxis among acutely ill patients to demonstrate the potential efficacy of implementing VTE prompts into standard admission policies.
Methods: A cross-sectional study was conducted in UHL and Nenagh hospital (NGH) from September to November 2019. Exclusion criteria included patients on therapeutic anticoagulation or those with anticoagulation contraindications. Risk was calculated using the Padua Score.[3] Anticoagulation prescriptions from medication lists were then recorded.
Results: Two-hundred and forty-three patients (193 UHL, 50 NGH) were identified. One-hundred and eight-two were included after exclusion criteria. In UHL, the medical proforma was used in all patients. Seventy-three (50.7%) thromboprophylaxis prompts were completed compared with 25% in 2018. Twenty-one patients were low risk, forty-seven moderate risk and seventy-six were high risk. Of low-moderate risk patients and high risk patients 60.3% and 76% received thromboprophylaxis respectively. This was a statistically significant increase in the high risk group from 63% in 2018 (p=0.071). Overall thromboprophylaxis rates were significantly higher than in 2008 (10% in low-moderate risk, 24% in high risk). Thromboprophylaxis rates in NGH were 76% in low-moderate risk and 80% in high risk patients.
Conclusions: VTE is a preventable life-threatening condition. Completion of the thromboprophylaxis proforma increased thromboprophylaxis rates in this group of high risk patients. Implementation of VTE prompts can therefore contribute to optimisation of VTE prevention strategies. This vital to reduce patient exposure to potentially devastating outcomes.

PB2354 | Prevention of Thrombogenesis in the Development of Metabolic Syndrome

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Background: Previously, it was found that the development of metabolic syndrome (MS) is accompanied by hypercoagulation with the transition to thrombogenesis. It is shown that regulatory proline-containing peptides prevent thrombosis during the development of atherosclerotic changes in blood vessels.
Aims: To show the preventive antithrombotic effect of arginine and lysine-containing peptides in the development of MS.
Methods: Male Wistar rats were used in the experiments. Experiments were conducted in accordance with the principles of the European Convention (Strasbourg). In a model of MS rats caused by keeping animals on a high-calorie diet (HCD) for 6 weeks, intranasal within 7 days administration of peptides RKKRPGP and

KKRRPGP was performed. After 20 h and 7 days samples of venous blood were analyzed for anticoagulant activity (APTT), enzymatic fibrinolytic activity (EFA), activity of tissue plasminogen activator (t-PA), euglobulin clot lysis time (ECLT), fibrindepolymerization activity (FDPA).

Results: In the blood of MS rats (control), a high degree of thrombogenesis was detected: APTT shortening by 50%, decrease in EFA, t-PA, ECLT, FDPA. 20 h after administration of peptides in the experimental rats an increase in fibrinolysis was found in comparison with the control: FDPA - by 70% (KKRRPGP), 206% (RKKRPGP), t-PA activity - by 24% ((RKKRPGP) or no change (KKRRPGP), APTT increased by 15% (KKRRPGP) and 25% (RKKRPGP). 7 days after the discontinuation of peptides at continuing of HCD, there was a preservation of increased anticoagulant and fibrinolytic activity of the blood and an even greater shortening of ECLT by 61-62% for both peptides.

Conclusions: Thus, when modeling MS in rats, arginine-and lysine-containing peptides had a complex effect on haemostatic system parameters, increasing the anticoagulant, fibrindepolymerization and fibrinolytic properties of blood plasma during 7 days of the experiment, which indicated their preventive positive antithrombotic effects in thrombogenesis.

Study was supported by RFBR 18-04-00260.

PB2355 | Is Low Molecular Weight Heparin-Calibrated Chromogenic Anti-Xa Assay Suitable for Assessing Anticoagulant Effect of Apixaban in Adolescents?

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Background: Although clinical application of apixaban does not require coagulation monitoring, there are clinical situations in which measurement of drug concentration should be performed. Anti-activated factor Xa (anti-FXa) inhibition methods for all anti-FXa drugs are based on the same principle, so there are attempts to evaluate potential clinical application of heparin-calibrated anti-FXa assay as an alternative method for direct FXa inhibitors.

Aims: To assess whether commercial low molecular weight heparin (LMWH)-calibrated anti-FXa assay can be used for assessing anticoagulant effect of apixaban in adolescents.

Methods: LMWH-calibrated anti-FXa method (Innovance heparin, Siemens Healthineers, Germany) was used for measurement of LMWH anti-FXa activity. Innovance heparin (Siemens Healthineers, Germany) calibrated with apixaban calibrators (Hyphen BioMed, France) was used for quantitative determination of apixaban concentration. Agreement between LMWH and apixaban calibrated anti-FXa assays was tested using kappa statistics whereas receiver operating characteristics (ROC) analysis was performed for LMWH therapeutic range. The study was funded by the Croatian Science Foundation as part of the research project IP-2016-06-8208.

Results: In 36 plasma samples of 11 adolescents (13-22 years), concentrations of apixaban ranged from 20 ng/mL to 267 ng/mL (median 94 ng/mL). LMWH anti-FXa activity ranged from 0.11 to 1.50 (upper LMWH measuring range) (median 0.68 IU/mL) (Table 1).

Inter-rater aggrement analysis showed poor agreement (κ=0.16; 95%CI - 0.0327 to 0.3386) between therapeutic range of LMWH-calibrated anti-FXa activity and clinically relevant apixaban concentrations (>30 ng/mL) (Table 2). ROC analysis showed that only apixaban concentrations between 57 and 121 ng/mL could be expected with certainty within therapeutic range for LMWH anti-FXa activity. Cut-off value for LMWH anti-FXa activity for apixaban concentrations < 30 ng/mL is 0.12 IU/mL.

Conclusions: LMWH anti-FXa activity assay is not appropriate method for quantitative estimation of apixaban concentration and should not be used as an interchangeable method. Quantitative measurement of apixaban concentration should be performed in all clinical situations.

TABLE 1 Results of apixaban concentration and low molecular weight heparin (LMWH) activity in paired samples in adolescents

	Median (95%CI) IQR
Apixaban concentration (ng/mL)	94 (69 - 107) 52 - 136
LMWH anti-FXa activity (IU/mL)	0.68 (0.47 - 0.85) 0.28 - 1.00

TABLE 2 Results of agreement between therapeutic range for LMWH anti-FXa activity and apixaban concentration at cut-off value of 30 ng/mL

LMWH therapeutic range 0.4 - 1.1 IU/mL	Apixaban <30 ng/mL	Apixaban >30 ng/mL	Number (ratio)
< 0.4	2	8	10 (0.28)
0.4 -1.1	0	19	19 (0.53)
>1.1	0	7	7 (0.19)
Kappa = 0.16 95%CI = -0.0327 to 0.3386	2	34	36

PB2356 | Thromboprophylaxis to International Conferences and Number of Thromboses (PINOT) study

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Background: Prevention of air travel-related venous thromboembolism (VTE) remains an area of clinical equipoise and current approaches require further research to ensure they are beneficial and safe for travellers. Previous data from *Kuipers et al.* suggest that 25% of long-haul air travellers use pharmacological thromboprophylaxis, however this research was performed prior to routine availability of direct oral anticoagulants (DOACs).

Aims:

- To evaluate the preventative strategies used by attendees to international conferences to reduce the risk of VTE.
- To determine if the use of thromboprophylaxis by attendees to international conferences affects the rate of VTE or increases bleeding events when compared to attendees who do not use prophylaxis.

Methods: Attendees of International Society of Thrombosis and Haemostasis (ISTH) Annual Congress 2019 were invited to participate in two online questionnaires. The first questionnaire evaluated risk factors for VTE and thromboprophylaxis used during air travel. Eight weeks after ISTH Congress, a second questionnaire was emailed and evaluated safety outcomes including VTE and bleeding as per ISTH definitions. Ethics approval was obtained from the local ethics review board.

TABLE 1 Baseline demographics

Age, median	**47**
Range	**21 – 82**
Female gender (%)	220 (54)
Weight in kilograms, median	72
Range	41 - 180
Height in centimetres, median	170
Range	130 - 201
Travel duration in hours, median	22
Class of travel (%)	
Economy	117 (29)
Business	279 (69)
First	7 (2)
Profession (%)	
Medical doctor	281 (69)
Scientist	74 (18)
Nurse	18 (5)
Student	12 (3)
Other	18 (5)
Number of risk factors for venous thromboembolism* (%)	
0	305 (76)
1	81 (20)
2	15 (4)
3	2 (<1)
Regular use of antiplatelet/anticoagulation prior to flight (%)	37 (9)

*Risk factors collected include: Recent surgery <8 weeks, lower limb injury/immobility, oestrogen use, pregnancy, active malignancy, personal history of VTE, first degree relative with VTE, presence of thrombophilia or none of the above.

TABLE 2 Results

Use of preventative measures (n=403) (%)	
Non-pharmacological prophylaxis only	184 (46)
Pharmacological prophylaxis only	22 (5)
Both pharmacological and non-pharmacological	73 (18)
No prophylaxis	123 (31)
Missing data	1 (<1)
Number of non-pharmacological measures* used (n=257) (%)	
1	149 (58)
2	90 (35)
3	18 (7)
Pharmacological measures (n=95) (%)	
Anti-platelet agent	39 (41)
Low molecular weight heparin	19 (20)
Rivaroxaban	21 (22)
Apixaban	11 (12)
Edoxaban	3 (3)
Dabigatran	1 (<1)
Other	1 (<1)
Venous thromboembolism	
Deep vein thrombosis	0
Pulmonary embolism	0
Other - central venous sinus thrombosis	1
Bleeding episodes	
Major bleeding	0
Clinically relevant non-major bleeding	0
Minor bleeding	1

*Non pharmacological measures evaluated include: Elastic compression stockings, recommended in-flight exercises and walking every 2 hours.

Results: Of 4399 attendees at ISTH 2019, the initial questionnaire was completed by 470 people (response rate 10.6%). After exclusions for unfinished questionnaires and flight time < 3 hours, 403 valid responses were analysed. The second questionnaire was completed by 260 people. Baseline demographics are listed in table 1. Overall, 69% of responders used some form of thromboprophylaxis and 23% used pharmacological prophylaxis table 2. One central venous sinus thrombosis was reported in an attendee who did not use preventative measures. One minor bleed (epistaxis) occurred in a person taking aspirin.

Conclusions: The majority of survey responders used thromboprophylaxis to prevent travel-related VTE. The effect of these measures on rates of thrombosis or bleeding remains unclear and consequently this research will continue at further international medical conferences, including ISTH 2020.

PB2357 | A Prospective Observational Multicentre Study to Assess the Incidence of and Risk Factors for (T) Thrombosis (I) in Patients with (L) Lower (L) Limb (I) Injuries (R) Requiring (I) Immobilisation in Ireland (TILLIRI)

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Background: Temporary lower limb immobilization (TLLI) after injury confers an increased venous thromboembolism (VTE) risk. The baseline risk is variable in published studies and remains unknown in Irish patients. Moreover, although promising VTE risk-prediction tools have been developed, no large study has prospectively characterized VTE risk factors in this cohort.

Aims: We have initiated a prospective National multicentre observational study in Ireland with the following aims:

1. To characterize VTE risk following TLLI in Ireland and
2. To prospectively gather data on VTE risk factors in this patient cohort.

Methods: This will be a multicentre study including 15 participating sites within the Irish Network for VTE Research (INViTE). Consecutive patients aged ≥18 years with a requirement for TLLI of any type/duration following lower limb trauma of any type will be eligible for inclusion. Clinical variables will be recorded. Patients will be followed up by telephone at 12 weeks. The primary outcome will be the cumulative incidence of symptomatic VTE at 12 weeks. We aim to recruit 4000 patients. We will conduct descriptive analyses to characterize the variables. All potential VTE predictors will be included in a multivariable mixed logistic regression model, with VTE occurrence as the outcome variable. Weak predictors will be excluded using the bootstrap backward elimination method. C-index and ROC will be reported. Model validation will be performed by a site-based internal-external validation framework.

TABLE 1 TILLIRI inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age ≥ 18 years	Unable to give consent
Lower limb trauma	
Requirement for lower limb immobilisation	

TABLE 2 Variables collected at initial visit and at 12 weeks

Initial VTE Risk Assessment Form:	12-week Phone Follow-up:
1. Height & Weight; 2. Gender;	1. Confirm Name & DOB; 2. Confirm relationship to subject
3. Performance status; 4. Pregnancy status;	3. Confirm body part injured; 4. Confirm injury type;
6. Family history - first degree relative with VTE;	5. Confirm immobilisation device type; 6. Confirm duration of immobilisation 7. Confirm if surgery was required;
7. Presence of co-morbidities; 8. Cancer in the past 5 years;	8. Confirm if chemical prophylaxis was used; 9. Confirm what chemical prophylaxis agent was chosen, if any;
9. Active cancer; 10. Treatment for cancer;	10. Confirm duration of chemical prophylaxis, if any; 11. Confirm if VTE investigations were required (DVT & PE);
11. Hormone replacement therapy (HRT)/Oral contraceptive pill (OCP); 12. Bed ridden (for 3 days or more) in the last 4 weeks;	12. Confirm if DVT was diagnosed; 13. Confirm if PE was diagnosed;
13. Surgery in last 3 months in the last 3 months; 14. Smoking status;	14. Confirm the VTE treatment used; 15. Confirm the name of the hospital where investigations and treatment took place.
15. Known thrombophilia; 16. Injury type;	
17. Method of immobilisation; 18. Weight-bearing status; 19. Medications - currently taking;	

Results: Since commencement in March 2019, 10 study sites (representing six Irish Hospital Groups) have consented and enrolled 512 patients.

Conclusions: Upon completion, this large prospective cohort study will estimate VTE risk following TLLI in Ireland and may permit characterization of VTE predictors in this patient group. These data will also support ongoing National efforts to raise VTE awareness in patients who have experienced TLLI.

PB2358 | The Application of the Lymphoma International Prognostic Index (IPI) to Predict Venous Thromboembolic Events (VTE) in Patients with Diffuse Large B-Cell Lymphoma (DLBL)

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Background: Several risk assessments models (RAM), including the Khorana and the ThroLy, attempted to identify lymphoma patients at higher risk for VTE with varying success. The International Prognostic Index (IPI) is a clinicopathological tool developed to help predict prognosis and response to treatment of patients with diffuse large B-Cell lymphoma (DLBL). Two modified versions of the IPI are also used in clinical practice: the age-adjusted IPI and the revised-IPI.

Aims: In this study, we utilize the IPI index to identify group(s) of patients with DLBL at higher risk of VTE.

Methods: Patients with pathologically-confirmed diagnosis of DLBL were included. Low IPI is considered for scores of 0-1, intermediate: 2-3 and high if > 3. Rates of VTE were examined among patients with low-IPI against those with intermediate and high-IPI, combined.

Results: Between 2006 and 2017, a total of 373 patients (52.5% males), median age: 49 (range: 18-90) years were included. At diagnosis, 157(42.1%) were stage IV and 189 (50.7%) had high LDH. All patients were treated on unified guidelines; 272 (72.9%) achieved CR while 66 (17.7%) had salvage chemotherapy.

Venous thromboembolic events were reported in 56 (15.0%) patients; 29 (51.8%) were ambulatory. Thirty-eight (18.4%) of 206 patients with intermediate-high IPI developed VTE, compared to 18 (10.8%) of 167 patients with low IPI, p=0.027. The application of the revised-IPI revealed similar results; VTE developed in 16.1% and 7.0% in the intermediate-high and low-IPI, respectively (p= 0.032). The age-adjusted IPI showed also similar trends with VTE rates of 16.7% among those the intermediate-high group compared to 6.8% in those with low-IPI, p=0.027.

Conclusions: The original IPI and its modified versions, routinely used at diagnosis as a prognostic and predictive tool, can also be utilized to define high risk patients for VTE. More work is needed to refine and improve currently available RAMs.

PB2359 | Can an Effective anticoagulation Clinic Reduce Bleeding Risks?

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Background: The risk of upper gastrointestinal (GI) bleeding in patients taking anticoagulation is well documented. Ray et al (2018) estimated that 1% to 1.5% of patients on oral anticoagulants will experience upper GI bleeds every year, and anticoagulant choice and PPI co-therapy could affect the risk of upper GI tract bleeding. For patients on Proton Pump Inhibitor (PPI) had a reduced overall bleeding across all anticoagulants, for an incidence rate ratio of 0.66 and a risk reduction of 34%.

Aims: To review risks of upper GI bleeding whilst on anticoagulation.

Methods: In Barnet District General Hospital there are 7700 patients on anticoagulation and are under the care of the anticoagulation clinic. We undertook a retrospective study from Sept 2018 to May 2019 to look at the number of patients presenting with an upper GI bleed.

Results: Between September 2018 and May 2019, 153 patients presented with an upper GI bleed of those only 17 patients were on anticoagulation (this includes 4 on warfarin and 11 on DOAC therapy). Of those patients who developed bleeds all were older than 80 apart from one who was 69 years old. This equates to 0.2% of patients on anticoagulation at Barnet Hospital presenting with upper GI bleeds during this period.

Conclusions: A factor that may explain the reduced incidence of bleeding whilst on anticoagulation is the involvement of the Barnet anticoagulation clinic. The clinic assesses and educates all patients on anticoagulation. This includes selecting out high risk patients and modifying risk factors such as ensuring PPI cover. Additionally, the anticoagulation clinic at Barnet, has the highest Time in Therapeutic Range (TTR) benchmarked nationally.

Though trial data shows an increased incidence of GI bleeds whilst on anticoagulation, this audit suggests that the role of an anticoagulation clinic could be significant in decreasing the incidence of bleeding events.

PB2360 | Weight-Adjusted versus Fixed Dose Heparin Thromboprophylaxis in Hospitalized Obese Patients: A Systematic Review and Meta-Analysis

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Background: Fixed-dose unfractionated heparin or low molecular weight heparin is the recommended treatment for

venous thromboembolism (VTE) prevention in hospitalized patients. However, its efficacy has been questioned in obese population (BMI>30 kg/m2). Results of previous studies on weight-adjusted doses of heparin for VTE prevention are contradictory. Different anticoagulant regimens are used in clinical practice but their role remains to be elucidated.

Aims: To clarify the efficacy and safety of weight-adjusted-dose heparin for VTE prevention in obese medical and surgical patients by means of a meta-analysis of relevant studies.

Methods: Pubmed, Scopus and Google scholar were searched up to December 31, 2019. Twelve studies reporting VTE occurrence, bleeding events and anti-Xa levels were included. A random-effect meta-analysis was conducted to derive odds ratios (OR) comparing fixed vs weight adjusted-doses heparins on VTE occurrence (6320/13317 patients), bleeding (5840/10906 patients), anti-Xa levels (284/294 patients). Age, BMI, female patients, medical-surgical patients, design and quality of studies were extracted for moderators and meta-regression analysis.

Results: Weight-adjusted-dose heparin administration was not associated with reduced VTE occurrence (OR 1.03, 95% C.I. 0.79 to 1.35, k=14, p=NS) or increased bleeding (OR 0.84, 95% C.I. 0.65 to 1.08, k=14, p=NS) (Figure 1) but it was associated with higher anti-Xa levels (ES 2.04, 95% C.I. 1.16 to 2.92, p< 0.0001) (Figure 2). A significant heterogeneity was present for comparison of anti-Xa levels (I2=94%, p< 0.0001) but not for VTE occurrence or bleeding (I2=7.6% and 12.8% respectively, both p=ns). None of the moderators explained the heterogeneity of results.

Conclusions: Although weight-adjusted doses of heparin for VTE prevention are associated with higher levels of anti-Xa levels, neither VTE occurence nor bleeding are modified in obese patients hospitalized for medical or surgical reasons. Large prospectic studies are needed to better understand the efficacy and safety of different heparin regimens on primary VTE prevention in obese patients.

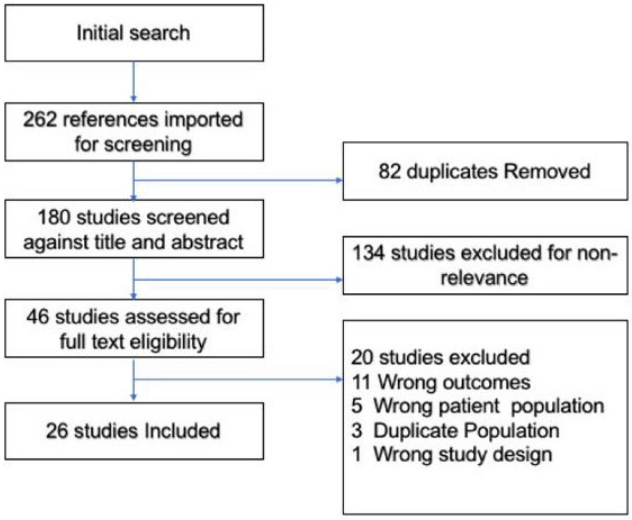

FIGURE 1 QUORUM Diagram of literature search

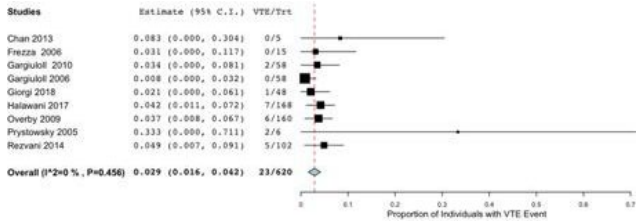

FIGURE 2 Meta-analysis of VTE Rate for High Quality Studies (IVC Group)

PB2361 | Efficacy and Safety of Pre-Operative Insertion of Inferior Vena Cava Filter in Patients Undergoing Bariatric Surgery: A Systematic Review

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Background: Prophylactic placement of inferior vena cava (IVC) filters has become a common intervention prior to performing bariatric surgery due to concerns about the high risk of venous thromboembolism (VTE) in this population and the unclear efficacy of standard dose pharmacologic prophylaxis in this high body weight population.

Aims: The objective of this systematic review and meta-analysis was to better characterize the risk and benefit of IVC filter insertion prior to bariatric surgery and to clarify the appropriate context for their use.

Methods: A systematic review of the literature of patients with prophylactic IVC filter insertion prior to bariatric surgery was performed using EMBASE, MEDLINE, CENTRAL (Cochrane) up to and including March 2018. The protocol was published in PROSPERO (CRD42018103411). Studies were independently assessed for inclusion and extracted for data by the authors. Quality was assessed using the Newcastle-Ottawa Scale with meta-analysis conducted on data from studies with Newcastle-Ottawa score >5.

Results: 26 studies were identified for inclusion into the review, of which none were randomized controlled trials. Meta-analysis was performed for the high-quality included studies. Nine high quality studies reported VTE outcomes and four reported major bleeding. The meta-analysis in those receiving IVC filters gave a pooled rate of VTE of 2.9% (95% C.I, 1.6to 4.2) and 1.1% (95%CI -0.9 to 3.1) for major bleeding compared to a rate of VTE of 1.6% (95% C.I, 0.7% to 2.5%) in those not receiving IVC filters. Six studies reported on IVC filter complications with a pooled rate of 0.6% (95% C.I, -0.1% to 1.1% I^2= 0%).

Conclusions: Overall the rate of venous thrombosis, major bleeding and device complication rate using IVC filters for prophylaxis in bariatric surgery patients is substantial. Use of IVC filters for prophylaxis remains a concern particularly given increasing evidence that

pharmacological prophylaxis is both safe and effective in these patients.

PB2362 | Incidence and Risk Factors of Venous Thromboembolism in Patients Undergoing Bariatric Surgery

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Background: Obesity is a major risk factor for venous thromboembolism (VTE). The incidence of VTE in bariatric surgery patients is low, but it contributes significantly to morbidity and mortality. Strategies for ideal prophylaxis are unclear due to the lack of evidence for risk stratification.
Aims: This study aims to determine the incidence of VTE in patients undergoing bariatric surgery in the DC metro area and to identify risk factors for VTE specific to this population.
Methods: We conducted a retrospective multicentric descriptive study, including all the patients who had bariatric surgery from Jan 1, 2017, to Dec 31, 2018. Statistical analysis was reported using means for descriptive data, t-test for continuous variables, and chi-square for categorical variables.
Results: A total of 1,175 patients underwent bariatric surgery, of which 3.91% (46) had VTE. The mean BMI was 44.69 (SD=9.10). The mean time to VTE was 34 days (SD=25). Older age was a risk factor (50 vs. 44 years, p=0.0002). Statistical differences were found in patients with the following comorbidities: diabetes (60% vs. 30%), history of VTE (32% vs. 4.34%), chronic kidney disease 21% vs. 5.49%), heart failure (15% vs. 5%) when comparing patients with VTE to those without VTE, respectively. BMI, race, length of stay, and duration of the operation were not significantly different. Caprini score was used to calculate perioperative VTE risk. Mean scores were significantly higher in patients with VTE (3.86; intermediate-risk) than those without a VTE event (2.75; low risk) (p=0.0021).
Conclusions: VTE incidence was higher in our bariatric patients (3.91%) compared to the literature (0.3-2.02%) and the general population (1-2 per thousand). VTE events were noted to occur at an average of 30 days following the procedure and in older patients with multiple comorbidities. Including the Caprini score to stratify the risk, we will design a prospective trial to achieve optimal thromboprophylaxis.

PB2363 | The Added Benefit of Mechanical Compression to Prevent VTE after Hip or Knee Arthroplasty: Post-Hoc Analysis of the EPCATII Study

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Background: Patients undergoing knee or hip arthroplasty are high risk for postoperative VTE. American Society of Hematology guidelines (2019) recommend combining mechanical compression with pharmacologic prophylaxis to prevent postoperative VTE. This recommendation however, is based on low-quality evidence, and there is a need for more data on whether mechanical compression reduces VTE in patients receiving pharmacologic prophylaxis.
Aims: To determine whether, following hip or knee arthroplasty, the use of mechanical compression reduces the rate of VTE in patients already receiving pharmacologic prophylaxis.
Methods: This was a post-hoc analysis of the EPCAT II study. EPCAT II was a multicentre, double-blind, randomized, controlled trial comparing rivaroxaban versus rivaroxaban with transition to ASA for the prevention of VTE following hip or knee arthroplasty. Among participating centres, there was heterogeneity in the use of postoperative mechanical compression, including a mix of graduated compression stockings, intermittent pneumatic compression devices, and no mechanical compression.
All patients in the original primary analysis of EPCATII were included. Patients in whom any post-operative mechanical compression was used were compared to patients that received no mechanical compression. The primary endpoint was symptomatic VTE (proximal DVT and/or PE). Significance was assessed using Fisher's exact test.
Results: A total of 3424 patients were included in the analysis. Of these, 550 patients received some form of mechanical compression postoperatively, while 2874 patients received no compression stockings. The rate of VTE was 0.36% (2/550) in patients that received mechanical compression, and 0.73% (21/2874) in patients that did not (RR 0.50, 95% CI 0.12-2.12 | p = 0.56). Among patients who received graduated compression stockings (n = 269), there were no VTE events.
Conclusions: Following hip or knee arthroplasty, in patients already receiving pharmacologic VTE prophylaxis, this analysis did not demonstrate a significant reduction in VTE with the addition of compression stockings.

PB2364 | Weight-Adjusted Tinzaparin for Venous Thromboembolism Prophylaxis in Bariatric Surgery Patients Weighing 160kg or More

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Background: Patients undergoing bariatric surgery are at an increased risk of venous thromboembolism (VTE), however, the optimal dose of low-molecular-weight heparin for prophylaxis remains uncertain.

Aims: St. Joseph's Health Care Hamilton utilizes a weight-adjusted tinzaparin dosage (75 units/kg rounded to nearest pre-filled syringe) for post-operative VTE prophylaxis. This study analyzed the safety of weight-adjusted tinzaparin for VTE prophylaxis in bariatric surgery patients at the highest weight class (> 160 kg).

Methods: This was a single center retrospective study involving patients weighing 160 kg or more who underwent bariatric surgery at St. Joseph's from January 2017 to September 2019. Patients received a single dose of weight-adjusted subcutaneous unfractionated heparin (UFH) [5000 or 7500 IU] immediately prior to surgery, either no prophylaxis or subcutaneous UFH [5000 or 7500 IU] post-operatively and either 10,000 or 14,000 IU Tinzaparin, beginning on the day after surgery, for 10 days. Additionally, intra-operative sequential compression devices could be used at the attending surgeon's discretion. Outcomes assessed were the occurrence of VTE or major bleeding over 30 days.

Results: Patient characteristics are summarized in Table 1. Of 287 eligible patients, 14 patients did not receive the standard dose of tinzaparin. Reasons for unexpected dosing were due to post-operative complication (n=1), had an indication for an alternate anticoagulant (n=5), and unspecified reasons (n=8). Pulmonary embolism occurred in one patient on post-operative day 1, prior to their first tinzaparin dose; major bleeding events occurred in 6 patients (2%). 24 patients received at least one dose of protocol appropriate tinzaparin but then received at least one dose of an alternative anticoagulant.

Conclusions: In this study of obese individuals undergoing bariatric surgery the use of weight-adjusted tinzaparin was associated with a lower risk of bleeding (2%) compared to general bariatric surgery (4.4%). These findings support the use of dose-adjusted tinzaparin for VTE prevention in such patients.

TABLE 1 Patient Characteristics

Variable	All patients (n=273)
Age (years), mean (SD)	44 (10)
Weight (kg), mean (SD)	182 (21)
Female (%)	129 (47)
Body Mass Index, mean (kg/m^2) (SD)	59 (8)
Received estrogen therapy (%)	27 (10)
Procedure (%)	
Sleeve Gastrectomy	171 (*63*)
Gastric Bypass	80 (*29*)
Duodenal Switch	21 (*8*)
Biliopancreatic Diversion	1 (*0.4*)
Pre-operative Unfractionated Heparin (%)	
7500 IU	242 (*89*)
5000 IU	30 (*11*)
Post-operative Unfractionated Heparin (day of surgery) (%)	
5000 IU	260 (*95*)
Unspecified	13 (*5*)
Tinzaparin Dosage (%)	
10,000 IU	54 (*20*)
14,000 IU	219 (*80*)

PB2366 | The Application of the Original and a Modified Version of the *ThroLy* Risk Assessment Model (RAM) to Predict Venous Thromboembolic Events (VTE) in Patients with Diffuse Large B-Cell Lymphoma (DLBL)

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Background: Patients with aggressive lymphomas are at higher risk for venous thromboembolic events (VTE) and mostly in ambulatory settings where thromboprophylaxis is not routinely offered. *ThroLy* is a risk assessment model (RAM) derived to predict the occurrence of VTE in a mixed basket of lymphomas.

Aims: In this study, we assess the clinical application of the original and a modified version of the model in a unified group of patients with diffuse large B-cell lymphoma (DLBL).

Methods: Hospital databases were searched for patients with DLBL and radiologically-confirmed VTE. Items in the *ThroLy* index were reviewed including previous VTE, reduced mobility, obesity, extranodal disease, mediastinal involvement, low neutrophil counts and Hemoglobin < 10.0 g/dL.

Results: A total of 378 patients, median age 48 (range: 18-90) years were recruited. Patients had high disease burden, including stage IV

(42.1%) and bulky disease (29.9%). All were treated on unified guidelines; 69 (18.3%) had primary refractory disease or early relapse for which they underwent salvage chemotherapy and autologous stem cell transplantation.

Venous thromboembolic events were reported in 57 (15.1%) patients. Among 58 patients with high (>3) *ThroLy* score, 16 (27.6%) developed VTE compared to 30 (16.7%) among 180 patients with intermediate (2-3) risk score and only 10 (7.5%) of 134 patients with low (0-1) score, p= 0.008. Modifying the *ThroLy* into two risk groups; high-risk (score ≥3) and low risk (score < 3) can still segregate patients; VTE developed in 30 (20.5%) high-risk patients (n=146) compared to 26 (11.5%) in the low-risk group (n=226), p=0.042. Neutropenia (ANC < 1000), a component of the *ThroLy*, was encountered in only 9 (2.4%) patients.

Conclusions: *ThroLy* RAM can identify patients with DLBL at high risk for VTE to justify thromboprophylaxis in ambulatory settings. Model can be modified by dividing patients into two, rather than three risk groups and further simplified by omitting ANC.

PB2367 | Antithrombotic Offices of the Arkhangelsk Region Russia

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Background: The main aims of arranging ACC in Arkhangelsk region for patients on AVK were the following: to provide the state of the art INR monit oring in the regional hospitals; Inter disciplinary approach to management of patients with VTE; registry manageme.

Aims: Creation of anti coagulant clinics.

Methods: The main AC was arranged in Arkhangelsk, it coordinates and controls the work of the remote clinics and also provides consulting work. The main antithrombotic clinic was arranged in Arkhangelsk, it coordinates and controls the work of the remote clinics and also provides consulting work. All parts of the project are fully compliant with Federal

Law - Personal Data Protection, data transfer protection, licens, medica l standards. All types of equipment are professional series that is: could be disinfected, have Regular QC and IT solution (IT1000) has been registered as Medical Device.

Results: The pharmacoeconomical analysis of the comparison of usual and new practice with ACC network showed double increase of the number of saved lives- from 25 to 50. At the same time the cost estimating relationship (CER) of saved lives with ACC network is reduced by twice. It is important to note that in the 5-year term, the economic effect of the implementation of ACC in Arkhangelsk region will reach 76 million rubles as a reduced costs for rebilitation, disability with prescription of AVK to all patients with indications. First experience of effect evedently demonstrates increase of TTR% (78-85%).

Conclusions: The main roles of central AC C are management, education, monitoring, reporting, TTR analysis, SE monitoring.

PB2368 | Lysophosphatidic Acid Promotes Thrombus Stability by Inducing Rapid Formation of Neutrophil Extracellular Traps via a Peptidylarginine Deiminase 4-Dependent Pathway: A New Mechanism of Thrombosis

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Background: Lysophosphatidic acid (LPA), a bioactive phospholipid, released by activated platelets, could further induce platelet shape change and aggregation, which plays an important role in thrombosis. However, the interaction of LPA with neutrophils in thrombosis has not been studied. Recently, neutrophil extracellular traps (NETs) have been demonstrated to bind plasma proteins and activate platelets, which promotes thrombosis.

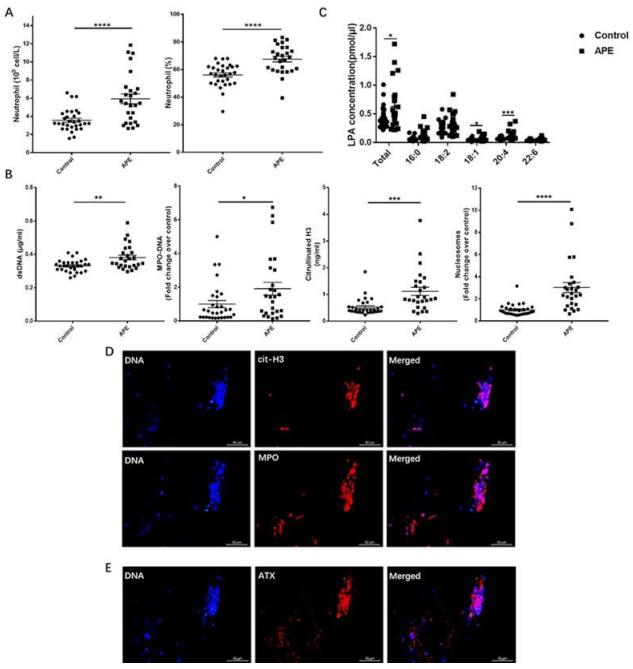

FIGURE 1 The increased levels of neutrophils, NETs and LPA in APE patients(A-C). ATX and NETs were present in intrapulmonary thrombus(D-E)

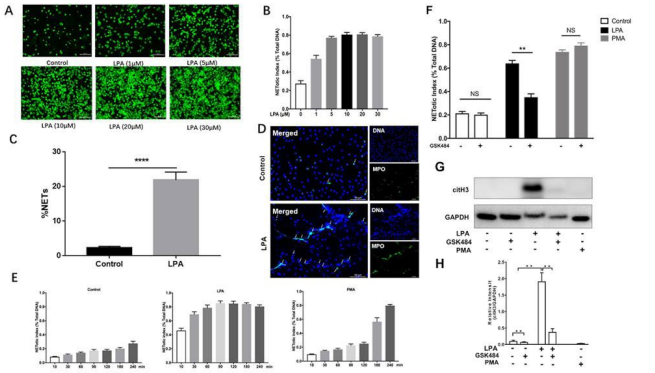

FIGURE 2 LPA induced rapid release of NETs(A-E).LPA induced release of NETs via a PAD4-dependent pathway(F-H)

Aims: To investigate whether LPA could activate neutrophils to release NETs, thereby predisposing to thrombosis and promoting thrombus stability.

Methods: Venous blood samples were obtained from 26 patients with acute pulmonary embolism (APE) and 30 control subjects. Immunofluorescence of NETs and autotaxin, the LPA-producing ectoenzyme, in human intrapulmonary thrombi were performed. The study was approved by medical ethics committee and informed consents were obtained. Induction of NETs and the signaling pathways were explored *in vitro*. Statistical significance was assessed by unpaired t test for normally distributed variables or Mann-Whitney test for skewed distributed variables.

Results: APE patients showed elevated levels of neutrophils, the markers of NETs (dsDNA, MPO-DNA, citrullinated histone H3, and nucleosomes), and LPA. NETs existed in human intrapulmonary thrombi and were surrounded by autotaxin(Figure 1). We showed that LPA induced rapid release of NETs from human neutrophils via a peptidylarginine deiminase 4-dependent pathway (Figure 2). LPA-induced NETs provided a scaffolding for plasma protein binding and generated a tPA-resistant blood clot. Addition of deoxyribonuclease I to tPA significantly accelerated the lysis of clots and human intrapulmonary thrombus. Furthermore, LPA-induced NETs could activate platelets to release LPA.

Conclusions: We are the first to implicate LPA in regulating the stability of thrombus by inducing rapid release of NETs via a PAD4-dependent pathway *in vitro* and *ex vivo* which could be a new mechanism of thrombosis. These findings provide a new insight into the prevention and therapy of venous thromboembolic disease by targeting the LPA-NETs signaling pathway.

PB2369 | Prevalence and Predictors of Thromboembolic Events in Patients with Gastric Cancer

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Background: Patients with gastric cancer are at higher risk for venous thromboembolic events (VTE). Majority of such patients are treated in ambulatory settings where thromboprophylaxis is not routinely offered.

Aims: In this study, we search for predictors that may help identify patients at higher risk to justify VTE-prophylaxis in ambulatory settings.

Methods: Patients with pathologically-confirmed gastric adenocarcinoma were retrospectively reviewed for radiologically-detected VTE. Clinical and pathological features known to increase the risk of VTE were studied.

Results: A total of 671 patients; median age (range) 55 (18-95) years, were recruited. During course of their disease, 150 (22.4%) patients had VTE, including 42 (28.0%) pulmonary embolism (PE) and 18 (12.0%) upper extremity DVT. Majority (n=112, 74.7%) had their clot in ambulatory settings and none had been on thromboprophylaxis. Venous thromboembolic rate was higher (n=99, 27.1%) in patients with metastatic disease (n=365), compared to 51 (16.7%) of those with nonmetastatic disease (n=306), p=0.010. Additionally, VTE rate was higher (n=93, 31.3%) among patients with high Khorana risk score (n=297) compared to 54 (17.3%) in those with intermediate score (n=312), p=0.001. However, rates of VTE were similar in active smokers (20.1%) and those who never smoked (21.6%), p=0.485 and in obese (BMI ≥30) patients (29.0%) compared to 21.9% among those with BMI < 30, p=0.282. Chemotherapy, including platinum, in patient with metastatic or nonmetastatic disease had no significant impact on VTE rates. In multivariate analysis, stage IV disease (Odd Ratio [OR], 2.084; 95% CI: 1.28-3.39, p=0.003) and high Khorana risk score (OR, 2.63; 95% CI: 1.64-4.23, p< 0.0001) were associated with significantly high VTE rates.

Conclusions: Patients with gastric cancer have notably high rate of VTE. Metastatic disease and high Khorana risk score are associated with even higher rates that may justify VTE prophylaxis, even in ambulatory settings, when the risk of bleeding is not high.

PB2371 | Real Life Experience of an Antithrombotic Stewardship Program for Thrombosis Prophylaxis during Hospitalisation in a Large Teaching Hospital

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Background: The risk factors associated with developing thrombosis, e.g. immobility or active malignancy are more prevalent in hospitalized patients. These patients need to be treated with adequate thrombosis prophylaxis (TP) during hospitalisation.
Aims: To determine, in a real life hospitalized population setting, the proportion of patients without TP and the proportion of these patients at high risk to develop thrombosis. Furthermore, the feasibility and effectiveness of manually reviewing and intervening in 'high-risk' patients by an antithrombotic stewardship program (ASP) was studied.
Methods: Prospectively, over a period of 3 months, all patients who were hospitalized for more than 72 hours and did not receive TP (heparins, DOAC nor VKA) enrolled in this observational study. The risk of thrombosis was assessed daily, using the PADUA scoring system. Patients with a PADUA score ≥4 were assigned as being at high risk of developing thrombosis. The characteristics of these patients were studied. Furthermore, these patients were manually reviewed by one nurse and discussed in the ASP to assess if interventions were needed.
Results: From February to May 2019, there were 586 consecutive patients enrolled. Of these, 389 (66%) patients were scored as low risk of thrombosis, while 197 (44%) patients were considered as at high risk. In this period, the ASP was able to review 60 (30%) of the high risk patients. Intervention by means of starting TP was carried out in 19 (32%) out of the 60 reviewed patients.
Conclusions: Our study shows that still a high number of patients at high risk of thrombosis do not receive appropriate TP during hospitalisation. However, manually reviewing these high risk patients is time consuming as only 30% of high risk patients could be reviewed by the ASP.

PB2372 | Venous Thromboembolism in Patients with Renal Transplantation

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TABLE 1 Site and type of VTE, treatment of VTE

Patients	112
DVT	90(85.7%)
EP	4 (3.8%)
DVT/EP	11 (10.4%)
Unprovoked	78 (73.5%)
Secondary to transient risk factors	28 (26.5%)
LMWH/warfarin	55 (52.4%)
Warfarin	41 (39.0%
DOACs	9 (8.6%)
Median (range) duration of treatment, months	6 (1-144)

Background: Scant data are available about the characteristics and optimal treatment of the episode of venous thromboembolism (VTE) occurring in renal transplant (RT) recipients.
Aims: Our study was undertaken to evaluate the characteristics of VTE in RT patients, the type of treatment used in clinical practice and the risk of recurrent VTE.
Methods: We performed a multicentric retrospective observational study among 7 Italian centers. Data were collected in a web based clinical report form with the use of REDCAP system.
Results: 112 patients who received renal transplantation (74 males 64%) and who developed a first episode of VTE after transplantation. Median age (range) at transplantation was 57 (22-76) years. Median time of VTE recurrence from RT was 6 (0.1-213) months; 783% of patients received RT from deceased donor; 10 patients (9.0%) ha kidney and pancreas transplantation. Characteristics of site and type of VTE and treatment of VTE are reported in table 1. During follow-up 84/112 patients stopped anticoagulant treatment. Among them 25 (30%) had recurrent VTE episodes.
Conclusions: We confirm the low rate of pulmonary embolism in RT patients with respect to general population. Instead, RT patients showed a high rate of recurrence accordingly with previous findings.

PB2373 | Is the Caprini Score a Reliable Tool to Predict the Risk of Venous Thrombosis in Orthopedic Patients?

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Background: Patients-related risk factors combined with the type of surgery contribute to provide the individual risk for venous thromboembolism (VTE) for each patient at each intervention. Different risk assessment tools (RAM) were developed to predict the risk of a patient to develop VTE.
Aims: The purpose of this study is to validate the Caprini score in population with different orthopedic interventions.

Methods: Data from a large population-based case-control study (the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis [MEGA] study) on the etiology of venous thrombosis were used. The Caprini variables were identified for each patient in order to calculate the score. Missing data was imputed by multiple imputation. The sensibility, specificity, positive predict values (PPV) and negative predict values (NPV) were calculate to each of the Caprini score stratum.

Results: Of 4,721 cases and 5,638 controls, 263 cases and 94 controls underwent an orthopedic intervention. A total of 20,9% of the cases and 41.5% of the controls were estimated to be on the lowest group (Caprini < 5 points). The relative risk of developing VTE, calculated as OR between the Caprini stratums increases with increasing Caprini scale. Patients assigned with 9-10 points and 11 points or more shown three-fold and six-fold increased risk compared to patients with Caprini score 0-2 (OR 3.5, CI 95% 1.2-10.3; OR 6.3, CI 95% 1.7-22.9), respectively. The discrimination of the test was moderate (c-statistic: 0.64). For the cutoff of 5-6 and 7-8 points, the sensitivity was 79% and 59% and the specificity was 41% and 60%, respectively.

Conclusions: The Caprini score is a useful tool to predict the risk of VTE in orthopedic patients, with a moderate ability to discriminate the patients at risk. The prevalence of VTE in each intervention should be include in the rational to decide which patient should receive the prophylaxis.

PB2374 | Visualization of Thrombin Formation on Activated Platelets and Fibrin Formation in Venous Flow Condition

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Background: Venous thromboembolism (VTE) is one of major causes of sudden death. Prevention of VTE is hard because of lacking understanding of mechanical and physical property of venous thrombus. Thrombin generation is most important phase for fibrin clot formation. Thrombin is explosively generated by prothrombinase complex on activated platelets.

Aims: Our aim of this study is visualization of time course of thrombin generation on activated platelets and fibrin formation in high magnification to elucidate extract physical property of venous thrombus generation at the point of view of fibrin formation in low shear stress.

Methods: Human venous blood was obtained from healthy volunteers. We separated platelets by centrifugation to stain with Alexa Fluor 488 and mixed them into the whole blood composition again. Alexa fluor 546-conjugated fibrinogen was added to visualize fibrin formation. BOC-Val-Pro-Arg-AMC (Boc-VPA), a sensitive fluorogenic substrate for alpha-thrombin, was added to visualize thrombin generation. To meet our aim, we made a microfluidic device system to observe thrombus structure formed under venous shear rate conditions. The blood sample was introduced to a home build microfluidic channels that ware fully siliconized and partly coated with collagen. The thrombus image and thrombin were observed by a fluorescence microscope with 60X magnification.

Results:

1. Thrombin was generated at a same time explosively on every cluster of activated platelets.
2. Thrombin generation was accelerated shortly before formation of fibrin network.

Conclusions: Thrombin generation on activated platelets was visualized by a fluorescence microscope.

PB2375 | Prevalence and Predictors of Venous Thromboembolic Events (VTE) in Patients Undergoing Salvage Chemotherapy and Autologous Stem Cell Transplantation for Relapsed Lymphomas

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Background: Lymphoma patients are at higher risk for venous thromboembolic events (VTE). Almost 25% of patients will have refractory disease or early relapse and majority of such patients undergo salvage chemotherapy and autologous stem cell transplantation.

Aims: Data on VTE in this setting is scarce. In this study, we investigate the prevalence and factors that may increase the risk of VTE in such patients.

Methods: Medical records were searched for patients with a lymphoma diagnosis and radiologically-confirmed VTE. Known risk factors for VTE were reviewed.

Results: A total of 216 patients, median age: 31 (range: 19-60) years were recruited. Most patients (n=140: 64.8%) had Hodgkin's lymphoma while 50 (23.1%) others had diffuse large B-cell lymphoma (DLBL). In total, 36 (16.7%) patients had VTE mostly as upper extremity DVT (n=28, 77.8%); 13 (46.4%) of them were related to central venous catheter (CVC). Thrombosis rates were higher among patients with high LDH (29.2% vs. 5.9%, p< 0.001), those with mediastinal involvement (25.9% vs. 11.5%, p=0.025) and in patients with longer hospital stay (22.1% vs.9.5%, p=0.036). Patients who failed to achieve complete remission (CR) post-transplant and patients with high ThroLy risk score had higher rates of VTE, but was not statistically significant; (25.0% vs. 12.8%, p=0.064) and (21.3% vs. 13.1%, p=0.094), respectively. Type of lymphoma (Hodgkin's vs. Non-Hodgkin's), tumor size, body mass index, mobilizing regimen and disease status prior to transplant had no impact on VTE rates. In multivariate analysis, high LDH (OR: 7.66; 95% CI: 2.79-21.04, p< 0.001) and mediastinal involvement (OR, 3.64; 95% CI:1.53-8.70, p=0.002) were associated with significantly higher rates of VTE.

Conclusions: Patients with relapsed Hodgkin's and Non-Hodgkin's lymphoma undergoing salvage chemotherapy and stem cell transplantation are at higher risk for VTE especially in those with high LDH and mediastinal involvement. If no contraindications exist, thromboprophylaxis might be considered in these settings.

PB2376 | Dilemma in Initiating Thromboprophylaxis in Critically Ill Patients

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Background: Critically patients had higher risk for developing deep vein thrombosis (DVT), which lead to increased mortality rate. DVT prophylaxis become modality to reduce number of DVT cases. Decision to initiate anticoagulant can be challenging in critically patients such as the presence of coagulation disorder due to sepsis or stress ulcer.
Aims: There are controversies regarding the VTE prophylaxis in patients. This study aims to determine the benefits of DVT prophylaxis in critically ill patients.
Methods: Case report.
Results: A-53 y.o female presented to ED with loss of consciousness. Her past medical history was diabetes. Initial examination showed sepsis from pneumonia, hyperglycemia without ketoacidosis, acute kidney injury, and hyponatremia. Sepsis protocol and renal supporting therapy were initiated. On the fourth day, she developed diabetic ketoacidosis. She regained consciousness on sixth day. On seventh day, she had hematemesis melena. Esomeprazole was administered. Sepsis and gastrointestinal bleeding was controlled on tenth day, she was prepared for discharged. However, she suddenly complained left leg swelling with warmth, pain, and tightness. The examination showed diffusely red, painful, and grossly swollen on the left lower extremity. Doppler ultrasonography revealed an acute DVT encompassing the popliteal and posterior tibial, proximal great saphenous, and peroneal vein. Sixty-milligram enoxaparin was administered twice daily for three days and 2 mg warfarin was given. She was discharged on fifteenth day with reduced swelling, pain, warmth on the left leg, and INR of 2.1. Follow up visit after discharged, revealed further reduction on size and pain on the left leg.
Conclusions: Hospitalized patients with critical illness and long immobilization should be screened for DVT and administered DVT prophylaxis. In some conditions, recent gastrointestinal bleeding due to stress ulcer may become dilemma to initiate anticoagulant.

PB2377 | Predictors of Venous Thromboembolic Events (VTE) in Patients with Testicular Cancer

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Background: Patients with testicular cancer are at higher risk for venous thromboembolic events (VTE) and mostly in ambulatory settings.
Aims: In this study, we search for clinical and pathological predictors that may help identify subgroup of patients at higher risk to justify thromboprophylaxis.
Methods: Patients with pathologically-confirmed testicular cancer were retrospectively reviewed for a diagnosis of radiologically-proven VTE. Clinical and pathological features possibly associated with VTE were analyzed.
Results: A total of 322 patients; median age (range) 31 (18-76) years were recruited. Pathological features include: non-seminoma (n= 194, 60.2%), tumors > T1 (n=147 45.7%), node-positive (n=148, 46.0%) and 56 (17.4%) with metastatic disease. Among patients with known smoking history, 166 (51.6%) were smokers and 59 (20.1%) were obese with BMI ≥30. Thromboembolic events were diagnosed in 26 (13.8%) of 188 who received 1st line cisplatin-based chemotherapy and among 11 (27.5%) of 40 patients who received 2nd line chemotherapy, compared to only one episode among 131 patients who never received any chemotherapy, p< 0.0001. Thromboembolism was also higher among a group of 75 patients with high LDH (n=16, 21.3%) compared to 11 (4.5%) of 247 patients with normal values (p< 0.0001), in patients with metastatic disease (23.2% vs. 5.3%, p< 0.001), patients ≥30 years (11.8% vs. 4.7%, p=0.024), >T1 disease (12.2% vs. 5.1%, p=0.022) and in those with node-positive disease (16.9% vs. 1.1%, p< 0.0001). In multivariate analysis, chemotherapy (Odds ratio [OR], 14.86; 95% CI: 1.94-114.08, p=0.009), high LDH (OR 3.80; 95% CI: 1.61-8.97, p= 0.002) and age> 30 years (OR 3.09; 95% CI: 1.22-7.85, p=0.018) were associated with significantly higher rates of VTE.
Conclusions: This is one of the largest studies attempts to identify factors associated with higher risk of VTE in patients with testicular cancer. Chemotherapy, high LDH, and age above 30 years are associated with significantly higher risk of VTE to justify thromboprophylaxis.

PB2378 | Efficacy and Safety of Apixaban for the Primary Prevention of VTE among Patients with Gastrointestinal Cancers: A Post-hoc Analysis of the AVERT Trial

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Background: The risk of venous thromboembolism (VTE) in cancer patients is well-recognized and is associated with an increase in

TABLE 1 Tumor-specific outcomes of patients with gastrointestinal cancers

Table 1 Tumor-Specific Outcomes of Patients with Gastrointestinal Cancers							
		No. of events(%)					
	Outcome Description	Treatment	Control	HR	95% CI		p-value
ALL (N=130)	VTE within 6 months	3 (4.6%)	13 (20%)	0.27	0.13	0.54	0.0002
Stomach cancer (N=44)		1 (4.0%)	6 (31.6%)	0.14	0.05	0.42	0.0005
Pancreas Cancer (N=75)		2 (5.4%)	6 (15.8%)	0.45	0.21	0.96	0.039
Colon Cancer(N=11)		0 (0%)	1 (12.5%)				
ALL (N=130)	Major bleeding within 6 months	2 (3.1%)	1 (1.5%)	2.39	0.29	19.78	0.42
Stomach cancer (N=44)		0 (0%)	0 (0%)				
Pancreas Cancer (N=75)		2 (5.4%)	1 (2.6%)	2.55	0.30	21.56	0.39
Colon Cancer(N=11)		0 (0%)	0 (0%)				
ALL (N=130)	Clinically-relevant bleeding within 6 months	5 (7.7%)	3 (4.6%)	1.97	0.64	6.06	0.23
Stomach cancer (N=44)		2 (8.0%)	0 (0.0%)				
Pancreas Cancer (N=75)		3 (8.1%)	2 (5.3%)	2.14	0.55	8.32	0.27
Colon Cancer(N=11)		0 (0.0%)	1 (12.5%)				

morbidity, mortality, and healthcare expenditures. The recent AVERT trial (N Engl J Med 2019 Feb 21;380(8): 711-719) demonstrated that prophylactic apixaban significantly lowered the rate of VTE when compared to placebo in intermediate-to-high risk (Khorana score ≥2) patients starting chemotherapy. However, the benefit of thromboprophylaxis with apixaban in gastrointestinal cancers specifically is unknown.

Aims: We aim to assess the efficacy and safety of apixaban in preventing VTE in patients with gastrointestinal cancers.

Methods: This is a post-hoc analysis of the AVERT trial, which was a randomized, placebo-controlled, double-blind clinical trial. The primary efficacy outcome was an episode of VTE within the first 180 days after randomization. The primary safety outcome was major bleeding, as defined by the ISTH. The secondary outcome measure was clinically relevant bleeding which combined major and clinically-relevant non-major bleeding episodes. Time-to-event analyses were performed on the different outcomes in patients with gastrointestinal cancers.

Results: A total of 133 patients were assessed in this post-hoc analysis. 130 patients were included in the modified intention-to-treat analysis, with 65 patients being allocated to each of the apixaban and placebo groups. The use of apixaban was associated with a significantly lower incidence of VTE (HR: 0.27; 95% CI: 0.13-0.54; p = 0.0002) and non-significant higher rates of major (HR: 2.39, 95% CI: 0.20-19.78, p = 0.42) and clinically-relevant bleeding (HR: 1.97; 95% CI: 0.64-6.06; p = 0.24) at 6 months. Tumor-specific outcomes are depicted in Table 1.

Conclusions: Apixaban therapy resulted in a significantly lower rate of VTE than did placebo among intermediate-to-high risk ambulatory patients with gastrointestinal cancers who were starting chemotherapy. The rates of major and clinically-relevant bleeding were higher with apixaban than with placebo. (Funded by the CIHR and Bristol-Myers-Squibb-Pfizer Alliance; NCT02048865.)

PB2379 | An Audit Examining Information Provided to Patients Regarding Venous Thromboembolism (VTE) at Discharge

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Background: VTE is a known complication of hospital stay. In 2017, an audit was performed, looking at the quality of the information provided to patients at discharge. Guidelines state that all patients should receive information, normally in the form a leaflet. This audit showed that 22% of the discharged patients recalled the information given regarding VTE. It went on to state only 21% of patients were being given the information on discharge.

Aims: To determine whether information deliverance has improved and examining the factors that influence recall of that information. Specifically, seeing if there has been an improvement since the 2017 audit.

Methods: Two-step evaluation of information deliverance and recall;

1). An observational study looking at inpatients that were discharged and the documentation of the information given

2). A telephone survey of patients that were discharged (within a month) looking at their recall of the information provided

Results:

1). In the Observational Study- 100% of patients were given the information verbally and via a leaflet

2). In the Telephone Survey- Only 46% of patients recalled the VTE information

Conclusions: The study has shown that since 2017, there has been a significant change in practice. Almost all patients receive VTE information on discharge. However, worryingly this has not changed the percentage of patients who retain and understand this information. This audit has shown that while telling patients about VTE is easily achievable, helping them to understand a foreign concept is far more difficult. The solution resides in not only simplifying the information in lay terms but also making it relatable to the general population. Currently, it is clear that solely delivering the information is inadequate; changes must also be made to its quality.

PB2380 | Preventing Maternal Death and Morbidity from Venous Thromboembolism (VTE): Results from a VTE Risk Score Trial during Hospitalization

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Background: Hospitalization in pregnancy and childbirth greatly increases the thromboembolic risk of these patients. The application of a protocol for assessing the risk of VTE reduces mortality and morbidity of these phenomena.

Aims: The goal of this study was to apply a thromboprophylaxis protocol with a VTE risk score for all hospitalized pregnant women.

Methods: This was a longitudinal, interventional and prospective study of hospitalized pregnant women in a single, high risk pregnancy reference Center. Patients were classified as low or high risk according to the VTE risk score (https://clinicaltrials.gov/ct2/show/NCT02600260). The high-risk group (score ≥3) received thromboprophylaxis with enoxaparin unless the patient had a contraindication for anticoagulation, such as active bleeding or a high bleeding risk. The collected data were descriptively analysed to identify the profile of pregnant women using percentages and absolute values. One patient could have undergone more than one evaluation. Statistic analysis by SPSS.

Results: The data of 11794 cases (8192 patients) were descriptively analysed:2411(20.4%) were classified as high-risk and 775(32.1%) were already on anticoagulation(pre natal anticoagulation); 1636 (15.28% scored ≥ 3) and were compared to the group that scored < 3 (n=9068). Protocol failure in the treated group: 7/1636 (0.4%) presented with VTE until 3 months post discharge and 6/9068(0.06%) in the low risk group. One patient had a severe adverse effect of anticoagulation. No patient died of VTE. The main risk factors were: age ≥35 and ≤39y- 437/1636(26.7%),OR1.6, (1.4-1.8), parity≥ 3 - 419(25.6%),OR3.5 (3.0-4.0), age ≥ 40y- 321(19.6%),OR 4.8 (4.1-5.6), multiple pregnancy 182(11.1%), OR 2.1 (1.7-2.5), BMI≥40 kg/m^2-375(18%), OR5.0(4.3-5.9), severe infections 144(8.8%), OR4.1(3.3-5.1) and cancer 110(6.7%), OR12.2(8.7-17.1).

Conclusions: This VTE risk score had a cost effective 15% incidence of high risk patients, maternal age, great multiparity, obesity, severe infections and cancer were the main risk factors.

PB2381 | The Incidence of Venous Thromboembolism along with Adherence to Guideline in Major Orthopedic Surgery

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Background: Prevention of venous thromboembolism (VTE) is important in undergoing major orthopedic surgery. It is not well-known how the physicians are adhering to the guidelines for thromboprophylaxis in real life practice.

Aims: This study aims to assess adherence rate to the thromboprophylaxis guideline affects the incidence of VTE.

Methods: This retrospective study using medical chart review the admitted patients underwent THA, TKA or HFS from March 2009 through February 2011. Compliance was defined adherence of orthopedic surgeons for chemical prophylaxis or mechanical prophylaxis. We assessed the adherence rate of thromboprophylaxis guideline, as well as analyzed incidence of VTE, major bleeding and all cause mortality in major orthopedic surgery within 3 months.

Results: The study population was total 391 patients (THA 80, TKA 95, and HFS 216 patients). The chemical compliance rate was 56% before surgery, 67% after surgery in total patients. The mechanical compliance rate was 100%. VTE occurred in 8 patients (8/391, 2.0%). Eight patients with pulmonary embolism (1/391, 0.26%), and 1 patient was pulmonary embolism with deep vein thrombosis (8/391, 2%). Major bleeding did not occur. The all-cause mortality was 3.6%(14/391)(THA 2.4%, TKA 1.1%, and HFS 5.1%). VTE related death was in one patient (1/391, 0.26%).

Conclusions: In this study, incidence of VTE in major orthopedic surgery within 3 months was similar with outcome of previous studies when preoperative and postoperative adherence rate of chemical prophylaxis was 56%, 67%, respectively.

PB2382 | Effectiveness of Implementing a Prophylaxis Protocol for Thromboembolic Events in Patients Admitted to a Tertiary Hospital

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Background: Hospitalization increases the risk of venous thromboembolism (VTE) by eight times, which can have important clinical consequences such as deep venous thrombosis (DVT) and pulmonary embolism (PE), in addition to promoting an increase in hospital stay and death. VTE is the most common preventable cause of death in hospitalized patients, thromboprophylaxis is the most appropriate mechanism to prevent VTE and its complications, so it is important to identify the risk of VTE in hospitalized patients and establish the recommended prophylaxis.

Aims: To evaluate, through a systematized protocol, the rate of thromboembolic events in hospitalized, clinical and surgical patients.

Methods: Patients admitted consecutively to a private tertiary hospital from January 1, 2018 to November 30, 2019, were evaluated for clinical and surgical hospitalization for at least 48 hours.

Patients admitted with DVT and / or PE, events of arterial thrombosis and those in palliative care were excluded from the analysis.

The events of DVT (distal and proximal), thrombosis of catheter and PE were recorded. These outcomes were confirmed with imaging tests.

Results: In a universe of 81445 units hospitalized in the described period, 114 events of venous thromboembolism. Among these events, 51 (44.74%) were distal DVT, 42 (36.84%) proximal DVT, 17 (14.91%) PE and 4 (3.5%) from catheter.

Conclusions: In the present study, in the period described above, 114 events were identified, corresponding to approximately 0.14% of hospitalized patients, below what is found in the literature.

This demonstrates that, in a highly complex hospital, the increase in adherence to the protocol, identification of the risk of VTE and an appropriate approach contributes to a constant search for the

reduction of preventable events, thus reducing hospital stay and morbidity and mortality.

PB2383 | Prevention of Catheter Complications in Patients with Cystic Fibrosis: Enoxaparin vs. a Direct Oral Anticoagulant

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Background: Nonpediatric cystic fibrosis (CF) patients are frequently hospitalized, often requiring placement of a central-venous catheter (CVC). Enoxaparin is often used for prevention of catheter-thrombosis, but requires daily injections. Direct oral anticoagulants (DOACs) provide a potential alternative that may be better accepted by patients.

Aims: Compare the efficacy, safety, and patient acceptance of enoxaparin or a DOAC in prevention of catheter complications in nonpediatric CF patients.

Methods: This retrospective study included CF patients at our hospital with placement of a CVC and received prophylaxis with either enoxaparin or DOAC between 1/2016 and 6/2019. Patients receiving both types of anticoagulants during the same admission were excluded. Patient demographics, medication use, laboratory values, and outcomes were collected from patient charts. Catheter complications included catheter replacement due to thrombosis or need for fibrinolytic therapy. All descriptive data are shown as mean ± standard deviation. Comparisons of categorical data used Fisher's exact or Chi Square and continuous data by student *t* test.

Results: We identified 149 CF patients with a CVC receiving enoxaparin (n=70) or DOAC (n=79) for prophylaxis. Of the patients who got enoxaparin, 81% got 40mg daily, and 91% of DOAC use was rivaroxaban 10mg daily. Patient demographics were similar between the groups (Table). Patients receiving a DOAC had a smaller CVC lumen diameter and had more prior catheters compared to enoxaparin patients. More patients receiving enoxaparin refused their doses compared to DOACs (59% vs. 10%; p< 0.001), as well as having more refused doses per patient (6.7±4.8 vs. 1.4±1.1; p=0.003). There were more CVC complications in patients receiving enoxaparin vs. DOACs (17% vs. 6%; p=0.043). No patients experienced major bleeding.

Conclusions: DOACs were better accepted by the patients compared to enoxaparin. DOACs also provided a reduction in catheter complications without increasing the risk of bleeding, making DOACS a promising alternative for CF patients.

PB2384 | Effectiveness of Thromboprophylaxis in the Prevention of Peripherally Inserted Central Catheter (PICC) Associated Venous Thromboembolism in Pediatric Patients: A Case-control Study

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Background: CHEO, a tertiary pediatric hospital in Ottawa, Canada, administers prophylactic anticoagulation to patients with PICCs based on the presence of elevated biochemical and hematological markers of inflammation. There has been no review of the effect of thromboprophylaxis on the burden of catheter associated VTE at our institution.

Aims: The primary objective of this study was to evaluate the effect of thromboprophylaxis on the risk of PICC associated VTE at our institution.

Methods: This is a single-centre retrospective case-control study at a medium-sized pediatric hospital. We included all patients with PICC line associated VTE at CHEO between January 1, 2005 and June 30, 2018, excluding patients with previous thrombosis or known thrombophilia. Information about PICC line insertion, VTE characteristics and inflammatory markers were abstracted from patient charts.

Results: 99 patients had PICC-associated VTE between January 1, 2005 and June 30, 2018. Most VTEs occurred in patients less than 3 months of age(49.5%), followed by adolescents over 12 years of

TABLE 1 Patient and catheter characteristics

Variable	Enoxaparin (n=70)	DOAC (n=79)	p-value
Age (years)	31.3 ± 10.5	30.2 ± 9.0	0.454
Female	53%	39%	0.133
BMI	21.2 ± 4.0	20.7 ± 3.8	0.436
Length of stay	10.4 ± 6.0	10.9 ± 5.6	0.600
Arterial thrombosis risk factors	0.94 ± 0.99	0.63 ± 0.79	0.035
Venous thrombosis risk factors	1.2 ± 1.0	1.3 ± 0.8	0.477
PICC line	86%	92%	0.293
Catheter lumen size (cm)	5.6±1.1	4.9±0.3	0.010
Prior catheters	5.7±7.0	10.9±8.3	<0.001

age(22.2%). 52.5% of patients had symptomatic VTE and 58.6% of VTEs were occlusive on imaging. Of 199 patients in our sample 13.6% received thromboprophylaxis with a similar number of recipients in both the VTE case and non-VTE control groups. Thromboprophylaxis was commonly prescribed to adolescents(78%) and rarely given to infants under 3 months of age(3.7%). In our multi-variable regression model we observed no statistically significant association between VTE and thromboprophylaxis, age, female sex, year of placement, ultrasound guidance or raised inflammatory markers. There was an observed association between increasing age and odds of receiving thromboprophylaxis.

Conclusions: This study did not demonstrate a reduction in the probability of PICC-line associated VTE for patients that were prescribed thromboprophylaxis and in our sample, neonates represented the largest population of patients with PICC line associated VTE but were the least likely to receive thromboprophylaxis.

PB2385 | Point-of-Care Monitoring of Unfractionated Heparin Treatment in the Intensive Care Unit: Preliminary Results of a Comparative Study with Laboratory-based APTT and Anti-Xa Activity

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Background: Intravenous unfractionated heparin (UFH) is administered routinely in the intensive care unit (ICU) for anticoagulation of patients. UFH doses are adjusted based on the monitoring of activated partial thromboplastin time (APTT) or anti-Xa activity. However, these strategies are associated with potentially large time intervals before dose adjustments, which could be detrimental to the patient.

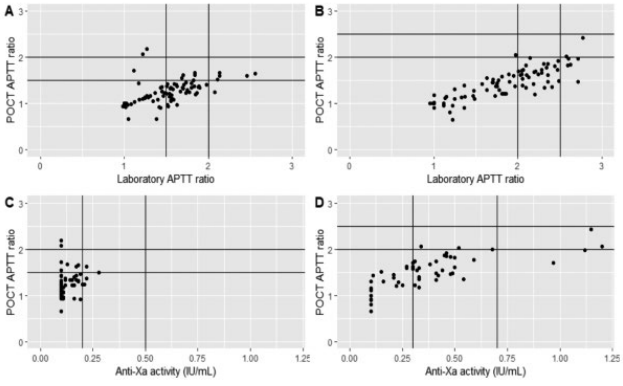
Aims: To compare a point-of-care (POC) version of APTT to (i) laboratory-based APTT and (ii) measurement of anti-Xa activity in terms of agreement and turnaround time (TAT).

Methods: Seventeen ICU patients requiring UFH therapy for at least 72 hours either for preventive anticoagulation (n=10) or therapeutic anticoagulation (n=7) were prospectively included after giving their written informed consent. UFH was administered according to a local adaptation of Raschke and Amanzadeh algorithms. UFH doses were adjusted on laboratory APTT for therapeutic (2.0-2.5 times baseline APTT) or prophylactic (1.5-2.0 times baseline APTT) anticoagulation. Simultaneous measurements of POCT-APTT (CoaguChek® Pro II, Roche Diagnostics) on a drop of fresh whole blood, laboratory-based APTT (C.K. Prest®, Stago) and Anti-Xa activity (STA®Liquid anti-Xa, Stago) on 3.2% buffered citrated plasma were systematically measured before dose adjustment. Agreement was evaluated in ten patients for whom both POCT and laboratory APTT before UFH administration were available.

Results: The relationship between POCT-APTT vs laboratory APTT (n=162 pairs) and POCT-APTT vs Anti-Xa (n= 117 pairs) for both therapeutic and prophylactic ranges are presented in Figure 1. There was no inter-method agreement between POCT vs laboratory APTT (Cohen's kappa -0.031 (95%CI -0.12-0.06; p=0.51), and between POCT vs Anti-Xa activity (Cohen's kappa -0.033 (IC95% -0.18;0.11; p=0.66). Global TAT from blood collection until dose adjustment based on laboratory APTT was 101.3 ± 44.8 minutes (mean±sd; Table 1).

Conclusions: Based on these preliminary results, no agreement between POCT-APTT and either laboratory APTT or Anti-Xa activity was identified. However, these latter methods were associated with high TATs.

Figure 1: Relationship between POCT APTT vs laboratory APTT and vs anti-Xa activity. (A-B) Relationship between POCT-APTT and laboratory APTT for prophyalctic (A) and therapeutic (B) anticoagulation. (C-D) Relationship between POCT-APTT and anti-Xa activity for prophylactic (C) and therapeutic (D) anticoagulation. POCT and laboratory APTT are expressed as ratio to baseline APTT. Solid lines indicate target therapeutic ranges. Anti-Xa activity range for prophylactic anticoagulation was set up at 0.2-0.5 IU/mL according to Thomas J et al*.



*Thomas J et al. Bleeding and Thrombotic Complications in the Use of Extracorporeal Membrane Oxygenation. Semin Thromb Hemost. 2018;44:20–29.

FIGURE 1

TABLE 1

Table 1: Comparison of the different tests used to monitor unfractionated heparin therapy. Times are expressed as mean ± standard deviation.

	Point-of-care testing APTT (CoaguChek® Pro II, Roche)	**Laboratory-based APTT** (C.K. Prest®, Stago)	**Laboratory-based Anti-Xa Activity** (STA®Liquid anti-Xa, Stago)
Time to results (mean±SD)	< 5 minutes.	56.8 ± 33.3 minutes.	71.1 ± 47.8 minutes.
Global TAT from blood collection until dose adjustment (mean±SD)	Not evaluated.	101.3 ± 44.8 minutes.	Not evaluated.
Sample matrix (minimal volume required)	A drop of fresh whole blood (≥ 8µl).	3.2% buffered citrated plasma (>500 µl).	3.2% buffered citrated plasma (>500µl).
Analysis principle	Electrochemical measurement of the APTT using celite as activator.	Coagulometric measurement of the APTT using kaolin as activator.	Chromogenic assay without antithrombin supplementation.
Measurement of UFH Acitivity	Indirect.	Indirect.	Direct.
Major benefit	Time savings.	Availability and familiarity of clinicians.	Direct measurement of heparin activity.

PB2386 | Thromboprophylaxis Audit in a Public Hospital - The Impact of Protocol Implementation

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Background: This clinical audit consists in an assessment of medical practices at Santa Casa de Misericórdia in Belo Horizonte (SCMBH) regarding the use of thromboprophylaxis in patients hospitalized at Internal Medicine Departament.

Aims: The objective is to evaluate the quality of the service provided and compare it with the ideal assistance. This is a third audit on the same topic that evaluates the impact of the measures suggested by the previous studies and electronic protocol implementation.

Methods: This is a retrospective and observational study whose data were obtained from the electronic records available at SCMBH between August and December 2019. Ninety patients were randomly selected admitted to clinical wards evaluating risk of thromboembolism and bleeding, as well as the prescription of thromboprophylaxis The risk assessment of VTE was based on the PADUA score (score greater than or equal to 4: considered high. The risk of Bleeding was assessed using IMPROVE score and was defined as high risk score greater than or equal to 7. The data collected were processed and compared with other audits.The recommendations were made using the GRADE methodology (The Grading of Recommendations Assessment, Development and Evaluation).

Results: There is an important increase in numbers, revealing that there was an positive impact in the implementation of the PADUA and IMPROVE scores in record. About correct use of thromboprophylaxis: There was a decrease in prescription misuse of medication and increased adherence and correct use of thromboprophilaxis with mandatory filling in the electronic medical chart.

Conclusions: We verified that the implementation of the mandatory questionnaire at admission had a strong positive impact implementing adequate thromboprophylaxis in patients admitted at SCMBH and reduced the iatrogenic use of heparins. The audit supports the importance of spreading the risk assessment routine in the service, maintaining the institutional protocol and also the importance of Electronic Chart and Protocol implementation.

VTE TREATMENT

PB2387 | Two Weeks of Low Molecular Weight Heparin for Isolated Symptomatic Distal Vein Thrombosis (TWISTER Study): Final Analysis of 242 Patients

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Background: Most deep vein thromboses (DVT) originate from distal (calf) vein thrombi, however 80-90% of these lyse spontaneously. The management of isolated distal DVT(IDDVT) is controversial.

Aims: Examine the efficacy/safety of two weeks of anticoagulation for a first symptomatic IDDVT.

Methods: Multicentre prospective cohort study in Australia/New Zealand for patients with confirmed first symptomatic IDDVT. Exclusions included active malignancy, bleeding risk factors, pregnancy/lactation, immobility, plaster casts or non-weight-bearing. Patients received therapeutic anticoagulation (enoxaparin or rivaroxaban) with repeat complete compression leg vein ultrasound (CUS) at the end of 2 weeks. Those with extension into proximal veins on two-week CUS were treated off study (group A). For those with ongoing symptoms, treatment was continued for a further 4 weeks (group B). If the patient was asymptomatic and there was no proximal DVT extension on CUS, treatment was stopped (group C). The primary outcome was the symptomatic recurrence of venous thromboembolism, VTE (DVT and pulmonary embolism, PE) within 3 months, in group C patients.

Results: 242 patients were enrolled at twelve sites; one was withdrawn on day five due to clinically relevant non-major bleeding. Of the remaining 241, 167(69%) received two weeks anticoagulation; 71(30%) six weeks anticoagulation; and three patients(1%) had proximal extension on 2-week CUS. Eleven patients in group C were lost to follow-up. Of the remaining 156 in the two-week anticoagulation arm, two had symptomatic distal DVT recurrence; VTE recurrence

rate 2/156; 1.3%(95% CI 0.19-5.41%). No patients, 0/156; 0%(95% CI 0.00-2.34) had proximal DVT or PE recurrence.

Conclusions: For IDDVT patients, two weeks of anticoagulation is associated with a low risk of VTE recurrence (1.3%), if patients are symptom-free at two weeks and repeat CUS at two weeks does not show thrombus extension into proximal veins. Based on this data, the previous standard of care, 6-12 weeks anticoagulation, is not required for most IDDVT patients.

PB2389 | Fixed Dose Rivaroxaban Can Be Prescribed in Extremes of Bodyweight, Results of a Population Pharmacokinetic Analysis

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Background: To date, there has been no data from clinical practice to suggest that pharmacokinetic (PK) parameters are not substantially altered at the extremes of bodyweight. The International Society of Thrombosis and Haemostasis Scientific and Standardisation Committee (ISTH SSC) suggest avoiding direct oral anticoagulants (DOACs) in patients weighing >120kg or BMI >40kg/m^2 and give no recommendation in those < 50kg. Emerging safety and efficacy data for rivaroxaban in morbid obesity suggests traditional therapy and rivaroxaban are comparable.

Aims: To develop a population PK model to understand the influence of bodyweight on rivaroxaban exposure.

Methods: Between 2013 and 2018, rivaroxaban plasma concentrations, laboratory markers and patient characteristics were collated from patients prescribed rivaroxaban attending the anticoagulation clinics at KCH. Patients were eligible if a time of last rivaroxaban dose was recorded. A population PK model was developed using a non-linear mixed effects approach, and then used to simulate rivaroxaban concentrations in patient subgroups of interest such as extremes of bodyweight and stages of renal impairment.

Results: A population PK model was developed from 913 patients weighing between 39kg and 172kg (table 1). Notably, 86 patients weighed >120kg, 74 patients had a BMI >40kg/m^2, and 30 patients weighed < 50kg.

A one compartment-model with between subject variability on apparent clearance and a proportional error model best described the data. Creatinine clearance, calculated using Cockcroft Gault with lean bodyweight as the weight descriptor in the equation, was the most significant covariate influencing rivaroxaban exposure (figure 1).

TABLE 1 Patient characteristics and indications for anticoagulation

Patient characteristics	N = 913
Gender, male (%)	522 (57.2)
Age, years (mean [range])	67 [19 - 96]
Weight, (kg) (mean [range])	85.8 [39.0 - 171.6]
BMI, kg/m^2 mean [range])	29.7 [15.6 - 55.8]
CRCL, ml/min (mean [range])	91.5 [15.9 - 297.2]
Indication for anticoagulation (%)	AF = 629 (68.9)
	VTE = 267 (29.2)
	Other = 17 (1.9)

Abbreviations: AF, atrial fibrillation; BMI, body mass index; CRCL, creatinine clearance calculated using total bodyweight; VTE, venous thromboembolism *Other indications for anticoagulation - Left ventricular thrombus, cardioembolic stroke of unknown aetiology.

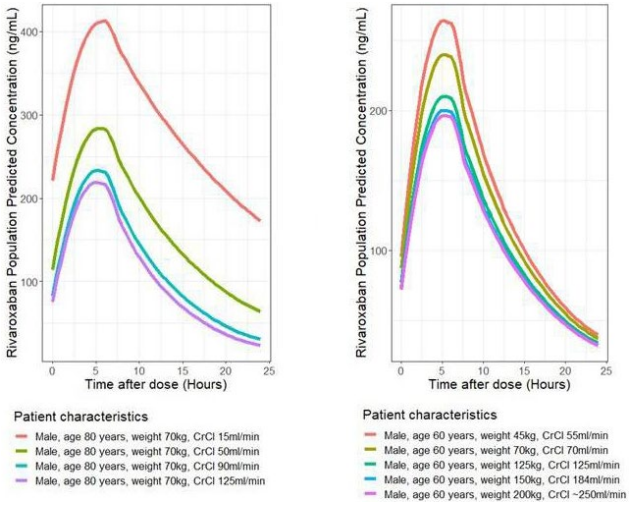

FIGURE 1 Simulation plots derived from steady state data for rivaroxaban 20mg once daily

Conclusions: Our work suggests that if renal function is adequate then rivaroxaban can be prescribed at the extremes of bodyweight. Current guidance with respect to rivaroxaban should be revised, recommending it as a suitable option for patients with a BMI >40kg/m^2 or weight >120kg, and those < 50kg. Further research is required to establish whether the same is true for other DOACs.

PB2390 | Management and Outcomes of Idiopathic Axillosubclavian Vein Thrombosis: Impact of the Treating Specialty

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Background: Anticoagulation is the standard of care for the management of idiopathic axillosubclavian vein thrombosis (IASVT) but it is unclear how the uptake of ancillary modalities such as thrombolysis

TABLE 1 Patient characteristics and treatment

* p<0.05	Haematology service n=35	Vascular surgery n=12
Patients, %	74	26
Age (median, range, years)	40 (17-86)	42.5 (17-60)
Male, %	49	69
Right-sided IASVT, %*	49	83
Concomitant PE%	31	8
Treated with anticoagulation alone, %	100	50
Anticoagulation duration (median, range, months)	6 (3-32)	6.6 (4-15)
Treated with additional/ ancillary intervention, %*	0	84

TABLE 2 Treatment outcomes

	Haematology service n=35	Vascular surgery n=12
Follow-up duration median, range, months	17 (0-121)	11 (0-85)
Thrombus resolution,% (95%CI)	26 (13-43)	42 (15-72)
Recurrent VTE, % (95%CI)	3 (0.1-15)	0
CRNMB,% (95%CI)	3 (0.1-15)	0
Major bleeding, % (95%CI)	0	17 (2-48)

and decompressive surgery, and subsequent outcomes vary according to the initial treating speciality.

Aims: To determine the impact of the treating specialty on the management and outcomes of IASVT.

Methods: Retrospective chart review of patients diagnosed with IASVT from January 2006 to April 2019 at Monash Health, Melbourne. The outcome measures were the effect of the initial treating specialty on anticoagulation duration, and the rates of additional interventions, residual vein thrombosis, VTE recurrence, and ISTH-defined bleeding.

Results: Patient characteristics, treatment and outcomes are shown in Table 1 and 2. Of 47 patients who presented acutely to the emergency department, 12 (26%) and 35 (74%) were initially referred to the vascular surgery (VS) and haematology service (HS), respectively, for further management. Both services were subsequently involved in 9 (75%) and 9 (26%), respectively. Compared with patients referred to HS, those referred to VS were more likely to undergo additional interventions (84% vs. 0%, p= 0.0001). Anticoagulation duration was similar between the two groups. The proportions of thrombus resolution (42%vs26%, RR1.27; 95%CI: 0.76-2.1) and VTE recurrence rates were similar in VS- and HS-referred patients. (0%vs3%, RR0.92; 95%CI: 0.04-21.3), but rates of clinically relevant bleeding (CRNMB and major bleeding) were numerically higher in the VS group (17%vs3%, RR13.8; 95%CI: 0.7-270). The one VTE recurrence (LLDVT and PE) occurred 3 months post IASVT whilst on anticoagulation, likely secondary to non-compliance.

Conclusions: Patients with IASVT who were referred to VS were more likely to undergo additional interventional management than those referred to HS. Despite this, anticoagulation duration and thrombus resolution rates were similar. Although both groups had low VTE recurrence rates, bleeding appeared numerically higher in patients referred to VS.

PB2391 | Rivaroxaban for Extended Thromboprophylaxis in Acutely Ill Medical Patients 75 Years of Age or Older

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Background: Elderly patients are at increased risk for major and fatal thromboembolic events. Thromboprophylaxis is underutilized because of bleeding concerns. The MARINER trial (NCT02111564) evaluated whether rivaroxaban reduced symptomatic venous thromboembolism (VTE) or VTE-related death in acutely ill medical patients at risk for VTE.

Aims: We hypothesized that rivaroxaban, especially at 10mg daily, would have a favorable benefit/risk profile in patients ≥75 years of age.

Methods: After informed consent, patients were randomized in a double-blind manner at hospital discharge to rivaroxaban (10mg/day-creatinine clearance ≥50 ml/min; 7.5mg/day - ≥30-< 50ml/min) or placebo for 45 days. In this pre-specified subgroup analysis, using a Cox proportional hazard model including treatment as a covariate, we compared the risk of the primary efficacy (symptomatic VTE and

TABLE 1 Efficacy results in MARINER by age and treatment group

Efficacy (ITT)		Age <75			Age ≥75		
		Rivaroxaban N=3853 (%)	Placebo N=3872 (%)	HR and 95% CIs	Rivaroxaban N=2154 (%)	Placebo N=2140 (%)	HR and 95% CIs
Overall Population	Primary Efficacy Outcome	25 (0.6)	32 (0.8)	0.78 (0.46, 1.32)	25 (1.2)	34 (1.6)	0.73 (0.43, 1.22)
	Symptomatic VTE	5 (0.1)	11 (0.3)	0.46 (0.16, 1.31)	6 (0.3)	14 (0.7)	0.43 (0.16, 1.11)
	VTE-related Death	21 (0.5)	23 (0.6)	0.91 (0.50, 1.65)	22 (1.0)	23 (1.1)	0.95 (0.53, 1.71)
		Rivaroxaban N=3575 (%)	Placebo N=3603 (%)	HR and 95% CIs	Rivaroxaban N=1334 (%)	Placebo N=1310 (%)	HR and 95% CIs
10mg	Primary Efficacy Outcome	20 (0.6)	27 (0.7)	0.75 (0.42, 1.33)	12 (0.9)	21 (1.6)	0.56 (0.28, 1.14)
	Symptomatic VTE	5 (0.1)	10 (0.3)	0.50 (0.17, 1.47)	1 (0.1)	9 (0.7)	0.11 (0.01, 0.86)
	VTE-related Death	16 (0.4)	18 (0.5)	0.90 (0.46, 1.76)	12 (0.9)	13 (1.0)	0.91 (0.41, 1.99)

TABLE 2 Safety Results in MARINER by Age and Treatment Group

Safety (Safety Set)		Age <75			Age ≥75		
		Rivaroxaban N=3837 (%)	Placebo N=3855 (%)	HR and 95% CIs	Rivaroxaban N=2145 (%)	Placebo N=2125 (%)	HR and 95% CIs
Overall Population	ISTH Major Bleeding	10 (0.3)	7 (0.2)	1.44 (0.55, 3.77)	7 (0.3	2 (0.1)	3.45 (0.72, 16.61)
		Rivaroxaban N=3561 (%)	Placebo N=3589 (%)	HR and 95% CIs	Rivaroxaban N=1329 (%)	Placebo N=1301 (%)	HR and 95% CIs
10mg	ISTH Major Bleeding	9 (0.3)	7 (0.2)	1.30 (0.48, 3.48)	4 (0.3)	2 (0.2)	1.95 (0.36, 10.67)

VTE-related death in the intent to treat population) and safety (ISTH major bleeding in the safety population) outcome events in patients ≥ and < 75 assigned to rivaroxaban or placebo in the overall study population and in the 10mg stratum.

Results: The primary event rate was 2-fold higher in patients ≥75 compared with those < 75. (Table). Compared with placebo, rivaroxaban had a numerically lower incidence of primary outcome events in both age groups (≥75: 1.2% and 1.6%, HR 0.73 95% CI 0.43, 1.22; < 75 0.6% and 0.8%, HR 0.78 95% CI 0.46, 1.32; interaction p-value for age =0.85). Similar results were observed in the 10mg stratum. The incidence of major bleeding was low and similar in the two age groups and treatment groups in the overall population (interaction p-value for age =0.35) and in the 10mg stratum.

Conclusions: Symptomatic VTE and VTE-related death occur frequently in elderly patients with acute medical illness. Rivaroxaban, particularly at 10mg daily, may reduce such events without a significant increase in major bleeding, suggesting a favorable benefit/risk profile in the elderly.

Funding: Janssen Research and Development

PB2392 | Variability of Management of Peripheral Catheter-Related Upper Extremity Superficial Vein Thrombosis

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Background: Despite peripheral catheter-related upper extremity superficial vein thrombosis (PCRUESVT) being relatively common, the optimal treatment strategy to avoid thrombus extension is not known.

Aims: To describe PCRUESVT risk factors, management strategies, complications and rates of radiologically confirmed extension.

Methods: We reviewed all patients diagnosed with symptomatic, PCRUESVT from March 2015 to December 2017 at Monash Health, Melbourne. Patients with a central catheter or concurrent indication for anticoagulation were excluded. The primary outcomes were the effect of identified risk factors and PCRUESVT management on rates of ISTH-defined bleeding and radiologically confirmed thrombus resolution or extension.

Results: Of 93 patients included, 54 were male (51%), median age 57 years (range 20-91). PCRUESVT characteristics, management and outcomes are shown in Table 1. PCRUESVT risk factors identified included underlying cancer (25%) and infection (28%). 73% involved a single segment ≥5cm and 63% were proximal to the cubital fossa. 69% were admitted at diagnosis and therefore continued on prophylactic anticoagulation. 28 cases (30%) had 1-3 repeat scans. Compared to group 1, all thrombus resolution occurred in group 2 (0vs25% RR1.44 95%CI: 0.2-10.7) and group 3 (0vs36% RR1.27 95%CI: 0.2-9.3) whilst only group 3 had radiologically confirmed extension (0vs8% RR1.67 95%CI: 0.2-12) and bleeding (0vs4% RR1.9 95%CI: 0.26-13.4). All cases of extension/bleeding had active malignancy and extension only occurred in thrombi ≥ 5cm in length.

Conclusions: PCRUESVT management is variable and ranged from observation/symptomatic treatment to prophylactic and intermediate/therapeutic dose anticoagulation. The rates of superficial and deep extension were both low at 1% (95%CI: 0.2-5.9). Both thrombus extension and bleeding only occurred in patients with active cancer who received intermediate/therapeutic anticoagulation.

PB2393 | Rivaroxaban Plus Aspirin for Extended Thromboprophylaxis in Acutely Ill Medical Patients: Insights from the Mariner Trial

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Background: Acutely ill medical patients are at greatest risk for venous thromboembolism (VTE) within 45 days after hospital discharge. The MARINER trial (NCT02111564) evaluated whether rivaroxaban reduces symptomatic VTE or VTE-related death in acutely ill medical patients at risk for VTE. Although aspirin was not randomized, approximately half of the enrolled subjects were receiving aspirin at baseline.

TABLE 1 PCRUESVT characteristics, management and outcomes

	Group 1 Observation or symptomatic relief n=34	Group 2 Prophylactic dose anticoagulation n=35	Group 3 Intermediate or therapeutic dose anticoagulation n=24
Patients,%	37	38	26
Cancer, %	48	17	35
Infection, %	15	58	27
No cancer or infection, %	43	34	23
Thrombus single segment ≥5cm, %	27	69	77
Thrombus proximal to cubital fossa, %	64	72	42
Treatment duration (weeks, median range)	NA	1.07 (0.1-25)	3 (0.6-31.9)
Bleeding, % (95%CI)	0	0	4 (0.1-21)
Extension, % (95%CI)	0	0	8 (1-27)

TABLE 1 Efficacy and Safety Endpoints in MARINER by Treatment Group

Endpoint (up to Day 45 in ITT Population)	Rivaroxaban Alone (N=2848)	Aspirin Alone (N=3046)	Rivaroxaban + Aspirin (N=3159)	No Rivaroxaban No Aspirin N=2966	P Value (Rivaroxaban + Aspirin vs No Rivaroxaban No Aspirin)
Sx VTE + VTE-related Death	0.91%	0.92%	0.76%	1.28%	0.042
VTE-related Death	0.84%	0.59%	0.60%	0.94%	0.125
Sx VTE	0.18%	0.43%	0.19%	0.40%	0.121
Sx VTE and All-cause Mortality	1.40%	1.44%	1.20%	2.12%	0.005
Sx VTE, MI, Non-hemorrhagic Stroke and CV Death	1.54%	1.87%	1.58%	2.12%	0.116
All-cause Mortality	1.33%	1.15%	1.04%	1.82%	0.010
Endpoint (on Treatmnet +2 days in Safety Population	Rivaroxaban Alone (N=2833)	Aspirin Alone (N=3032)	Rivaroxaban + Aspirin (N=3149)	No Rivaroxaban No Aspirin (N=2948)	P Value (Rivaroxaban + Aspirin vs No Rivaroxaban No Aspirin)
Major Bleeding	0.28%	0.20%	0.29%	0.10%	0.105
Non-major Clinically Relevant Bleeding	1.34%	0.92%	1.49%	0.78%	0.009

Aims: We hypothesized that treatment with rivaroxaban on background aspirin therapy would be superior to placebo without background aspirin therapy in preventing thrombotic outcomes.

Methods: Primary and major secondary outcomes were compared in the intention to treat population in 4 subgroups: (1) rivaroxaban alone (10mg - creatinine clearance ≥50ml/min; 7.5mg - ≥30-< 50ml/min); (2) aspirin (≤162mg daily) alone; (3) rivaroxaban plus aspirin; (4) neither rivaroxaban nor aspirin. Incidences were compared using Chi-square testing, without adjustment for multiplicity. Major bleeding (MB) and non-major clinically relevant (NMCR) bleeding were assessed in the safety population. Analyses were post hoc and we did not investigate aspirin use beyond baseline.

Results: Rivaroxaban with background aspirin was associated with lower rates of symptomatic VTE and VTE-related death than treatment without rivaroxaban or background aspirin (0.76% vs 1.28%, p=0.042). Event incidences for rivaroxaban alone (0.91%) or aspirin alone (0.92%) were similar and numerically higher than with the combination therapy (Table). The combination of rivaroxaban and aspirin reduced symptomatic VTE and all-cause mortality (ACM) (p=0.005) and ACM alone (p=0.01). MB was similar in the rivaroxaban versus rivaroxaban+aspirin groups. NMCR bleeding was increased with combination therapy (p=0.009).

Conclusions: Rivaroxaban plus aspirin use at baseline appears superior to placebo with no aspirin use at baseline in preventing key efficacy outcomes including ACM. Efficacy results were similar in the rivaroxaban alone or aspirin alone groups. MB was similar with combination therapy but NMCR bleeding was increased compared with no therapy.

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PB2394 | Reasons and Associated Outcomes in Delayed Anticoagulation in Venous Thromboembolism

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Background: Delayed initiation of anticoagulation for acute venous thromboembolism (VTE) is associated with increased risk of thrombus propagation, embolization and death. Identification and analysis of reasons for anticoagulation delay (AD) are important safety measures.

Aims: Describe barriers to initiation of anticoagulation and outcomes of patient with AD for over 24 hours.

Methods: Consecutive patients enrolled in the Mayo Clinic VTE Registry between March 2013 and November 2019 with acute VTE were followed prospectively. Patient outcomes were assessed in person, by mailed questionnaire, or scripted phone interview. Outcomes were reported at 90 days.

Results: Of the 2355 patients enrolled, 92 (3.9%) experienced AD of >24h. Among the AD group, the mean time to anticoagulation initiation was 7.6±10.4 days, and median of 4 days. Patients with AD more often had thrombosis at an unusual location (38% vs. 9.7% p< 0.001) and were more likely to have a provoked VTE (p=0.01) due to cancer or immobility (Table 1).

TABLE 1 Demographics and outcomes in patients with 3 months of follow up

Variable	No delay (n=2017)	Anticoagulation Delayed (n=86)	p value
Age, mean (±SD)	61.0 (14.4)	62.4 (13.0)	0.39
Female gender, n (%)	878 (43.6)	35 (40.7)	0.60
Lower extremity DVT and/or PE, n (%)	1676 (83.1)	47 (54.7)	**<0.001**
Upper extremity DVT, n (%)	209 (10.4)	13 (15.1)	0.16
Atypical location, n (%)	195 (9.7)	32 (37.2)	**<0.001**
Provoked, n (%)	1620 (80.4)	79 (91.9)	**0.03**
Recurrence at 90 days	34 (1.7)	5 (5.8)	**0.006**
Major bleeding at 90 days	54 (2.7)	5 (5.8)	0.08
CRNMB at 90 days	58 (2.9)	6 (7.0)	**0.03**

TABLE 2 Reasons for delay in initiation of anticoagulation in 92 patients

Reason for AD (n=92)	n (%)
Deferred decision to expert consult	36 (39.1)
Recent or upcoming procedure	14 (15.2)
Current or recent bleeding	11(12.0)
Thrombocytopenia	9 (9.8)
Missed diagnosis of VTE	6 (6.5)
Logistics (Insurance, availability, etc.)	5 (5.4)
Patient choice	4 (4.3)
No reason documented	7 (7.6)

Most common reasons for AD (Table 2) were waiting for expert opinion, proximity to surgery, recent bleeding, thrombocytopenia, and missed diagnosis. AD could have been avoided in 60% (access, workflow, misdiagnosis and education).

In patients with 3 months of follow up (n=86), patients with AD had higher frequency of VTE recurrence (5.8% vs. 1.7% p=0.006) and clinically relevant non-major bleeding (CRNMB) (7.0% vs. 2.9% p=0.03) compared to the patients with immediate onset of anticoagulation. There was no difference in mortality (9.3% vs 5.1% p=0.09) or major bleeding (5.8% vs. 2.7% p=0.08). After adjusting for age and cancer, differences remained significant for recurrence (p=0.02) and CRNMB (p=0.04) and not significant for mortality or major bleeding.

Conclusions: AD was associated with worse clinical outcomes. Although uncommon, most delays could have been avoided. Most AD happened in patients with thrombus in unusual locations, reflecting current confusion regarding management for VTE of these locations.

PB2395 | Effect of Antiplatelet Therapy on Clinical Outcome of Patients Treated with Anticoagulation for Acute Venous Thromboembolism

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Background: Some patients treated for acute venous thromboembolism (VTE) with anticoagulation (AC) are also prescribed antiplatelet therapy (APLT) medications and the impact on clinical outcomes in this group of patients is not well understood.

Aims: Assess clinical outcomes such as, major bleeding, clinically relevant non major bleeding (CRNMB), VTE recurrence and mortality in patients anticoagulated for acute VTE who are also on APLT.

Methods: Consecutive patients enrolled in the Mayo Clinic VTE Registry between March 1, 2013 and November 30, 2019 for acute

TABLE 1 Anticoagulant (AC) and antiplatelet therapy (APLT) used by group

Treatment	AC (n=2025)	AC & APLT (n=773)
Apixaban, n (%)	761 (37.6)	327 (42.3)
Rivaroxaban, n (%)	517 (25.5)	161 (20.8)
LMWH, n (%)	453 (22.4)	143 (18.5)
Warfarin, n (%)	242 (12.0)	121 (15.7)
Heparin, n (%)	34 (1.7)	16 (2.1)
Dabigatran/ Edoxaban, n (%)	11 (05) / 7 (0.3)	3 (0.4) / 2 (0.3)
Aspirin or NSAID, n (% of APLT)		720 (93.1)
P2Y12 inhibitor, n (% of APLT)		98 (12.7)
NSAID/aspirin and P2Y12 inhibitor, n (% of APLT)		45 (5.8)

TABLE 2 Rate of adverse outcomes by therapy type

Outcome (rate per 100 person-years)	AC (n=2025)	AC & APLT (n=773)	p-value
Major bleeding	5.11	5.02	0.92
CRNMB	6.71	7.31	0.67
VTE recurrence	4.47	4.22	0.87
Mortality	30.34	31.35	0.82

VTE were followed prospectively. Patient outcomes were assessed in person, by mailed questionnaire, or by a scripted phone interview.
Results: During the study period, 2798 patients were treated with anticoagulation for acute VTE of whom 773 (27.6%) individuals were also treated with APLT for other indications (see Table 1 for AC and APLT used). Patients treated with APLT were significantly older (66.2 vs 58.9 years, p< 0.001), had more DVT (79.2% vs 75.4%, p=0.031), and were less likely to have cancer (40.4% vs 46.6%, p=0.003) compared to the patients treated with AC only. APLT was associated with an identical rate (per 100 person-years) of major bleeding (5.11 vs 5.02, p=0.92) and similar rate of CRNMB (6.71 vs 7.31, p=0.67) compared to AC only. Also, VTE recurrence (4.47 vs 4.22, p=0.87) and mortality (30.34 vs 31.35, p=0.82) were similar in both study groups (Table 2).
Conclusions: APLT is commonly used in patients anticoagulated for acute VTE but this combined treatment with AC has no detrimental effect on safety and does not improve effectiveness of therapy.

PB2396 | Preliminary Investigation of Venous Thromboembolism Risk Assessment and Prophylaxis for Medical In-patients: A Multicenter Study in Nigeria

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Background: Venous thromboembolism (VTE) is a preventable cause of mortality among hospitalized patients in medical wards. Data is lacking on risk assessment and prophylaxis for medical in-patients in low resource setting.
Aims: This study aimed to assess the practice of VTE risk assessment and institution of prophylaxis for patients admitted into the medical wards of University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla, Enugu, Nigeria.
Methods: This was a retrospective study of 426 patients admitted into the medical ward of six different teaching hospitals in the country. each representing a geopolitical region in Nigeria. This was done over a four months period from July 1 to Oct 31, 2019. The case notes of these in-patients were reviewed and information on age, VTE risk assessment score, and anticoagulation use were obtained.
Results: The age of the participants ranged from 23 to 89 years (median, 58 years). Out of the 426 patients, 146 (34.2%) were on anticoagulation using either prophylaxis or therapeutic, and 74 (17.4%) had a record of VTE risk assessment score on their case files. Majority of those on anticoagulation, 130 (89%) were placed on LMWH (Clexane) only. No patient was commenced on compression stockings as a form of DVT prophylaxis.
Conclusions: There is a very poor practice of VTE risk assessment of hospitalized patients in UNTH, Nigeria. It is pertinent to identify patients at risk of developing DVT and/or pulmonary embolism, and prompt intervention instituted. This practice should be made a routine for all in-patients in related low resource settings.
Keywords: Deep Vein Thrombosis, anticoagulation, In-patients, VTE risk assessment

PB2398 | Direct Oral anti-Xa Inhibitors for Treatment of Venous Thromboembolism after Gastrectomy: Report on 3 Cases

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Background: Direct oral anticoagulants (DOACs) including the anti-Xa inhibitors apixaban, edoxaban and rivaroxaban are effective and safe for treatment of venous thromboembolism (VTE). They are absorbed in the upper gastrointestinal tract and in case of rivaroxaban must be taken with meals due to its lipophilicity. In patients after gastrectomy data on their absorption are scarce and anti-Xa inhibitors are generally not recommended. However, some clinical situations might prompt their use as they have advantages including lower bleeding rates and ease of application compared to vitamin-K-antagonists or heparin.
Aims: To evaluate the absorption rate of oral anti-Xa inhibitors in patients after gastrectomy and their clinical outcome.
Methods: We measured levels of anti-Xa inhibitors in plasma of three patients who required anticoagulation after total gastrectomy or Roux-en-Y bypass surgery. Chromogenic anti-Xa assays specifically calibrated for rivaroxaban and edoxaban (STA Liquid Anti-Xa, Diagnostica Stago) were used.
Results: Patients' characteristics, medical history and anti-Xa levels at different time points are shown in table 1. All patients had anti-Xa levels well above the detection limit 2 hours after intake. In patient 2 edoxaban trough levels after 24 hours were below the detection limit. The patient was switched to rivaroxaban, with trough levels

TABLE 1 Patients' characteristics, medical history and anti-Xa levels

	Baseline	2 h after intake	4 h after intake	24 h after intake
Pat 1 (female, 55 years, gastrectomy for gastric cancer, pulmonary embolism 5 days after surgery)				
Rivaroxaban (ng/mL)	< 20	351	143	168
Pat 2 (female, 37 years, gastrectomy for pancreatic cancer, incidental pulmonary embolism)				
Edoxaban (ng/mL)		83	67	< 20
Rivaroxaban (ng/mL)		61	39	22
Pat 3 (male, 34 years, Roux-en-Y bypass, recurrent deep vein thrombosis)				
Edoxaban (ng/mL)	< 20	129	109	

again close to the detection limit. Patient 3 had recurrent superficial venous thrombosis during vitamin-K-antagonists and edoxaban despite anti-Xa levels well within normally expected levels. He was switched to apixaban without recurrent phlebitis ever since. None of the patients had recurrent VTE during observation time of 13, 10 and 12 months.

Conclusions: In patients after gastrectomy, direct oral anti-Xa inhibitors are absorbed but levels may vary considerably. Systematic analyses with clinical endpoints are warranted to evaluate efficacy and safety in these patients.

PB2399 | Apixaban versus Heparin/Warfarin Treatment in Pulmonary Embolism: Results from the UK Apixaban Length-of-Stay Pulmonary Embolism - Hospital Admissions Study (ALPHA-PE)

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Background: Outpatient treatment of patients with low-risk pulmonary embolism (PE) is recommended by the British Thoracic Society

TABLE 1 LOS and total LOS in patients hospitalised with acute symptomatic PE, by study cohort

	Cohort 1 (January to June 2013), n=262*	Cohort 2 (January to June 2017), n=269	Difference in mean LOS
Heparin/warfarin, n (%)	214 (81.7)	14 (5.2)	-
Apixaban, n (%)	NA	194 (72.1)	-
Other anticoagulation, n (%)	48 (28.3)	61 (22.7)	-
LOS (days) - Overall, mean (SD)	7.4 (9.4)	4.6 (7.3)	- 2.8†
LOS (days) - Heparin/warfarin, mean (SD)	7.3 (8.1)	7.2 (6.0)	-0.1
LOS (days) - Apixaban, mean (SD)	NA	4.5 (7.8)	NA
Total LOS (days) - Heparin/warfarin, mean (SD)	7.5 (8.2)	7.2 (6.0)	-0.3
Total LOS (days) - Apixaban, mean (SD)	NA	5.0 (8.5)	NA

*Seven out of 269 patients were excluded from analyses: six patients who died during their hospital stay and one patient who received dabigatran etexilate. †P-value <0.005 LOS: Length of stay; NA: Not applicable; SD: Standard deviation Total LOS includes readmissions within 30 days of initial discharge.

TABLE 2 LOS in patients hospitalised with acute symptomatic PE, by sPESI risk stratification and anticoagulation type, Cohorts 1 and 2 combined

sPESI risk stratification	Warfarin (n = 228)	Apixaban (n = 194)	Difference in mean LOS
Low risk			
Total, n	91	96	-
LOS (days), mean (SD)	4.7 (3.8)	2.7 (4.3)	-.20*
High risk			
Total, n	122	91	-
LOS (days), mean (SD)	9.2 (9.6)	6.5 (10.1)	-2.7

*P-value <0.005 LOS: Length of stay; SD: standard deviation; sPESI: simplified Pulmonary Embolism Severity Index.

and European Society of Cardiology.[1,2] However, PE treatment often takes place in hospital.

Aims: The ALPHA-PE study compared length of stay (LOS) in patients treated with anticoagulation for acute PE in hospital before and after apixaban was introduced in the UK, and total LOS (including readmissions ≤30 days of initial discharge). LOS was stratified by risk according to the simplified Pulmonary Embolism Severity Index (sPESI).

Methods: A retrospective chart review at five UK hospitals included patients aged ≥18 years, admitted with acute symptomatic PE and initiated on anticoagulation pre- and post-licensing of apixaban for treating VTE in the UK. The study used Cohort 1 before apixaban licensing (C1), 01 January to 30 June 2013 and Cohort 2 after apixaban licensing (C2), 01 January to 30 June 2017. The study was powered to detect LOS difference of ≥1.0 day between C1 and C2.

Ethical and research approvals were obtained from the Office Research Ethics Committees Northern Ireland (Reference: 17/NI/0128) and the Health Regulatory Authority (Reference: 223148). As this was a retrospective review of patient medical records without direct patient contact, informed consent was not sought.

Results: The study included 538 patients; 269 (50.0%) in Cohort 1 (C1) and 269 (50%) in Cohort 2 (C2). Mean age was 66.9 years (C1) and 64.2 years, (C2) with 51.3% male (C1) and 44.6% male (C2). Demographic and clinical characteristics were well matched between C1 and C2. Patients hospitalised with PE and treated with apixaban had shorter mean LOS of 2.7 days compared with patients on warfarin, and shorter total LOS of 2.2 days (Tables 1 and 2).

Conclusions: Patients hospitalised with PE and treated with apixaban, compared with warfarin, had shorter LOS regardless of sPESI risk profile.

PB2400 | Demographics and Clinical Outcomes of Patients Younger than 40 Years Treated for Acute Venous Thromboembolism

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Background: Patients younger than 40 years with acute venous thromboembolism (VTE) are thought to have different demographic characteristics and profile of risk factors compared to patients 40 to 75 years of age, which could be associated with different clinical outcomes.

Aims: Assess demographic profile, risk factors, and clinical outcomes such as VTE recurrence, major bleeding, clinically relevant non major bleeding (CRNMB) and survival in young patients treated with any anticoagulation (AC) for acute VTE and those treated only with direct oral anticoagulants (DOACs).

Methods: Consecutive patients enrolled in the Mayo Clinic VTE Registry between March 1, 2013 and November 30, 2019 for acute

TABLE 1 Demographics and risk factors

Variable	Age <40 (n=257)	Age 40-75 (n=2123)	p-value	Age <40, DOACs (n=168)	Age 40-75, DOACs (n=1224)	p-value
Female, n (%)	150 (58.4)	915 (43.1)	<0.001	93 (55.4)	540 (44.1)	0.006
PE, n (%)	99 (38.5)	934 (44.0)	0.095	57 (33.9)	508 (41.5)	0.061
DVT and PE, n (%)	36 (14.0)	455 (21.4)	0.006	22 (13.1)	234 (19.1)	0.059
Provoked VTE, n (%)	207 (80.9)	1734 (81.8)	0.762	128 (76.6)	943 (77.1)	0.800
Active cancer, n (%)	49 (19.1)	1024 (48.2)	<0.001	24 (14.3)	474 (38.6)	<0.001
Trauma, n (%)	25(9.7)	118 (5.6)	0.008	22 (13.1)	81 (6.6)	0.003
Hormonal therapy/ pregnancy, n (%)	61 (23.7)	50 (2.4)	<0.001	35 (20.8)	38 (3.1)	<0.001
Other provoking factors, n (%)	72 (28.0)	439 (20.7)	0.007	39 (23.2)	256 (20.9)	0.494
Patients receiving DOACs, n (%)	197 (76.7)	1554 (73.2)	0.235	—	—	

TABLE 2 Clinical outcomes during the total treatment period (rates per 100 person-years)

Outcome	Age <40 years	Age 40-75 years	p-value	Age <40, DOACs	Age 40-75, DOACs	p-value
Mortality	12.02	30.37	<0.001	10.64	23.89	0.008
VTE recurrence	2.43	4.77	0.16	2.94	3.52	0.70
Major bleeding	3.04	4.92	0.24	2.94	3.05	0.90
CRNMB	4.28	7.00	0.16	4.97	8.32	0.21

VTE were followed prospectively. Patient outcomes were assessed in person, by mailed questionnaire, or by a scripted phone interview.
Results: During the study period, there were 257 patients with age < 40 years and 2123 aged 40-75 years. Patients younger than 40 years were more often women with VTE provoked by trauma, hormonal therapy or pregnancy, or by other provoking factors, but far less often with underlying malignancy compared to patients with the age of 40-75 years (Table 1). Patients < 40 years old had lower mortality but bleeding clinical outcomes were not different than the reference age group (Table 2). VTE recurrence was also similar (2.43 versus 4.77 per 100 person-years, p=0.16). When comparing study groups for those patients treated with DOACs (n= 1392), clinical outcomes were still not statistically different with the exception of mortality.
Conclusions: Patients younger than 40 with acute VTE have a different demographic profile and set of risk factors but similar clinical outcomes compared to the reference age range. This does not change when patients are treated with DOACs. As expected, the age group < 40 had better survival compared to the group of patients between 40 and 75 years of age.

PB2401 | Pre-Treatment with Heparinoids Does Not Impact Clinical Outcome of Apixaban and Rivaroxaban in Patients Treated for Venous Thromboembolism

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Background: Apixaban and rivaroxaban, the two most commonly prescribed direct oral anticoagulants (DOACs), were assessed in large clinical trials and approved for acute venous thromboembolism (VTE) therapy without pre-treatment with heparinoids (PrHep). Therefore, it remains unclear whether PrHep can change clinical outcomes related to these anticoagulants.
Aims: Evaluate the effects of PrHep on clinical outcome of patients treated with apixaban or rivaroxaban for VTE.
Methods: Consecutive patients enrolled in the Mayo Clinic VTE Registry between March 2013 and November 2019 with acute VTE were followed prospectively. Patient outcomes were assessed in person, by mailed questionnaire, or scripted phone interview.
Results: Of the 2798 patients enrolled, 1468 were treated with apixaban (n=931) or rivaroxaban (n=584) of which 655 (426 apixaban, 229 rivaroxaban) had PrHep (404 low molecular weight heparin and 251 unfractionated heparin). The mean duration of PrHep was 3.1±3.5 days (median 2.0, range 0.5-14 days). PrHep group had a higher percentage of pulmonary embolism (51.0% vs 30.4%, p< 0.001) and cancer (40.9% vs 31.6%, p=0.001) compared to the no pre-treatment (nPrHep) group. VTE recurrence (rate per 100 person-years) was not different in PrHep versus nPrHep therapies when analyzed in the total patient group (1.72 vs 2.48, p=0.45), apixaban group (1.80 vs 3.45, p=0.28), or rivaroxaban (1.64 vs 1.52, p=0.83) group (Table 1 and Figure 1). Death, major bleeding, and clinically relevant non-major bleeding (CRNMB) were not different in these three groups when PrHep to nPrHep managements were compared (Table 1).
Conclusions: PrHep does not change clinical outcomes of apixaban or rivaroxaban therapy. More patients with pulmonary embolism and with cancer had heparinoid pre-treatment reflecting frequent hospitalization for pulmonary embolus management and only recently changed recommendations for cancer-associated VTE therapy.

TABLE 1 Outcomes in PrHep versus nPrHep in the total patient group and separately for apixaban and rivaroxaban (rates per 100 person-years)

Outcome	Total group, PrHep vs nPrHep (p-value)	Apixaban, PrHep vs nPrHep (p-value)	Rivaroxaban, PrHep vs nPrHep (p-value)
VTE recurrence	1.72 vs 2.48 (0.45)	1.80 vs 3.45 (0.28)	1.64 vs 1.52 (0.83)
Death	26.11 vs 22.90 (0.34)	29.38 vs 25.18 (0.15)	22.15 vs 20.62 (0.76)
Major bleeding	2.97 vs 3.25 (0.82)	3.62 vs 3.41 (0.88)	2.19 vs 3.09 (0.60)
CRNMB	6.71 vs 8.21 (0.38)	5.42 vs 8.54 (0.23)	8.30 vs 7.87 (0.97)

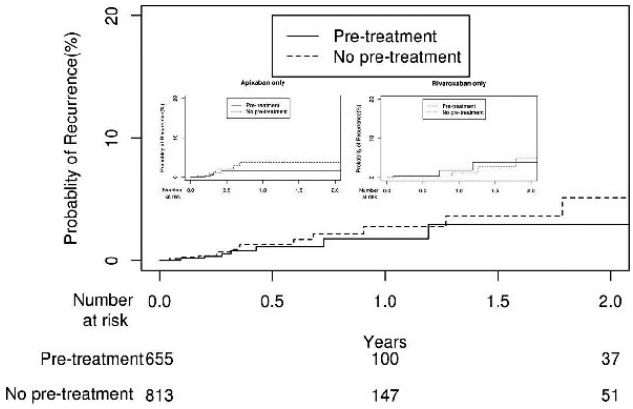

FIGURE 1 Kaplan-Meier curves: VTE recurrence in the total patient, apixaban, & rivaroxaban groups comparing pre-treatment versus no pre-treatment

PB2402 | Demographics and Clinical Outcomes of Older Adults Treated for Acute Venous Thromboembolism

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Background: Older adults (more than 75 years) with acute venous thromboembolism (VTE) are thought to have different demographic characteristics and profile of risk factors compared to the group of patients between the ages of 40 and 75, which could be associated with different clinical outcomes.

Aims: Assess demographic profile, risk factors, and clinical outcomes such as VTE recurrence, major bleeding, clinically relevant non major bleeding (CRNMB) and survival in older adults treated with anticoagulation for acute VTE.

Methods: Consecutive patients treated at the Mayo Clinic VTE Registry between March 1, 2013 and November 30, 2019 for acute VTE were followed prospectively. Patient outcomes were assessed in person, by mailed questionnaire, or by a scripted phone interview.

Results: During the study period, there were 242 older adults with age >75 years and 1224 with age 40-75 years. Older adults had less VTE provoked by hormonal therapy/pregnancy or by other provoking factors (e.g. reduced mobility from recent travel or prolonged hospitalization, acute infections or catheter insertion) compared to the patients with the age of 40-75 years (Table 1). Older adults had significantly worse survival and higher major bleeding. VTE recurrence and CRNMB rates did not differ by age group (Table 2).

Conclusions: Older adults with acute VTE have similar demographic profile but have significantly higher major bleeding rate compared to the group of patients between 40 and 75 years of age. Survival is lower as expected in the older age group.

TABLE 1 Type of PE-specific therapy used

Type of therapy	N, %
Anticoagulation alone	59, 54
Catheter-directed thrombolysis	20, 18.2
Aspiration thrombectomy	19, 17.3
Surgical embolectomy	1, 0.9
Full-dose tPA	5, 4.5
Half-dose tPA	5, 4.5
Quarter-dose tPA	2, 1.8
More than 1 intervention	12, 24

TABLE 2 Biomarkers and outcomes compared between type of therapies used

	Anticoagulation alone	Anticoagulation + PE specific treatment	p-value
N	59	51	
Age (mean, SD)	70.7, 17.1	57.5, 15.6	<0.01
Alive at discharge (%)	55 (93)	47 (92)	0.76
Death unrelated to PE (%)	6 (10)	1 (2)	" "
Death related to PE (%)	1 (1.7)	3 (6)	" "
Max heart rate (mean, SD)	106, 19.7	116, 20.1	0.01
Peak troponin (ng/mL; mean, SD)	0.66, 1.6	0.9, 2.2	0.51
BNP (pg/mL; mean, SD)	568, 703.6	656, 1070.1	0.62
D-dimer (ug/mL; mean, SD)	10.1, 7.5	12.3, 7.7	0.31

PB2404 | Management of Intermediate-High Risk Pulmonary Embolism Utilizing a PE Response Team

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Background: Intermediate-high risk pulmonary embolism (PE), with hemodynamic stability and evidence of right ventricular dysfunction, is common and treatment is controversial. Whether therapy more aggressive than anticoagulation alone improves outcomes remains uncertain. We report our experience with intermediate-high risk PE (ESC 2019 definition) utilizing our multidisciplinary PE Response Team).

Aims:

1. To present intermediate-high risk PE treated with different approaches and report outcomes.
2. To see whether specific clinical factors correlate with therapeutic decisions.

Methods: Retrospective case series of patients treated between 2017-2019, evaluating clinical and laboratory parameters at time of diagnosis. Chi-square testing was used for discrete variables and one-way ANOVA for continuous variables.

Results: A total of 472 patients were treated by the PE Response Team, of which 110 were intermediate-high risk. A comparison of patients treated with anticoagulation alone versus anticoagulation plus additional PE-specific therapy (specified in Table 1) may be found in Table 2. All-cause mortality was 10%. Progression to massive PE was seen in 2.7% of patients. There was no statistical difference in any outcome between patients treated with anticoagulation alone and those treated with a PE-specific therapy. Patients were more likely to be treated with anticoagulation alone if they were older ($p< 0.01$) or had a lower heart rate ($p=0.01$). While patients treated with PE-specific therapy did have higher biomarkers, these were not statistically significant between groups.

Conclusions: We found no statistical difference in survival to discharge or progression to massive PE between patients treated with anticoagulation alone and patients treated with anticoagulation plus a PE-specific therapy. Patients appeared to be more likely to receive aggressive therapy if they were younger or had higher heart rate.

PB2405 | Inferior Vena Cava Filter (IVCF) Utilization: Quality Assurance at a Quaternary Care Medical Center

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Background: Pulmonary embolism (PE) is a deadly complication of deep vein thrombosis. Inferior vena cava filter (IVCF) utilization, one method of preventing PE, has been increasing significantly over the years. Although different guidelines have been established to promote judicious use of IVCFs, the degree of adherence is unclear.

Aims: Analyze indications for IVCF placement at Cedars-Sinai Medical Center (CSMC). Examine adherence to American College of Chest Physicians (ACCP) and Society of Interventional Radiology (SIR) guidelines for IVCF placement at CSMC.

Methods: In a single-center retrospective study, indications were analyzed for seventy-one IVCF placements at CSMC between February and December of 2017. Two experts adjudicated selected cases. Adherence rates for ACCP and SIR guidelines were calculated. Fisher's exact and t-tests were performed to detect factors that might explain differences in adherence rates.

Results: Adherence rates were 76.1% and 67.6% respectively for SIR and ACCP guidelines. There was a significant difference in adherence to ACCP guidelines when race was a factor ($p=0.024$). The most common indication for filter placement was acute venous thromboembolism (VTE) with contraindication to anticoagulation (81.5% and 91.7% respectively for SIR and ACCP). The most common reason for filter placement without an accepted indication was acute VTE with perceived contraindication to anticoagulation (52.9% and 39.1% respectively for SIR and ACCP). 31/71 (43.7%) cases required expert adjudication. 25.8% (8/31) of adjudicated cases were deemed to have an accepted indication, 38.7% (12/31) a reasonable indication, and 35.5% (11/31) no specific indication.

Conclusions: Our study found that adherence to established guidelines ranged from 67.6-76.1%. The most common reason for non-adherence was perceived contraindication to anticoagulation. The effect of race on guideline adherence is unclear and more data is needed. Possible measures to improve adherence to guidelines include:

1). consultation with VTE experts,
2). clarity of language in guidelines, and
3). multidisciplinary approach to guideline formulation.

PB2406 | Management and Outcomes Following Subsegmental Pulmonary Embolism: Single Centre Experience at King's College Hospital

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Background: Pulmonary embolism (PE) remains a significant cause of morbidity and mortality. Increased awareness of PE and advances in imaging resolution have led to increased diagnosis of subsegmental PE (SSPE). The need for anticoagulation to treat isolated SSPE remains uncertain.

Aims: Our aim was to review local management and outcomes following SSPE.

Methods: From the electronic patient record (EPR), all PE imaging reports (April 2017 to October 2019) were reviewed to identify isolated or multiple SSPE. Patient demographics, comorbidities, management and outcomes were also obtained from the EPR.

Results: PE was diagnosed in 649 patients, with 51 (7.8%) SSPE. Patient characteristics are presented in Table 1. Most patients (66.7%, n=34) presented acutely via the emergency department. Filling defects were bilateral in 15, unilateral in 15, with single vessel SSPE in 21 patients. Lower leg imaging was performed in 21 (41%) patients with three DVTs identified. Four (7.8%) patients were not anticoagulated, and two patients declined treatment. Overall, 88% (45/51) patients were treated with anticoagulation (DOAC 26, LMWH 15 and warfarin 4) with three IVC filters placed due to an initial contraindication. Seven patients were lost to follow-up, with median overall follow-up of 260 (range 12-1001) days. Of those with

TABLE 1 Patient characteristics

Mean age (SD), years	60 (17)
Male, n (%)	28 (55)
Mean BMI (SD), kg/m^{2*}	26 (6)
Median Charlson Comorbidity Index (range)	4 (0-13)
Ethnicity, White, n (%)	28 (55)
Previous VTE, n (%)	12 (24)
Family history VTE, n (%)	3 (6)
Malignancy, n (%)	11 (22)
Unprovoked, n (%)	23 (45)

*Missing data, n=6; BMI, body mass index; SD, standard deviation; VTE, venous thromboembolism;

follow-up, 88% (38/43) stopped anticoagulation at median of 91 days (range 31 -798) with the remainder continuing long term. Nine (18%) patients died (no PE deaths), at a median of 78 days post diagnosis. Eight (14%) patients experienced bleeding events, with two (4.5%) major bleeds. There were 2 (3.9%) recurrent events diagnosed 4 and 24 months post withdrawal of anticoagulation.

Conclusions: Despite limited evidence most SSPE were anticoagulated for a finite period, which led to bleeding complications in one-sixth of the cohort. Mortality was high but not directly attributable to SSPE. Our findings suggest a cautious approach to anticoagulation in SSPE is justified.

PB2407 | Edoxaban for the Long-Term Therapy of Venous Thromboembolism in Real-Life RIETE Registry

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Background: The recommended dose of edoxaban for venous thromboembolism (VTE) treatment is 60 mg daily, or 30 mg daily in particular clinical conditions. No real-life data are currently available about its use in long-term treatment.

Aims: To assess the efficacy and safety of edoxaban for long-term VTE treatment in real-life setting.

Methods: Prospective data from VTE patients receiving edoxaban 60 mg or 30 mg daily included in 204 centers participating in the RIETE registry from October 2015 to November 2019, aimed to validate the benefit of using different dosages. Outcome of the study was the cumulative rate of the composite of VTE recurrences or major bleeding in patients with or without criteria for dose reduction. Multivariable analysis was performed to identify independent predictors for the composite outcome.

Results: 562 patients received edoxaban for long-term therapy. Most (94%) of the 416 patients not meeting criteria for dose reduction received edoxaban 60 mg daily, and 92 patients meeting criteria (63%) received 30 mg daily. During treatment, 2 patients developed recurrent VTE, 6 had major bleeding and 9 died (fatal bleeding 2). Among patients not meeting criteria for dose reduction, those receiving 30 mg daily had a higher rate of the composite event (hazard ratio [HR] 8.37; 95%CI 1.12-42.4) and a significant higher mortality rate (HR 31.1; 95%CI 4.63-262) than those receiving 60 mg. On multivariable analysis, only patients with cancer or chronic heart failure were at increased risk for the composite event. Among patients meeting criteria for dose reduction, those receiving 60 mg daily had no VTE recurrences, no major bleeding, and a non-significantly higher mortality rate (HR 5.04;95%CI 0.54-133) than those receiving 30 mg daily.

Conclusions: Edoxaban seems to be effective and safe for long-term VTE treatment in real-life. The criteria for dose reduction should be reformulated to include other parameters.

PB2408 | Management of Acute Venous Thromboembolism in Patients Taking Antiplatelet Therapy: A Bi-Center Analysis

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Background: Concomitant use of anticoagulant and antiplatelet therapy increases the bleeding risk, but most of the data derived from patients with atrial fibrillation. Data in patients with venous thromboembolism (VTE) are limited.

Aims: To report the management and evolution of patients diagnosed with acute VTE while receiving antiplatelet treatment.

Methods: We performed a post-hoc analysis of patients included in two prospective registries, sharing the same case report form, from 2007 to 2017. The primary outcome was the proportion of patients discharged with concomitant anticoagulant and antiplatelet therapy. Secondary outcomes were clinically relevant bleeding, cardiovascular events (including myocardial ischemia, ischemic stroke and lower

TABLE 1 Clinical outcome during the follow-up

	anticoagulant and antiplatelet therapy	Anticoagulant therapy	OR (IC 95%)
Patients, n	n=61	n=193	
Clinically Relevant Bleeding	9 (14.8%)	16 (8.3%)	2.43 (1.06-5.57)
time (mean months ± SD)	8.6 ± 13.3	7.0 ± 12.9	
VTE recurrence	0	3 (1.6%	
Cardiovascular Event	5 (8.2%)	13 (6.7%)	1.24 (0.42-3.63)
Death	8 (13.1%)	29 (15.0%)	0.85 (0.37-1.97)
time (mean months ± SD)	6.8 ± 5.5	7.4 ± 6.2	
Fatal bleeding	1 (1.6%)	3 (1.6%)	
Fatal ischemic cardiovascular event	0	2 (1.0%)	

limb ischemia), recurrent VTE and death, during the follow-up, according to the continuation of antiplatelet therapy.

Results: Among the 1,702 patients with acute VTE, 272 (15.89%) were receiving antiplatelet treatment at the diagnosis, of whom 62 (22.7%) were discharged with concomitant anticoagulant and antiplatelet therapy. These patients were younger (mean age: 73.2 ±11.8 vs 77.2 ± 14.5, p=0.003), had more often history of dyslipidemia (55.7% vs 37.3%, p=0.017), myocardial ischemia (55.7% vs 21.8%, p< 0.001) and peripheral arterial diseases (36.1% vs 13.5%, p< 0.001). Clinically relevant bleedings rate (after a mean follow-up of 18.4 ± 19.1 months of follow up) was increased in patients discharged with concomitant anticoagulant and antiplatelet therapy (HR=2.43; 95%CI [1.06-5.57]) as compared with patients with antiplatelet discontinuation. There were no difference in terms of cardiovascular events, VTE recurrence and death among groups (table 1). On multivariable analysis, being discharged with concomitant anticoagulant and antiplatelet therapy was associated with an increased risk of clinically relevant bleeding (HR=6.5; 95%CI [2.8-15.7]).

Conclusions: Among the 15% of patients who presented VTE under antiplatelet, one in five are discharged with concomitant anticoagulant and antiplatelet therapy. These patients had an increased risk of clinically relevant bleeding during follow-up.

PB2409 | Edoxaban Treatment of Venous Thromboembolism in Routine Clinical Practice in the Non-Interventional Global ETNA-VTE Program: A Sub-Analysis by Age Groups

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Background: Edoxaban is approved for treatment of deep vein thrombosis (DVT) and pulmonary embolism (PE), and prevention of recurrent DVT and PE. The non-interventional, global ETNA-VTE program, conducted in Asia and Europe, investigates safety and effectiveness of edoxaban in unselected VTE patients in routine clinical practice.

Aims: Baseline patient characteristics and clinical events at 1-year follow up are reported for overall study population and by age groups.

Methods: This study enrolled patients with acute DVT and/or PE for whom edoxaban was initiated as part of routine clinical care. All patients provided written informed consent, and the study was approved by responsible Ethic Committees and/or Institutional Review Boards. Data were collected at baseline and during 12-month follow-up.

Results: Among the 3,908 patients, mean age was 64.9 ± 15.50 years; 43.3% of patients were ≤65 years, 24% were ≥65 to < 75 years, and 32.7% were ≥75 years old. Mean body weight and creatinine clearance, and the proportion of male gender were lower with advanced age. Thus, the percentage of patients on 60 mg edoxaban once daily was lower with advanced age (Table 1). With increasing age, DVT and PE recurrences trended towards lower incidences whereas major bleeding events trended higher. Total mortality was higher with advanced age and was not driven by cardiovascular mortality (Table 2).

Conclusions: VTE recurrences occurred less frequently and bleeding occurred more frequently in older patients. In routine clinical practice, edoxaban treatment for VTE demonstrated effectiveness and safety across all age groups.

TABLE 1 Baseline characteristics

	Overall [N=3908]	Age < 65 years [N=1692]	Age in [65; 75) years [N=938]	Age ≥ 75 years [N=1278]
Age, years, mean ± SD	64.9 ± 15.50	50.1 ± 10.52	69.6 ± 2.86	81.0 ± 4.51
Male, n (%)	1900 (48.6%)	949 (56.1%)	444 (47.3%)	507 (39.7%)
Weight, kg, mean ± SD	73.6 ± 19.05	79.7 ± 19.72	72.9 ± 18.09	66.3 ± 15.92
eGFR (Cockcroft-Gault), ml/min, mean ± SD	88.2 ± 38.44	115.7 ± 36.46	80.8 ± 25.72	58.4 ± 19.03
Geographic region, n (%)				
Europe	2407 (61.6%)	1173 (69.3%)	541 (57.7%)	693 (54.2%)
Japan	1254 (32.1%)	428 (25.3%)	338 (36.0%)	488 (38.2%)
South Korea /Taiwan	247 (6.3%)	91 (5.4%)	59 (6.3%)	97 (7.6%)
Type of index VTE event, n (%)				
DVT	2227 (57.0%)	1000 (59.1%)	500 (53.3%)	727 (56.9%)
PE with DVT	937 (24.0%)	400 (23.6%)	226 (24.1%)	311 (24.3%)
PE w/o DVT	744 (19.0%)	292 (17.3%)	212 (22.6%)	240 (18.8%)
Medical history, n (%)				
Hypertension	1713 (43.8%)	404 (23.9%)	489 (52.1%)	820 (64.2%)
Diabetes mellitus	506 (12.9%)	134 (7.9%)	154 (16.4%)	218 (17.1%)
Dys-/hyperlipidemia	849 (21.7%)	233 (13.8%)	231 (24.6%)	385 (30.1%)
COPD	218 (5.6%)	45 (2.7%)	59 (6.3%)	114 (8.9%)
Major surgery or trauma	559 (14.3%)	266 (15.7%)	129 (13.8%)	164 (12.8%)
Bleeding history	175 (4.5%)	59 (3.5%)	49 (5.2%)	67 (5.2%)
Major or CRNM bleeding	124 (3.2%)	42 (2.5%)	34 (3.6%)	48 (3.8%)
Major bleeding	80 (2.0%)	25 (1.5%)	24 (2.6%)	31 (2.4%)
Major GI bleeding	12 (0.3%)	4 (0.2%)	5 (0.5%)	3 (0.2%)
Ischemic stroke	156 (4.0%)	25 (1.5%)	39 (4.2%)	92 (7.2%)
Edoxaban 60 mg at baseline, n (%)	2716 (69.5%)	1433 (84.7%)	622 (66.3%)	661 (51.7%)

TABLE 2 Clinical events by age during 12-month follow-up

Events during 12-months follow-up, n (%/year)	Overall [N=3908]	Age < 65 years [N=1692]	Age in [65; 75) years [N=938]	Age ≥ 75 years [N=1278]
VTE recurrence	111 (3.45)	62 (4.23)	26 (3.37)	23 (2.35)
DVT only	77 (2.38)	43 (2.92)	19 (2.45)	15 (1.53)
PE with or w/o DVT	44 (1.36)	26 (1.75)	9 (1.16)	9 (0.92)
PE w/o DVT	37 (1.14)	19 (1.28)	9 (1.16)	9 (0.92)
PE with DVT	7 (0.21)	7 (0.47)	0 (0.00)	0 (0.00)
All–cause mortality	198 (6.07)	54 (3.61)	49 (6.28)	95 (9.63)
CV mortality	37 (1.13)	7 (0.47)	10 (1.28)	20 (2.03)
Any bleeding	459 (15.42)	196 (14.40)	107 (14.91)	156 (17.49)
Major bleeding [ISTH]	83 (2.57)	22 (1.48)	25 (3.24)	36 (3.70)
ICH	20 (0.61)	4 (0.27)	4 (0.51)	12 (1.22)
Major GI bleeding	23 (0.71)	2 (0.13)	10 (1.29)	11 (1.12)
CRNM bleeding	123 (3.86)	54 (3.70)	23 (2.99)	46 (4.80)

Abbreviations: CRNM, clinically relevant nonmajor; CV, cardiovascular; DVT, deep vein thrombosis; GI, gastrointestinal; ICH, intracranial haemorrhage, ISTH, International Society on Thrombosis and Haemostasis; PE, pulmonary embolism; VTE, venous thromboembolism.

PB2410 | The Use of Fondaparinux for Recurrent Thrombotic Events

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Background: There is limited evidence to guide the management of recurrent thrombosis occurring despite therapeutic anticoagulation. **Aims**: To review fondaparinux use in a single-centre, for recurrent thrombotic events despite therapeutic warfarin and/or low-molecular-weight-heparin (LMWH).

TABLE 1 Thrombotic histories and anticoagulation treatment

Patient details:	CrCl (ml/min	Weight (kg)	Underlying thrombotic diagnosis	Initial thrombosis (0 months)	1st re-thrombosis and treatment	2nd re-thrombosis and treatment	3rd re-thrombosis and treatment	4th re-thrombosis and treatment	5th re-thrombosis and treatment
Patient 1: 28-year-old female	112	87	Thrombotic APS: right popliteal DVT aged 28y, week 20 of first pregnancy (LMWH until 6w postpartum) Obstetric APS aged 30y: Pre-eclampsia 28 weeks' gestation, Triple positive	Left popliteal DVT, aged 40y Warfarin target 2.5, (range 2.0-3.0) APS diagnosed	4 months Extension of DVT to left common femoral vein while INR 2.5 Warfarin target INR increased to 3.5 (range 3.0-4.0)	12 months DVT extension to external iliac vein and common femoral vein while INR 3.4 ~20% increase on standard treatment dose LMWH	24 months Left sigmoid sinus thrombosis Acute frontal infarct following MRA ~30% increase on standard treatment dose LMWH	36 months Segmental PE Course of rituximab Switched to fondaparinux 7.5mg od	
Patient 2: 42-year-old male	127	124	Thrombotic APS: arterial and venous thrombosis Triple positive	Left MCA infarct: initial standard treatment with antiplatelet agents	1 month Right MCA infarct Triple positive aPL identified Warfarin target INR 3.5 (range 3.0-4.0) initiated	21 months Right popliteal vein DVT (INR 2.6 at time of event) Continued warfarin (target INR 3.5)	23 months Proximal extension of DVT to superficial femoral vein Standard treatment dose LMWH	23.5 months Segmental PEs ~20% increase on standard treatment dose LMWH	26 months Proximal extension of DVT to common femoral vein Switched to Fondaparinux 5mg bd Course of rituximab
Patient 3: 31-year-old female	101	94	Thrombotic APS: arterial and venous thrombosis Triple positive	Cerebral venous sinus thrombosis Warfarin target INR 2.5 (range 2.0-3.0)	5 months Cerebral venous sinus thrombosis (erratic INRs) Standard treatment dose LMWH	14 months Multiple anterior and posterior arterial circulation infarct ~20% increase on standard treatment dose LMWH	16 months Segmental PE Issues with patient adherence to LMWH IVC filter inserted Course of rituximab	24 months Bilateral iliofemoral and IVC thrombus Catheter directed thrombolysis Fondaparinux 5mg bd and clopidogrel started	
Patient 4: 22-year-old female	129	76	Venous Long term central venous access due to nutritional needs and electrolyte replacement No thrombophilia identified	PICC-associated DVT Standard treatment dose LMWH	2 months PICC-associated DVT ~20% increase in LMWH	6 months Venous: PICC-associated left brachial vein DVT ~30% increase on standard treatment dose LMWH	10 months PICC-associated right proximal cephalic vein and right atrial thrombus Switched to fondaparinux 2.5mg am, 5mg pm	40 months Right axillary and brachial vein PICC-associated DVT Fondaparinux dose increased to 5mg bd	41 months Right IJV subacute non-occlusive thrombus Fondaparinux 5mg bd continued and clopidogrel added

aPL: antiphospholipid antibodies; APS: antiphospholipid syndrome; bd: twice daily; CrCl: Cockcroft & Gault creatinine clearance; DVT: deep venous thrombosis; IJV: internal jugular vein; INR: international normalised ratio; IVC: inferior vena cava; LMWH: low-molecular-weight-heparin; m: month; MCA: middle cerebral artery; MRA: magnetic resonance angiography; od: once daily; PE: pulmonary embolism; PICC: peripherally inserted central catheter; w: weeks; y: years; Triple positive: presence of lupus anticoagulant, IgG/IgM anticardiolipin and anti-β2 glycoprotein I antibodies.

Methods: Patients receiving fondaparinux for recurrent thrombosis were identified from the complex anticoagulation clinic lists. A retrospective review of the notes was performed to identify the underlying thrombotic disorder, thrombotic events and their management.

Results: Thrombotic histories and anticoagulation treatment are detailed in Table 1. Four patients (three females, one male), median age 32 (range 22-42) years were on long-term fondaparinux. Three (patients 1-3) had triple positive thrombotic antiphospholipid syndrome (APS); patient 1 also had obstetric APS. Two APS patients experienced arterial and venous thromboembolic events (VTE), the other two patients had VTE alone. Initial management of recurrent thrombosis included assessment of anticoagulation intensity at the time of the event and patient adherence. In all four cases, LMWH was used up to approximately 30% above standard treatment dose for further thrombotic events, prior to switching to fondaparinux. Patient 1 received split-dose high-intensity dalteparin, approximately 20% above standard treatment dose, as the international normalised ratio (INR) was 3.6 at the time of re-thrombosis. Patients 3 and 4 received clopidogrel together with fondaparinux as additional antithrombotic treatment. Catheter-directed thrombolysis was undertaken in the acute situation in one patient. All three APS patients received a course of rituximab as empirical treatment for anticoagulant-refractory thrombosis. There were no subsequent thrombotic events on fondaparinux, plus clopidogrel in patients 3 and 4, over a median follow-up of 44 (range 19-54) months. There were no major bleeding events.

Conclusions: In this case series, fondaparinux provided an effective and safe option in patients, three APS and one non-APS, with recurrent arterial or venous thrombosis, despite the use of therapeutic warfarin and/or LMWH.

PB2411 | Role of Residual Pulmonary Vascular Obstruction on the Risk of Long-Term Recurrence of Venous Thromboembolism After Pulmonary Embolism: A Cohort Study

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Background: Residual vascular pulmonary obstruction (RVPO) after pulmonary embolism (PE) has been associated with recurrent venous thromboembolism (VTE) after 2-3 year follow-up. However, there is no standardized definition of RPVO and its predictive role on recurrent VTE requires confirmation.

Aims: To determine whether RPVO is associated with recurrent VTE after 10 years.

Methods: Consecutive patients were evaluated 6 to 12 months after objectively diagnosed PE at the Thrombosis Center of ASST Sette Laghi (Varese, Italy) and underwent lung perfusion scan between 2004 and 2008 to detect RPVO, as part of a previously published study. In 2019 patient electronic clinical records were searched or, if missing, patients were contacted by telephone, to detected objectively diagnosed recurrent VTE.

Results: 112 patients were included in the study. At the time of PE diagnosis, the mean age was 62.5 years (DS 16.3) and the proportion of females/males were 58/42%. PE was unprovoked in 62 patients (55.4%) and DVT was diagnosed in 72 patients (64.3%). As compared to diagnostic imaging, follow-up lung perfusion scan, performed after 12.9 months (DS 10.4), identified no change in 21 patients (19%), partial reperfusion in 25 (22%) and complete reperfusion in 66 (59%). Older age and unprovoked PE were more frequent in patients with RPVO as compared to those without. During a mean follow-up of 117.1 months (DS 59.6), 31 patients (27.7%) were diagnosed with recurrent VTE (23 PE, 8 isolated deep vein thrombosis). Recurrent VTE occurred in 26.1% of patients with RPVO as compared to 28.8% without RPVO (p=ns).

Conclusions: In this study, during 10-year follow-up, RPVO was not associated with recurrent VTE. Larger studies are needed to confirm the predictive role of RPVO, by using a standardized definitio.

PB2412 | Effectiveness and Safety of Apixaban for Treatment of Venous Thromboembolism in a Large Daily Practice Cohort

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Background: Phase 3 trials have shown comparable efficacy of direct oral anticoagulants (DOACs) and vitamin K antagonists in patients with acute venous thromboembolism (VTE), with less major bleeding events in patients randomized to DOAC treatment. With DOACs being increasingly used in clinical practice, evaluation of the DOACs in daily practice-based conditions is needed to confirm their safety and effectiveness.

Aims: To evaluate the effectiveness and safety of apixaban in VTE patients in daily practice.

Methods: In this retrospective cohort study, consecutive patients diagnosed with venous thromboembolism in two Dutch hospitals (Leiden University Medical Center, Leiden and Haga Teaching Hospital, The Hague) were identified based on administrative codes. We assessed recurrent VTE, major bleeding and mortality during a 3-month follow-up period in those treated with apixaban.

Results: Of 671 consecutive VTE patients treated with apixaban from January 2016 until July 2018, 371 presented with acute pulmonary embolism (PE) and 300 patients with deep-vein thrombosis (DVT). The baseline demographic are summarized in Table 1. Their mean age was 59.7 years (SD 16.4), 48% was female and 6% had active malignancy at time of diagnosis. Two patients were diagnosed with recurrent VTE (0.3%; 95%CI 0.08-1.1, Table 2) and 12 with

TABLE 1 Baseline characteristics of patients with VTE treated with apixaban

Demographics	N=671	Percentage/SD
Age, mean (SD)	59.7	16.4
Male sex, no (%)	347	51.7
Weight in kg, mean (SD)	84.7	18.6
VTE risk factors		
Previous venous thromboembolism — no. (%)	145	22
COPD — no. (%)	65	9.7
Estrogen use — no. (%)	67	10
Immobilisation — no. (%)	174	26
Active malignancy no. — no. (%)	42	6.3

TABLE 2 VTE-related adverse events of patients treated with apixaban

	frequency	Percentage	95% CI
1. Overall mortality	11	1.6	0.9 - 2.9
2. Major bleeding	12	1.8	1.0 - 3.2
3. Recurrent VTE	2	0.3	0.08 - 1.1

major bleeding (1.8%; 95%CI 1.0-3.2); 11 patients died (1.6%; 95%CI 0.9 - 2.9), of which one patient because of fatal PE and one because of a possible intracranial bleed. Other side effects of apixaban were mostly transient and included headache (2.5%), nausea (0.9%), hypersensitivity/rash (0.4%) and abdominal discomfort (2.4%), causing switch to an alternative anticoagulant in 13% of all patients with side effects.

Conclusions: In this large practice based cohort with patient level data, treatment of apixaban yielded a low incidence of recurrent VTE, comparable to the phase 3 Amplify study patients, but a higher incidence of major bleeding, reflecting the importance of daily practice evaluation.

PB2413 | Home Treatment Compared to Initial Hospitalization in Patients with Acute Pulmonary Embolism in the Netherlands: A Cost Analysis

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Background: Venous thromboembolism (VTE) constitutes a substantial economic burden amounting to about 60 million euros per year in the Netherlands. Compared to initial hospitalization, home treatment of pulmonary embolism (PE) is associated with lower healthcare costs. An accurate estimation of cost savings per patient treated at home is currently lacking.

Aims: The primary aim of this study was to compare resource utilization and medical costs during the first 3 months after a PE diagnosis in patients who are treated at home versus those who are initially hospitalized.

Methods: Average 3-months hospital costs were estimated, for patients initially treated at home or in hospital. Patient-level data of the YEARS study (van der Hulle T et al. Lancet 2017) included length of hospital stay, rates of PE-related readmissions and complications. Diagnostic and treatment costs were derived in accordance with the Dutch guidelines for economic evaluations in health care. Multivariate regression analysis was used to adjust for relevant patient characteristics.

Results: Mean hospitalization duration was 0.7 days for those treated initially at home (n=184) and 4.5 days for those initially treated in hospital (n=220); mean readmission rates were 9.8% and 8.7% respectively. Total average costs per hospitalized patient were €3.282,- and total average costs for those treated at home were €1.497,-. The adjusted mean difference was €1.576,- (95%CI €1220 - €1930).

Conclusions: Home treatment of hemodynamically stable patients with acute PE was associated with a cost reduction of €1.576. This

TABLE 1 Three-months hospital costs for initial home treatment or hospitalization

	Specification	Unit price	Home treatment	Hospitalization
Initial ER visit		€269,-	100%	100%
Laboratory costs		€35,-	100%	100%
Radiological costs	X-Ray	€43,-	88%	88%
	CTPA	€183,-	100%	100%
Room and board		€495,-	0.68 days	4.54 days
Pharmacy costs		€217,-	100%	100%
PE-related readmission	Admission	€2.475,-	4.9%	4.1%
	Emergency room	€269,	4.9%	3.6%
Outpatient clinic		€95,-	3 times	2 times

TABLE 2 Estimation of costs for Daily hospitalization costs and follow-up costs for a 3-month period

		Home treatment	Hospitalization	Difference	Adjusted difference	(95%CI)
Initial ER visit		€269,-	€269,-	-	-	-
Laboratory costs		€35,-	€35,-	-	-	-
Total radiological costs	Total	€221,-	€220,-	€0.58	€2.30	(-0.8 - 5.4)
Room and board		€337,-	€2.247,-	€-1.907,-	€-1.697,-	(-2.041 to -1.353)
Pharmacy costs		€217,-	€217,-	-	€-0.03	(-9.1 - 9.0)
PE-related readmission	Total	variable	variable	€27,-	€26,-	(-90 - 141)
Outpatient clinic		€285	€190,-	€95,-	-	-
Total costs		**€1.497**	**€3.282**	**€-1.785**	**€-1.576,-**	**(-1.930 to -1.222)**

Adjusted for: age, gender, COPD, heart failure, kidney injury, malignancy, tachycardia, need for O2 therapy.

difference underlines the advantage of triage-based home treatment of these patients.

PB2414 | Direct Oral Anticoagulants and Vitamin K Antagonists for Treatment of Cerebral Vein Thrombosis

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Background: Cerebral vein thrombosis (CVT) typically affects young adults and requires anticoagulation for a period that varies from 3 months to lifelong. Direct oral anticoagulants (DOAC), because of their safety profile and the lack of laboratory monitoring, may represent a valid alternative to vitamin K antagonists (VKA). To date, one phase III trial evaluated dabigatran compared to VKA, while the use of the anti-Xa have been described in case reports and series only.

Aims: To evaluate the efficacy and safety of DOAC in patients with CVST compared to VKA.

Methods: All consecutive patients with CVT from September 2013 to January 2019 were included in the study. Recurrent or progressive thrombosis and bleeding complications were the study outcomes, expressed in terms of cumulative incidence rates.

Results: Eighty-two patients with CVT were included, 17 treated with DOAC (all anti-Xa) and 65 with VKA. In both groups the median age at diagnosis was 40 years and male to female ratio 1:3. One patient treated with apixaban had a CVT progression and one on VKA had a superficial thrombophlebitis. Bleeding occurred in 5 patients (17%) treated with DOAC (all on apixaban or rivaroxaban) (1 severe and 3 mild menorrhagia and 1 subdural hematoma) and in 3 (5%) with VKA (1 severe and 2 mild menorrhagia). A time-to-event analysis was performed showing a cumulative incidence rate of bleeding and thrombotic episodes from start of anticoagulation until 12 months of treatment of 2% and 2% respectively in the VKA group and 31% and 9% respectively in the DOAC group.

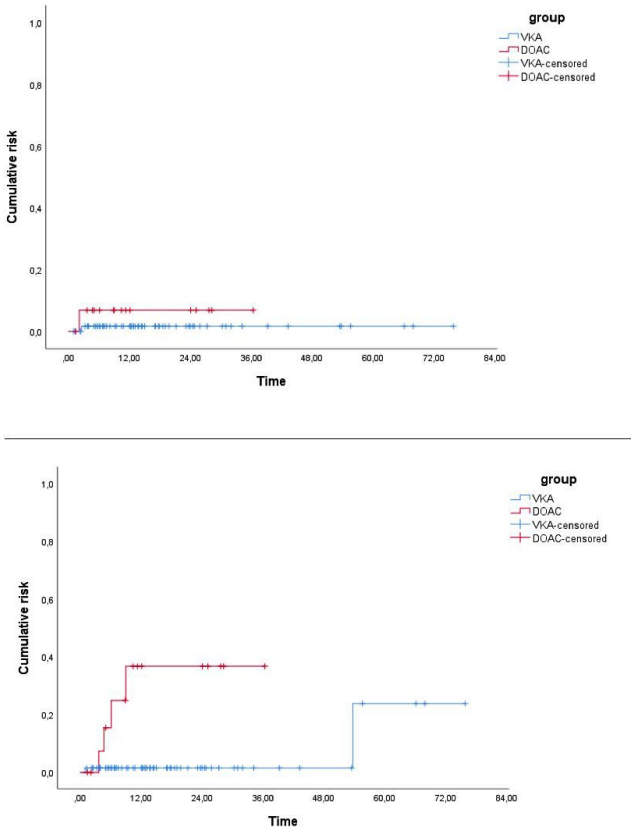

FIGURE 1 Kaplan-Meier estimates for thrombotic events (upper panel) and bleeding events (lower panel) in CVT patients treated with DOAC or VKA

Conclusions: Considering the limitation of the sample size, our results raise some concerns on efficacy and safety of anticoagulant treatment with anti-Xa DOAC in patients with CVST. Further data from randomized controlled trial are needed.

PB2415 | Risk of Bleeding of Concomitant Antiplatelet and Anticoagulation Therapy in Patient Treated for a Venous Thormboembolic Event

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Background: As antiplatelet therapy becomes widespread, physicians face the issue patients being treated with antiplatelet agents (APA) and anticoagulants for venous thromboembolism (VTE). It is well known that the association of aspirin with anticoagulant therapy significantly increase the risk of bleeding in patients under anticoagulants for VTE, but data are sparse.
Aims: To assess the risk of bleeding of patients treated with anticoagulant for VTE disease receiving APA and in patients not receiving APA.
Methods: We performed a research on Pubmed database, including clinical trials and observational studies, with following words: pulmonary embolism, deep vein thrombosis, VTE, anticoagulant. Between 1947 to December 2019; we selected 6 randomized clinicals trials (EINSTEIN DVT and PE, HOKUSAI, RECOVER I and II, AMPLIFY); and 19 observational studies.
The study outcomes were the occurrence of Major Bleeding (MB) and Clinical-Relevant-Non-Major-Bleeding (CRNMB). In each study, use of antiplatelet therapy was collected.
Results: *Observational studies*: Across all the nineteen observational eligible studies, 317419 patients were treated for a VTE disease, with 48231 (15%) treated with APA associated with a curative anticoagulation. In two observational studies, the risk of bleeding was reported : the use of APA significantly increased risks of bleeding (OR : 1.4 [95% CI : 1.0 - 1.9] and OR : 3.55 [95% CI : 2.73 - 4.61]).
RCT: Of the 27023 patients included, 3473 patients (12.8%) were treated with APA when anticoagulation is started for a VTE event. Use of APA significantly increased risk of MB and CRNMB (respectively OR: 2.25 [95% CI: 1.76 - 2.87]; and OR: 2.11 [95%CI: 1.16 - 3.83]).
Conclusions: Our study confirms that use of an APA with anticoagulant in case of VTE is a common occurrence. Our study provides an accurate estimate of the increase risk of both MB and CRNMB in both clinical trials and observational studies.

PB2416 | Long-Term Management of Anticoagulants in Patients with Cancer-Associated Thrombosis (CAT) Beyond 6 Months: A 432-Patient Retrospective Non-Interventional Study

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Background: Few data are available about anticoagulation management beyond 6 months in CAT patients.
Aims: Our objective was to describe anticoagulant modalities beyond 6 months and up to 12 months in CAT patients initially treated with low molecular weight heparin (LMWH).
Methods: CAT patients previously included in two prospective cohort studies (aXa-NCT02898051; PREDICARE - NCT03099031) and still alive at the end of the initial 6-month treatment period were eligible to participate in this retrospective non-interventional French multicenter study.
Main study outcome was to describe anticoagulant treatment in the management of CAT patients in usual medical care from 7th month to 12th month. Clinical outcomes including venous thromboembolism recurrence, bleeding and deaths were presented previously.
Results: Among the 432 patients (aged 66.5±12.7 years) alive after 6 months of anticoagulant therapy for CAT and included in the study, 332 were followed up to 12 months while 96 patients deceased before study end and 4 patients were lost-to follow-up. Anticoagulant modalities were missing for 10 patients.
At 6 months, anticoagulant therapy was stopped in 74 patients, 56 were switched to vitamin K antagonists (VKA) (16.1% [95%CI, 12.4%-20.4]), 30 to direct oral anticoagulants (DOAC) (8.6% [95%CI, 5.9%-12.1]) and 6 to others. LMWHs were maintained in 256 patients (73.6% [95%CI, 68.6-78.1]). Among them, beyond 6 months,

TABLE 1 Management of the anticoagulant treatment beyond 6 months (IQR: interquartile range; mo: months)

Treatment at inclusion (at 6 months from the index event)	LMWH (n = 256)	VKA (n = 56)	DOAC (n = 30)
Median duration beyond 6 months (IQR)	4.6 mo. (2.0-6.0)	6 mo. (5.2-6.0)	6 mo. (5.4-6.0)
Treatment discontinuation from 7 to 12 months [n (%)]	86 (33.7)	12 (21.8)	8 (27.6)
Treatment switch from 7 to 12 months to [n (%)]			
LMWH	7 (2.7)	7 (12.7)	0
VKA	20 (7.8)	-	0
DOAC	13 (5.1)	0	-
UFH	2 (0.8)	1 (1.8)	0

LMWHs were definitively discontinued in 86 patients (33.7%), the main reason being a favorable course of the cancer (16 patients, 18.6%), whereas concern about bleeding risk is low (2 patients, 2.3%) patients. Anticoagulation switches from 7 to 12 months are reported in the table.

Conclusions: Anticoagulation with LMWH was maintained beyond 6 months and up to 12 months in the vast majority of patients with CAT. They were in accordance with clinical practice guidelines suggesting that treatment should be maintained as long cancer is active or in the absence of bleeding risk.

PB2417 | Sodium Valproate-induced Drug Interactions with DOAC Anticoagulant Activity: A Case Report

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Background: Antiepileptic drugs (AEDs), as sodium valproate, inducing cytochrome P450 isoenzymes, especially CYP3A4/5, might increase the metabolism of anti-Xa DOACs (rivaroxaban, apixaban, edoxaban) and reduce their anticoagulant effect. Valproate induces also P-gp efflux pump activity reducing the intestinal absorption of DOACs. These interactions may lead to decreased antithrombotic efficacy.

Aims: To measure plasma levels of rivaroxaban and dabigatran on valproat treatment.

Methods: We are reporting a case of a 30-years old man, who had a history of symptomatic post-traumatic epilepsy, manifested by simple partial motor and complex seizures, some of which are generalized secondary. He received a chronic valproate treatment at a stable dose (1000 mg) for three years. During active sports he developed deep ileofemoral vein thrombosis. Thrombophilic screening revealed a *Prothrombin 20210 G/A heterozygous mutation*. Treatment with 2 x 15 mg rivaroxaban was started for 21 days, followed by 20 mg OD without significant clinical improvement after one month.

Results: Anti-Xa activity was measured twice at peak level (3rd hour) after administration of 20 mg rivaroxaban with values below 10 ng/ml in two consecutive days. Ultrasonography showed no withdrawal of venous thrombosis. Rivaroxaban was replaced with dabigatran 2 x 150 mg/d for 4 days. Again low anticoagulant activity was measured - trought level 40 ng/ml vs median value of 85 ng/ml (52 - 182 ng/ml). We used DTI (Hemoclot, Hyphen) assay for dabigatran and DiXaI (Hyphen) assay for rivaroxaban on Sysmex 2000i. We decided to switch the patient to vitamin K antagonist acenocoumarol and received a good anticoagulant response at a dose of 28 mg/weekly (INR 2.8). There was an improve of the clinical symptoms of edema and swelling in the next 4 weeks.

Conclusions: The pharmacokinetic interaction between valproate and DOACs significantly reduces plasma levels and anticoagulant activity of DOACs (rivaroxaban and dabigatran) and adversely affects antithrombotic efficacy.

PB2419 | Hypermenorrhea and Menometrorrhagia during Rivaroxaban Therapy

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Background: Anticoagulant therapy of DVT involves a risk of hemorrhagic complications. However, gender-related hemorrhages such as hypermenorrhea and menometrorrhagia is rarely taken into account.

Aims: Objective is assess the incidence of uterine bleeding during Rivaroxaban therapy.

Methods: In 3 years, we observe 72 women with DVT. All women are of reproductive age and have regular menstruation. The duration of thrombosis was from 3 to 36 months. All patients received Rivaroxaban 20mg/day.

The 19 women had the gynecological disorders such as endometriosis, uterus myoma and endometrial polyps. The 6 women had

hypermenorrhea. All patients denied the presence of active menometrorrhagia until the development of venous thrombosis.

During Rivaroxaban therapy 32 (44.4%) patients indicated the development or progression of hypermenorrhea; and menometrorrhagia was in 7 of them (gynecological disorders in 34%).

Results: Rivaroxaban dose was reduced to 10mg/day in 3 women with a positive effect. In 15 women, Rivaroxaban dose was reduced to 10 mg/day only for menstruation, and 9 patients noted the decrease blood loss by flexible mode of anticoagulation.

Apixaban 5mg/day in 2 patients, Apixaban 10mg/day in 2 patients and Enoxaparin 40mg/day once daily in 1 case replaced Rivaroxaban. The reduced dose of alternative anticoagulant had a positive effect in all women. However, the stop of hypermenorrhea was only in 1 case of Apixaban 10mg/day. There was no recurrence of DVT in these patients during the observation period.

The anticoagulation was completed prematurely in 9 patients. After stop of Rivaroxaban therapy, thrombosis developed again in 1 woman with history of recurrent DVT.

Conclusions: The EINSTEIN DVT/PE subanalysis showed the development of uterine bleeding in 30.7% of women during Rivaroxaban therapy. The first results of "real practice" show a higher incidence of gender-associated hemorrhages (44.4%). In our opinion, it is necessary to continue accumulating data, possibly by an appropriate register.

PB2420 | Rivaroxaban versus Warfarin for Treatment and Prevention of Recurrent Venous Thromboembolism in Obese Patients

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Background: Rivaroxaban has demonstrated consistent drug levels and anticoagulation activity in obese patients versus those of normal weight. Limited data evaluating the effectiveness and safety of rivaroxaban versus warfarin in obese patients with venous thromboembolism (VTE) exist.

Aims: To evaluate the effectiveness and safety of rivaroxaban versus warfarin for treatment and prevention of recurrent VTE in obese patients.

Methods: We performed a cohort analysis using Optum® De-Identified Electronic Health Record (EHR) data from 11/1/2012-9/30/2018. We included patients with a body mass index (BMI)≥30 kg/m^2 admitted to the hospital, emergency department or observation unit for VTE, who received rivaroxaban or warfarin as their first oral anticoagulant (OAC) within 7-days after and had ≥12-months of EHR activity prior to the acute event. We excluded patients with evidence of OAC use at baseline. Patients receiving rivaroxaban were 1:1 propensity-score matched to patients receiving warfarin (standard differences < 0.10 achieved for all covariates). Outcomes included recurrent VTE and major bleeding at 3-6- and 12-months using an intent-to-treat approach. Subanalyses stratified by BMI (30.0-34.9, 35.0-39.9 and ≥40 kg/m^2) were performed. Risk was compared using Cox regression.

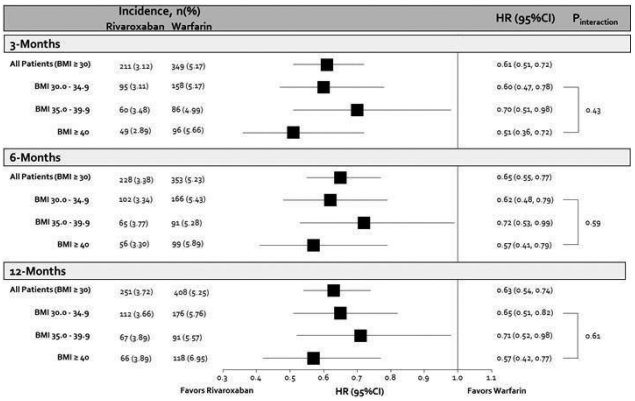

FIGURE 1 Incidence and hazard ratio of recurrent venous thromboembolism

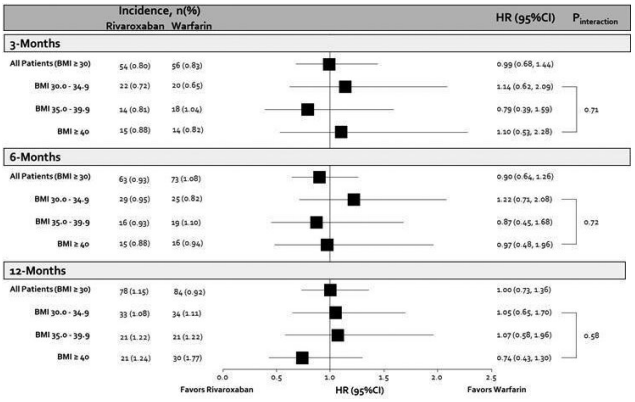

FIGURE 2 Incidence and hazard ratio of major bleeds

Results: We identified 6,755 rivaroxaban and 6,755 warfarin users with a BMI≥30 kg/m^2 experiencing an incident VTE. At 3-, 6- and 12-months, rivaroxaban was associated with a reduced risk of recurrent VTE compared to warfarin (Figure 1). There was no difference in major bleeding observed between rivaroxaban and warfarin at 3-, 6- or 12-months (Figure 2). Subanalyses did not show a statistically significant interaction across BMI categories for either the recurrent VTE (p-interaction≥0.43) or major bleeding (p-interaction≥0.58) outcomes at any time point.

Conclusions: Among obese patients experiencing an acute VTE, rivaroxaban was associated with a significantly reduced risk of recurrent VTE versus warfarin at 3-, 6- and 12-months, without impacting major bleeding. Our findings remained consistent across evaluated BMI classes.

Note: Funding by Janssen

PB2421 | Venous Thromboembolism Profile and Management Differences between Sexes in a RIETE's Hospital in Brazil

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Background: Reduction in venous thromboembolism (VTE) mortality rate in Brazil has been less marked in women and in Brazilian's North/Northeast regions, where VTE management data is scarce.
Aims: To evaluate the profile and management of patients with DVT and/or PE in a private hospital in Salvador, participating in the RIETE registry.
Methods: Consecutive patients from 5/2018 to 8/2019 were evaluated, comparing differences between sexes.
Results: Of the 68 patients, 67.8% were women, the mean age 52 years (± 19.43) and 34.7% of women, vs. 13.6% of men were ≤40 years-old (p=0.72). There were 54.5% PE, 27.9% DVT/PE and 14.7% DVT; more women had PE (58.7% vs. 54.5%) and more men had DVT/PE (42.1% vs. 23.9%). Among the risk factors for VTE, 65.0% were overweight, 29.4% had obesity, 26.4% cancer, 10.5% history of VTE, 7.3% family history of VTE, 11.7% surgery ≤ 2 months, 10.3% immobility >4 days, 5.8% smoking and 4.4% recent travel >6 hours; 32.6% of women used hormones. There were 33.8% of in-hospital VTE; 30.8% were admitted to intensive care unit; the mean length-of-stay was 8±5 days. Among the early discharged, 23.3% (< 48 hours), the initial choice of anticoagulant was enoxaparin, 95.2% and rivaroxaban, 4.8%; the choice for maintenance was rivaroxaban 61.9%, apixaban 28.6% and warfarin, 9.5% (P = 0.038). For those hospitalized ≥48 hours, 36.2% used rivaroxaban, 27.7% apixaban, 21.3% warfarin, 12.8% enoxaparin and 2.1% edoxaban. There were some differences between sexes in baseline characteristics, but none on the choice of anticoagulants or hospital length-of-stay.
Conclusions: This cohort in the Brazil's Northeast region had more women with VTE, high rates of PE and strong risk factors such as cancer, hormone use and obesity. Enoxaparin was still the main choice for initial treatment, but DOACs were more common in the early discharged without differences between sexes.

PB2422 | The Impact of an Educational Intervention in Patients with Atrial Fibrillation Treated with Warfarin

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Background: Educational interventions are of utmost importance for oral anticoagulation control of patients taking warfarin.
Aims: To assess the impact of a patient-centered educational strategy focused on low-income patients with poor anticoagulation control with warfarin.
Methods: Non-randomized controlled study. Adult patients with atrial fibrillation with time in therapeutic range (TTR) < 60% were recruited at two anticoagulation clinics (ACs) inBelo Horizonte, Southeast Brazil. Intervention group received 4 educational sessions based on patient-centered care approach and one telephone call between each session, in addition to usual care. Control group received usual care only. The intervention lasted four months. We assessed TTR and warfarin knowledge (Oral Anticoagulation Knowledge [OAK]test) before and after intervention. Health literacy was assessed on baseline using SHALPA-18. All participants signed an informed consent form (CAAE 65928316.3.0000.5149, REBEC RBR-9cy6ty and UTN U1111-1217-0151). Intention-to-treat analysis was performed.
Results: Through the study, 168 patients were included: 83 to intervention group (63±12 years-old, 35% male, 60% with inadequate health literacy) and 85 to control group (68±14 years-old, 52% male, 75% with inadequate health literacy). Most patients allocated to the intervention (92%) participated of at least one face-to-face meeting, 16% participated in all four sessions. When comparing intervention and control groups, there was an increase in the median change of hits in the OAK test (2[interqualite range 0-5] intervention vs.1[interqualite range -2-3] control, p=0.036). There was no significant difference in the median TTR change between groups (11[interquartile range 1-29]% vs. 20[interquartile range -7-43]%, p=0.69). The number of patients who increased TTR in intervention group was greater than the controls (77% vs. 65%), although this difference was not statistically significant (p=0.10).
Conclusions: The study suggests a patient-centered educational intervention may contribute to improve warfarin knowledge, but

further studies with large sample sizes are necessary to assess the impact on anticoagulation control.

Acknowledgments: CNPq, CAPES, FAPEMIG.

PB2423 | 90-Day Follow up in Patients with Suspected Deep Vein Thromboses (DVT) who Have Had a DVT Excluded: Should this Be Routine Clinical Practice?

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Background: Approximately 85% of individuals with suspected deep vein thromboses (DVT) have a DVT excluded. Investigational studies usually include a 90-day follow up on such patients to determine the percentage of false negative results. This outcome measure is not usual in routine clinical practice.

Aims: To evaluate the outcome and potential benefit of including a 90-day follow up in routine clinical practice for patients attending a primary care-based clinic with suspected DVT, who have had a DVT excluded.

Methods: All patients referred to our nurse-led, community hospital DVT assessment service were initially evaluated with a 2-level Wells score and Point of Care quantitative D-dimer (Cardiac D-dimer, Roche). A DVT was excluded if both of these were negative, or if either or both were positive by a negative Compression Ultrasound Scan (CUS) performed as per the NICE (National Institute for Health and Care Excellence) protocol. These patients had a 90-day telephone follow up to ascertain whether they had subsequently developed a DVT or pulmonary embolus or had been treated with an anticoagulant during the follow up period.

Results: Between January and October 2019, 266 patients were seen. Ten were diagnosed with a DVT and 26 with a superficial venous thrombosis. 230 (86.5%) were discharged with DVT excluded and had a 90-day follow up. Only 1 (0.4%) had developed a DVT after 6 weeks, associated with starting chemotherapy for cancer.

Conclusions: Our very low false negative rate on 90-day follow up showed that our nurse-led assessment and CUS results were reliable. This shows good clinical practice, was good for nursing staff morale and encouraging for patients discharged with a DVT excluded. We suggest that a 90-day follow up should be part of routine clinical practice as an important outcome measure in patients in whom a DVT has been excluded.

PB2424 | Hokusai Post-PE Study: A Follow-up Study on Long-Term Outcomes of Pulmonary Embolism in Patients Treated with Edoxaban vs Warfarin

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Background: Post-pulmonary embolism (Post-PE) syndrome, comprising persistent dyspnea, impaired exercise capacity and chronic thromboembolic pulmonary hypertension, is a long-term complication of pulmonary embolism (PE). The pathophysiological determinants of post-PE syndrome are still uncertain; however, there is an association with residual thrombosis. Nevertheless, post-PE syndrome is associated with decreased quality of life (QoL) and prevention is of great importance. Direct oral anticoagulants (DOACs) have a more stable pharmacologic profile than vitamin K antagonists (VKA) and may lead to improved thrombus resolution. We therefore hypothesized that treatment of PE with edoxaban compared to VKA (both preceded by heparin), would lead to an improved long-term QoL of PE patients.

Aims: To assess long-term generic and PE-specific QoL following PE, in patients treated with edoxaban vs. warfarin.

Methods: This is a follow-up study of the original HOKUSAI VTE trial (NCT00986154). We are collecting data from previously enrolled patients at least 5 years after the index PE. The primary outcome is the QoL, generated by the validated generic (SF-36) and PE-specific (PEmb-QoL) questionnaire. We will compare QoL between both

treatment arms. Ethical approval was granted in all participating centers and patients signed informed consent.

Results: Up to now, 24 of 76 (32%) of the centers that were invited managed to participate and we have currently included 245 patients. The analyses will be performed after completion of data collection (May 2020).

Conclusions: So far, we have included 245 patients. Inclusions will continue until May 2020 and at the ISTH 2020 we will be able to present our results on QoL after PE in patients treated with edoxaban compared to patients treated with warfarin.

PB2425 | Development of Cyclic Peptide Inhibitors of Coagulation Factor XIa for Safer Anticoagulation

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Background: Current anticoagulants are associated with a major side effect - they bring a risk of severe bleeding, a life-threatening condition. This strongly limits their potential and leads to events of inappropriate treatment. Thus, there is a strong unmet need for a safer class of anticoagulants. Coagulation factor XI (FXI) has been identified as a promising target for safer anticoagulation. Various studies have shown that FXI is involved in the pathogenesis of thrombosis, while not being essential for hemostasis. Peptide-based FXIa inhibitors have not yet been developed, despite the attractive properties of peptides as a drug format.

Aims: We aimed at developing potent and selective peptide-based FXIa inhibitors for safer anticoagulation.

Methods: We screened large peptide libraries against FXIa and isolated binders were characterized for their inhibitory activity and effect on ACT and aPTT. The stability and pharmacokinetic profile of a hit were improved. The optimized molecule was tested for anticoagulation efficacy ex vivo in a hemodialysis model and in vivo in rabbits to assess its therapeutic potential.

Results: We have identified a cyclic peptide that inhibits FXIa with single-digit nanomolar affinity and high selectivity over homologous proteases. The newly generated peptide is active in plasma and whole blood, and inhibits rabbit FXIa, which is key for in vivo evaluation and preclinical development. We found that the pharmacokinetic properties of the cyclic peptide could be modulated and tailored using various conjugation strategies. A conjugate showed an anticoagulation effect for several hours in rabbits, which would correspond to more than one day in human. The same molecule delayed blood clotting in an ex vivo model of hemodialysis to a similar extent as heparin applied at the maximal recommended dose.

Conclusions: In summary, we present the development of the first peptide-based FXIa inhibitor, which shows good affinity, selectivity, stability, and potent anticoagulation ex vivo and in vivo.

PB2426 | Risk Factors of Hemorrhagic Complications on the Anticoagulant Therapy

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Background: Anticoagulant therapy (ACT) by VKA, and DOAC, requires an assessment of all risk factors for the development of hemorrhagic complications.

Aims: To identify factors contributing to the development of neurological hemorrhagic complications (NHC) in the background of long-term ACT.

Methods: Retrospective study - analysis of case histories of 43 patients with hemorrhagic neurological complications on the ACT. We studied the presence of the causes of complications, outcomes, complications, their frequency, comorbidities, level of INR and BP during hospitalization.

Results: 43 patients, aged 46 to 83 years (Me = 67), receiving ACT, were hospitalized with diagnosis of "hemorrhagic stroke/intracranial bleeding", confirmed clinically and on CT scan. Fatal outcome in 39.5% patients. patients (83.7%) took Warfarin, patients (9.1%) took DOAC. 3 patients took Omeprazole, which can increase plasma concentration and increase the half-life of R-warfarin. 5 patients took Digoxin, 1 patient - Rosuvastatin. Taking these drugs with Warfarin requires dose adjustment and / or control over the degree of induced drug hypocoagulation. At the time of hospitalization, 31 patients with high BP. Uncontrolled BP. 34.9% patients had renal dysfunction, liver dysfunction - 18.6%, and thyroid gland disease - 7%. The value of INR, in the time of hospitalization, was more than 4 in 25% of patients taking VKA.

Conclusions: BP, atherosclerosis, impaired kidney and liver function, diabetes, and their therapy can increase the possibility of fatal bleeding. The reported study was funded by RFBR according to the research project No18-00-00814-KOMFI (18-00-0047818).

PB2427 | EmpoderACO: Elaboration and Validation of a Protocol for Behavioral Change in Patients on Warfarin

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Background: Warfarin is an oral anticoagulant widely used for primary and secondary thromprophylaxis. It has a narrow therapeutic index and wide variability in dose-response, requiring frequent monitoring and assistance. Anticoagulation control with warfarin remains a challenge. Patients on warfarin could benefit from educational strategies focusing on changing behavior, to improve individual's active participation in self-care and adherence to drug therapy.

Aims: To elaborate and validate the EmpoderACO, a protocol for behavioral change in patients on warfarin.

Methods: The instrument was developed as following: elaboration of the initial version of the protocol by specialists; multiple rounds of corrections of the instrument; pre-testand adaptations before releasing its final version. The relevance, adequacy and clarity of each item of the instrument were evaluated by a multiprofessional committee of judges using the E-Surv platform. The internal reliability was calculated using the content validity coefficient (CVC) of the evaluation of the judge's committee. The pre-test involved 30 patients on warfarin assisted at an anticoagulation clinic of a university hospital in Brazil. At this phase, patients made a comprehensive evaluation of the protocol items.

Results: The self-care domains covered in the protocol were: understanding and satisfaction of treatment, reduction of adverse events; and promoting well-being and healthy habits. Overall, 34 professionals participated of the committee of judges, including: physicians, nurses, pharmacists, nutritionists, linguistsand a pedagogue. The CVC was considered satisfactory with a compliance CVC>0.91 of the items for relevance, adequacy and clarity. The clarity of the instrument assessed by the patients, in the pre-test, obtained an average CVC of 0.96.

Conclusions: The protocol EmpoderACO may contribute to the design of educational strategies to improve patients' understanding of their own health problem and drug therapy, communication process between professionals and patients, and decision-making process.

Acknowledgment: This study received partial support from CNPq, CAPES and FAPEMIG.

PB2428 | Evaluation of Thrombin and Fibrin Generation in Venous Thromboembolism (VTE) Patients Receiving Anticoagulation

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Background: Current coagulation assays measure only the start of the clotting process, and do not reflect the in-vivo anticoagulant effect of direct oral anticoagulants (DOACs). Global coagulation assays such as the calibrated automated thrombogram (CAT) and overall haemostatic potential (OHP) may provide a better assessment.

Aims: As part of a larger prospective VTE biomarker study, we provide an interim analysis to explore the role of CAT and OHP in VTE patients receiving therapeutic anticoagulation.

Methods: Patients with symptomatic VTE were prospectively consented between Jan 2018 and Dec 2019. Blood was sampled whilst patients were receiving therapeutic anticoagulation and measured for rivaroxaban and apixaban specific anti-Xa, as well as CAT and OHP assays using manufacturer specifications.

Results: 193 (105 M, 88 F) patients with median age 57 years were recruited. Unprovoked events comprised 51.8% (n=100) and major VTE were 67.4% (n=130). Of patients who had been sampled, there were 65 on rivaroxaban, 58 on apixaban, 31 on warfarin and 13 on clexane. CAT assays demonstrated a reduction of thrombin generation parameters consistent with anticoagulation effect. Thrombin peak provided the best correlation displaying a logarithmic relationship between thrombin peak and rivaroxaban (R^2= 0.55) or apixaban anti-Xa

TABLE 1 OHP results (available at time of interim analysis) for study patients compared to normal controls

	Normal controls n=135	VTE patients n=138	Rivaroxaban n=63	Apixaban n=39	Warfarin n=27
OCP, mean (95%CI)	57.83 (55.84-59.82)	67.41 (65.32-69.5) p<0.001	65.1 (62.12-68.07) p<0.001	65.83 (62.33-69.33) p<0.001	74.51 (69.95-79.06) p<0.001
OHP, mean (95%CI)	28.52 (27.33-29.7)	35.81 (34.16-37.47) p<0.001	35.4 (32.89-37.92) p<0.001	34.85 (31.95-37.75) p<0.001	38.09 (34.27-41.91) p<0.001
OFP%, mean (95%CI)	49.93 (48.54-51.32)	47.06 (45.65-48.47) P=0.005	45.86 (43.72-48) P=0.002	47.05 (44.00-50.1) P=0.07	49.39 (46.75-52.04) P=0.75

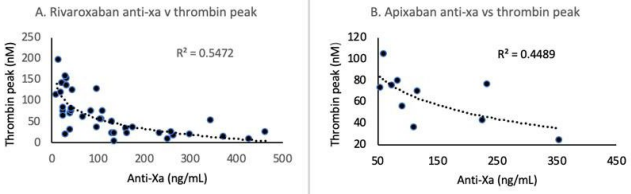

FIGURE 1 Relationship between thrombin peak and available anti-Xa levels (A) Rivaroxaban (n=31) and (B) Apixaban (n=10)

(R^2= 0.45) (Fig 1) with significant variation noted at low anti-Xa levels. Fibrin generation was not affected by anticoagulation (Table 1) and appeared significantly increased compared to normal controls.

Conclusions: The anticoagulant effect of rivaroxaban and apixaban can be measured using CAT, with significant individual variation particularly at low anti-Xa levels. Fibrin generation capacity is not affected by anticoagulation due to the addition of excess thrombin. However all fibrin generation parameters are significantly increased compared to normal controls, suggesting a hypercoagulable tendency in VTE patients. Further analysis of the fibrinolytic pathways in VTE as well as correlation with clinical outcome is suggested and is ongoing.

PB2430 | Variability in Exposure to Non-Vitamin K Antagonist Oral Anticoagulants (NOACs) in Hospitalised Patients

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Background: It is unclear whether elderly patients established on NOACs are at a greater risk of exposure to these drugs which could subsequently increase their risk of bleeding.

Aims: To assess exposure to NOACs in relation to age, renal function and gender.

Methods: Medically stable hospital inpatients ≥65 years, established on either apixaban, rivaroxaban or dabigatran, were recruited. Demographics, clinical data and two blood samples were collected for estimation of trough and peak plasma drug concentration using diluted thrombin time or anti-factor Xa assay, as appropriate. Plasma NOAC concentrations were compared against those reported from seminal clinical trials.

Results: 151 patients (76 on apixaban, 61 on rivaroxaban, 14 on dabigatran) were recruited. Overall, there was up to 48-fold and 13-fold variation in trough and peak plasma drug concentrations, respectively. A significantly greater proportion of patients on apixaban had peak plasma drug concentrations within the reported ranges compared to those on either rivaroxaban or dabigatran (82.9% v 44.3% v 64.3% respectively; p< 0.001). A significantly greater proportion of patients aged 65-74 had peak NOAC concentrations within the reported ranges compared to those aged ≥75 years (82.1% v 61.8% within range; p=0.04, and 14.3% v 31.7% above range). A significantly greater proportion of female patients compared to males (37.2% v 19.2%; p=0.014) and patients with severe renal impairment compared to those with normal renal function [40% (10/25) with CrCl< 30 ml/min v 10% (2/20) with CrCl≥80ml/min; p=0.024) had peak plasma NOAC concentration above published ranges.

Conclusions: There is large variability in exposure to NOACs in hospitalised patients. The proportion of patients exceeding the plasma NOAC concentration ranges reported in clinical trials increased with increasing age, reduced renal function and being female. Whether the observed increases in NOAC exposure translates to increased risk of bleeding in such patient populations requires further investigation.

PB2431 | Heparin Induced Thrombocytopenia in an Obese Pregnant Woman with Venous Thrombosis

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Background: Heparin induced thrombocytopenia (HIT) in pregnancy is a very rare adverse event of anticoagulant treatment with a high risk of thrombotic complications. Fondaparinux is considered a safe alternative to heparin in HIT. In very obese patients, however, data on appropriate dosing of fondaparinux is scarce.

Aims: To emphasize difficulties in management of obese pregnant women with HIT by presenting a case of a young patient who developed HIT in the 14th week of pregnancy.

Methods: The patient's chart was reviewed for details of diagnosis, treatment and outcome.

Results: 21- year-old, obese (146 kg, BMI 51.4 kg/m^2), otherwise healthy woman presented with left-sided iliofemoral venous thrombosis in the 10th week of her second pregnancy. Treatment with dalteparin 15,000 IU b.i.d was introduced (peak anti-Xa=0.87 IU/mL). After 27 days of treatment thrombocytopenia (28x10^9/L) was recorded. Simultaneously, iliofemoral venous thrombosis in the other leg was detected. She was switched from dalteparin to argatroban due to scarce data on fondaparinux dosing in obese patients. Diagnosis of HIT was confirmed in two separate laboratories. Upon normalization of platelet count, warfarin was initiated (2.0-3.0 INR, weekly dose 33 mg). In the 25th week of pregnancy she was switched from warfarin to fondaparinux 12.5 mg daily (anti-Xa = 1.24 mg/mL). A planned cesarean section was performed in the 38th week of pregnancy. Blood loss at delivery was 1700 mL mainly due to uterine atony. Neonatal birthweight was appropriate for gestational age (3.440 g). After delivery warfarin was reintroduced and continued for 6 weeks. There were no thromboembolic complications. HIT antibodies were still present before delivery and 13 weeks postpartum.

Conclusions: Our case highlights the problem of insufficient data on appropriate dosing of fondaparinux in obese pregnant patients,

which led us to use argatroban and later warfarin for initial HIT treatment and switch to fondaparinux only in later stages of pregnancy.

PB2432 | ETNA-VTE Europe: The Effect of Body Mass Index on 12-Month Outcomes in VTE Patients with Edoxaban

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Background: Obesity (defined as a body mass index [BMI] ≥30 kg/m^2) increases the risk for first spontaneous venous thromboembolism (VTE) two-fold. During the treatment of VTE, obesity may also affect bioavailability, distribution, and half-life of edoxaban, and consequently, patient outcomes. Dose reduction of edoxaban is recommended in patients with a bodyweight ≤60 kg.

Aims: To characterize obese patients and to investigate outcomes in patients treated with edoxaban categorised by BMI.

Methods: Patients in ETNA-VTE-Europe who had acute symptomatic VTE and received edoxaban were recruited across eight European countries. Patients were divided according to BMI [kg/m^2]: 18.5-25, 25-30 and ≥30.

Results: Of 2131 patients, 573 were normal weight, 849 overweight (BMI 25-30) and 560 obese (BMI ≥30); 149 patients had no BMI data and 18 patients had a BMI < 18.5. Obese patients were more often female, had higher rates of hypertension and diabetes, chronic venous insufficiency and a higher eGFR than non-obese patients. They also had higher rates of prior VTE and were receiving the 60 mg edoxaban dose more often (Table 1). At 1-year follow up, VTE recurrence was 2.67% and consistent across categories. Any bleeding was observed in 12.29% and major bleeding in 1.69% with no increase with BMI. While all-cause mortality was in the same order across BMI groups, there was a trend for a reduced cardiovascular mortality in obese patients (Table 2). Table 1: Baseli… Table 2: Clinic…

Conclusions: Obesity does not appear to substantially affect the risks of recurrent VTE and any bleeding complications in a contemporary cohort of edoxaban treated patients. Apart from all-cause mortality following a U-shape association and CV-mortality being

TABLE 1 Baseline characteristics of patients enrolled in the ETNA-VTE-Europe study according to BMI [N=2131]*

	Overall*† [N=2131]	BMI 18.5-25* [N=573]	BMI 25-30* [N=849]	BMI ≥30 * [N=560]
Female, n (%)	983 (46.1%)	297 (51.8%)	335 (39.5%)	322 (51.3%)
Age, years, mean ± SD	62.4 ± 16.07	61.9 ± 18.19	64.0 ± 14.93	61.9 ± 14.97
BMI, kg/m^2, mean ± SD	27.9 ± 5.00	22.7 ± 1.85	27.3 ± 1.37	34.2 ± 4.04
recalc. eGFR (Cockroft-Gault), ml/min, mean ± SD	95.7 ± 38.95	82.1 ± 31.85	92.3 ± 34.60	114.0 ± 44.88
Frailty (physician judgement), n (%)	261 (12.2%)	75 (13.1%)	106 (12.5%)	75 (11.9%)
Medical history, n (%)				
Hypertension	888 (41.7%)	165 (28.8%)	359 (42.3%)	60 (57.3%)
Diabetes Mellitus	235 (11.0%)	40 (7.0%)	81 (9.5%)	117 (18.6%)
Chronic venous insufficiency	234 (11.0%)	49 (8.6%)	104 (12.2%)	85 (13.5%)
History of VTE, n (%)				
Prior PE±DVT	160 (7.5%)	38 (6.6%)	55 (6.5%)	54 (8.6%)
Prior DVT	350 (16.4%)	89 (15.5%)	149 (17.6%)	108 (17.2%)
Edoxaban treatment at baseline, n (%)				
Edoxaban 60 mg	1873 (87.9%)	449 (78.4%)	771 (90.8%)	521 (93.0%)
Edoxaban 30 mg	258 (12.1%)	124 (21.6%)	78 (9.2%)	39 (7.0%)

DVT, deep vein thrombosis; eGFR, estimated glomerular filtration rate; PE, pulmonary embolism; SD, standard deviation; VTE, venous thromboembolism. * Patients with history of cancer, active cancer at baseline or new cancer within six months (182 days) after start of acute index VTE were excluded from analysis. †One-hundred forty nine patients had missing BMI.

TABLE 2 Clinical outcomes of patients enrolled in the ETNA-VTE-Europe study during 12-month follow-up according to BMI (N=2131)*

Outcomes during 12-months follow-up*, n (%)	Overall†‡ [N=2131]	BMI 18.5-25† [N=573]	BMI < 25† [N=573]	BMI ≥30† [N=560]
VTE recurrences	57 (2.67%)	14 (2.44%)	24 (2.83%)	17 (2.71%)
PE with or w/o DVT	24 (1.13%)	4 (0.70%)	11 (1.30%)	7 (1.11%)
DVT only	38 (1.78%)	10 (1.75%)	17 (2.00%)	12 (1.91%)
Any bleeding	262 (12.29%)	78 (13.61%)	97 (11.43%)	87 (13.85%)
ICH	8 (0.38%)	1 (0.17%)	5 (0.59%)	1 (0.16%)
Major bleeding [ISTH]	36 (1.69%)	10 (1.75%)	17 (2.00%)	7 (1.11%)
CRNM bleeding	69 (3.24%)	18 (3.14%)	25 (2.94%)	28 (4.46%)
Major GI bleeding	9 (0.42%)	3 (0.52%)	4 (0.47%)	1 (0.16%)
All–cause mortality	46 (2.16%)	17 (2.97%)	15 (1.77%)	15 (2.39%)
CV mortality	23 (1.08%)	9 (1.57%)	9 (1.06%)	5 (0.80%)
Any stroke	13 (0.61%)	3 (0.52%)	4 (0.47%)	5 (0.80%)
Ischaemic stroke	7 (0.33%)	2 (0.35%)	1 (0.12%)	4 (0.64%)
Haemorrhagic stroke	3 (0.14%)	0 (0.00%)	2 (0.24%)	0 (0.00%)
Stroke or systemic embolism	16 (0.75%)	3 (0.52%)	5 (0.59%)	8 (1.27%)

CRNM, clinically relevant nonmajor; CV, cardiovascular; DVT, deep vein thrombosis; GI, gastrointestinal; ICH, intracranial haemorrhage; ISTH, International Society on Thrombosis and Haemostasis; PE, pulmonary embolism; VTE, venous thromboembolism. *Overall population †Patients with history of cancer, active cancer at baseline or new cancer within six months (182 days) after start of acute index VTE were excluded from analysis. ‡One-hundred forty nine patients had missing BMI.

lowest in BMI ≥30, there were no appreciable differences in outcomes across the various subgroups.

PB2433 | Predicting Recurrence and Bleeding in Patients with Venous Thromboembolism: A Systematic Review and Critical Appraisal of Prediction Models

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Background: Currently available prediction models for recurrence and bleeding are infrequently used when deciding about anticoagulant treatment duration after venous thromboembolism (VTE) due to concerns about performance and validity.

Aims: Our aim was to critically appraise these models by systematically summarizing data from model derivation and validation studies in the setting of VTE.

Methods: MEDLINE and CENTRAL were searched up until November 15th, 2019. All studies using a design for developing, updating, validating, or evaluating prediction models for recurrence or bleeding in adult patients with VTE were included. The Prediction model Risk Of Bias Assessment Tool (PROBAST) was used to assess risk of bias and applicability of the included studies.

Results: Search and selection yielded 19 eligible studies evaluating 6 prediction models for recurrent VTE (Table 1), including 6 on model development, 8 on model validation, and 1 on updating an existing model. Four models were evaluated in independent datasets at least once. Overall, models for recurrent VTE appeared to perform poorly to moderately with c-statistics ranging between 0.39 to 0.83. Twenty-seven eligible studies that evaluated 18 prediction models for bleeding during anticoagulant treatment in patients with VTE were identified (Table 1), including 8 studies on model development and 16 on model validation. Although most models seemed promising in development studies, their predictive performance was poor to moderate in external validation studies; c-statistics ranged from 0.52 to 0.72, with the majority being below 0.60. All but two of the included studies were judged to be at high risk of bias, mainly due to limitations in the statistical analysis.

Conclusions: Currently available prognostic models for recurrent VTE and anticoagulation-related bleeding often have important methodological limitations and insufficient predictive accuracy. These findings do not support the use of these models to decide about continuing or discontinuing anticoagulant treatment after initial treatment of VTE.

TABLE 1 Identified prediction models for anticoagulation-related bleeding and recurrent venous thromboembolism

Recurrent VTE		Bleeding			
		Developed in patients with VTE receiving oral anticoagulation		Validated in VTE population	
Models	*External validation studies*	*Models*	*External validation studies*	*Models*	*External validation studies*
L-TRRiP	1	BLLADS	0	ORBIT	1
DAMOVES	1	Seiler	0	ATRIA	4
Huang	0	Hokusai	0	HAS-BLED	7
Vienna (updated)	1	EINSTEIN	0	HEMORR2HAGES	3
DASH	4	VTE-BLEED	4	Shireman	1
Vienna	2	ACCP 2016	1	Kearon	2
HERDOO2	1	ACCP 2012	2	mOBRI	2
		RIETE	8	Nieuwenhuis	1
		Kuijer	6	OBRI	3

Abbreviations: VTE venous thromboembolism

PB2434 | ETNA VTE Europe: A Contemporary Snapshot of VTE Patients Treated with Edoxaban in Clinical Practice across Eight European Countries

A.T. Cohen[1]; U. Hoffmann[2]; P. Hainaut[3]; S. Gaine[4]; C. Ay[5]; M. Coppens[6]; M. Schindewolf[7]; D. Jimenez[8]; B. Brüggenjürgen[9]; P. Levy[10]; P. Bramlage[11]; G. Agnelli[12]

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Background: Edoxaban, a direct oral anticoagulant inhibiting FXa, has proven its efficacy and safety in the ENGAGE AF-TIMI 48 and Hokusai-VTE clinical trials. Clinical practice patients, however, may differ from those of clinical trials.

Aims: Using an observational study design, we aimed to compare Hokusai-VTE patients with those treated in clinical practice, and expand the knowledge about edoxaban's clinical effectiveness and safety in the treatment and prevention of venous thromboembolism (VTE).

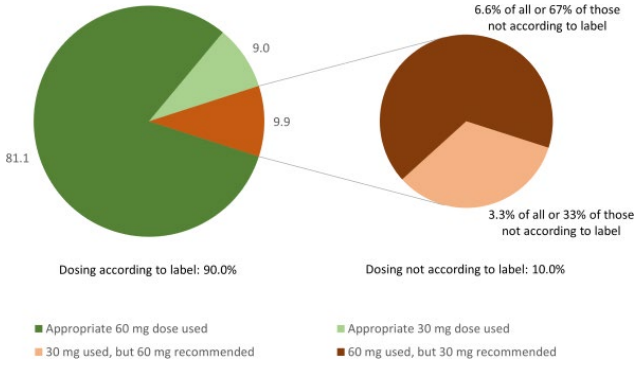

FIGURE 1 Dosing according to edoxaban label based on bodyweight, creatinine clearance and P-gp inhibitor use

Methods: ETNA-VTE-Europe is a prospective, non-interventional post-authorisation safety study conducted in eight European countries.

Results: A total of 2,879 patients presenting with acute VTE (median age 65 years, 46.5% female) were enrolled at 339 sites (133 office-based physicians and 206 hospitals). Of the 2,680 patients with complete data, 23.6% reported prior VTE and 2.8% had a history of bleeding.

Patients in ETNA-VTE were older (65 vs. 57 years), more likely to be female (46.5 vs. 39.8%) and had a higher prevalence of chronic venous insufficiency (11.1 vs. 1.6%) than in the European cohort of the Hokusai-VTE clinical trial (n=1,512). Body weight and creatinine clearance were substantially lower in clinical practice with more patients having a bodyweight ≤60 kg (9.3% vs. 5.6%) and a CrCl ≤50 ml/min (10.2% vs. 4.1%).

Edoxaban dosing was adherent to label in 90% of patients, with higher than recommended (60 mg) doses and lower than recommended doses (30 mg) used in 6.6% and 3.3% of the patients, respectively. Heparin lead-in was used in 84.7% of patients. It was more frequently used in PE than DVT patients (91.3% vs. 80.1%; p< 0.0001).

TABLE 1 Patient characteristics of those in clinical practice (ETNA-VTE) overall and by VTE presentation and those in clinical trials (HOKUSAI-VTE)

	HOKUSAI-VTE [N = 1,512]	ETNA-VTE [N = 2,680]	ETNA-VTE DVT only [N = 1,559]	ETNA-VTE PE ± DVT [N = 1,121]	p-value DVT vs. PE
Age, years/Female patients	57/39.8	65/46.5	64/45.3	66/48.2	0.0052/0.1577
Body weight, kg	84.0 (73.9-95.3)	80 (70-92)	80 (70-90)	81 (70-94)	0.0031
Acute DVT only	854 (56.5)	1,559 (58.2)	1,559 (100.0)	0 (0.0)	n.a.
PE with or without DVT	658 (43.5)	1,121 (41.8)	0 (0.0)	1,121 (100.0)	n.a. n.a.
Chronic Venous Insufficiency	24 (1.6)	297 (11.1)	214 (13.7)	83 (7.4)	<0.0001
Cancer	136 (9.0)	253 (9.4)	133 (8.5)	120 (10.7)	0.0566
Stroke	18 (1.2)	79 (2.9)	29 (1.9)	50 (4.5)	0.0001
CrCL*,**, ml/min	104.3 (79.0-128.9)	90.1 (65.7-117.7)	91.2 (65.5-120.4)	89.3 (65.9-115.0)	0.2238
Edoxaban 60 mg	1,371 (90.7)	2,351 (87.7)	1,349 (86.5)	1,002 (89.4)	0.0317

Conclusions: The data describe a clinical practice population of VTE patients that is partially different from prior randomised controlled trials. Edoxaban is largely used adequately in these patients, respecting the recommendations for treatment initiation, dosing, and dose adjustments in special patient populations.

Patient characteristics of those in clinical practice (ETNA-VTE) overall and by VTE presentation and those in clinical trials (HOKUSAI-VTE)

PB2435 | Upper Extremities Deep Vein Thrombosis and DOAC Treatment: a Prospective Cohort Study

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Background: Limited data are available on the use of direct oral anticoagulants (DOACs) in patients with upper extremities deep vein thrombosis (UEDVT).

Aims: To assess the effectiveness and safety of DOACs in the treatment of UEDVT.

Methods: Data on patients with an objective diagnosis of acute UEDVT treated with DOACs were merged from prospective cohorts of patients with venous thromboembolism to obtain a collaborative database. Study outcomes were recurrent venous thromboembolism (VTE) and major bleeding occurring during DOAC treatment.

Results: Overall, 132 patients were included: mean age was 47.7±18.0 years (range 18 to 97), males were 42.4%. Twenty-seven percent of patients had 2 or more risk factors for VTE, 29.5% had UEDVT complicating a central venous line or after pacemaker implantation (see Table).

Ninety-two patients (70%) were managed as outpatients. Increased age (OR 1.03, 95% CI 1.0-1.05) and anemia (OR 1.35, 95% CI 1.07-1.70) were associated with in-patient management. Among patients treated with apixaban (40) or rivaroxaban (85) loading dose was used in 72%; in patients treated with dabigatran (5) or edoxaban (2) mean heparin pre-treatment was 8 days. DOACs were started after 1 month from UEDVT diagnosis in 12.9% of patients; active cancer was a main predictor for delayed initiation (OR 22.2, 95% CI 5.8-84.4).

Mean treatment duration in patients with a scheduled stop was 4.9 months, while mean follow-up in those who continued DOACs was 9.3 months.

No recurrence of VTE nor major bleedings occurred during DOAC treatment; one patient had acute limb ischemia (1.38% patient-year), 7 clinically relevant non-major bleedings (3 genital, 2 epistaxis, 1 gingival, 1 genital bleeding plus epistaxis; 10.51% patient-year), 5 deaths (4 cancer, 1 advanced age; 9.69% patient-year).

Conclusions: Our data support the effectiveness and safety of DOACs for the treatment of acute UEDVT. Further studies are required to confirm these findings.

PB2436 | Saddle Pulmonary Embolism in the Era of Incidental Events: Incidence, Clinical Findings and Outcomes in a Single Center Cohort

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Background: Incidental or unsuspected PE (UPE) currently represents about half of the PEs diagnosed in the setting of cancer. The specific prognostic role of PE burden and more specifically 'saddle PE' remains controversial.

TABLE 1 Characteristics and risk factors of patients with UEDVT treated with DOACs

	Overall (n= 132)	Active cancer (n= 34)	Non-cancer risk factor (n= 62)	Unprovoked (n= 36)
Age, years mean±SD	47.7±18.0	55.4±13.2	46.1±18.1	43.3±19.9
Male, n (%)	56 (42.4)	14 (41.2)	23 (37.1)	19 (52.8)
Concomitant pulmonary embolism, n (%)	11 (8.3)	3 (8.8)	5 (8.1)	3 (8.3)
Weight, kg mean±SD	71.6±14.9	67.3±10.5	74.8±16.0	69.6±15.6
CVC, PICC or PMK, n (%)	39 (29.5)	19 (55.9)	20 (32.3)	-
Recent surgery or trauma, n (%)	19 (14.4)	2 (5.9)	17 (27.4)	-
Effort thrombosis or thoracic outlet syndrome, n (%)	12 (9.1)	0	12 (19.4)	-
Thrombophilia or estrogen-progestin therapy, n (%)	17 (12.9)	1 (2.9)	16 (25.8)	-
Patients with a scheduled stop treatment, n (%)	79 (59.8)	10 (29.4)	44 (70.9)	25 (69.4)

Aims: To assess the incidence, clinical findings and short-term outcomes of cancer patients with 'saddle PE' including acute symptomatic and incidental events.

Methods: Consecutive patients with cancer-associated PE diagnosed with either CT angiography specifically ordered to depict PE (acute suspected events) or scheduled conventional CT scan (UPE) included at the Hospital Clinic of Barcelona (Spain) were included (2005-2014). All CT scans were thoroughly reviewed by two senior chest radiologists. The clinical characteristics and PE-related outcomes within 30 days after PE diagnosis (overall and PE-related mortality, venous thromboembolism recurrence and major bleeding) were assessed according to saddle vs. non-saddle PE.

Results: A total of 310 cancer patients with PE were collected including 21 cases diagnosed by V/Q scintigraphy (excluded from the present study) and 289 cases diagnosed by CT scan. Saddle PE was diagnosed in 36/289 cases (12.5%) including 21 (58%) acute symptomatic events and 15 (42%) UPE. Table 1&2 show the main baseline characteristics and outcomes according to saddle vs. non-saddle PE. Patients with saddle PE were more likely to have PE symptoms (69% vs. 51%; p=0.042), tachycardia (44% vs. 24%; p=0.01) and oxygen saturation < 95% (56% vs. 31%; p=0.004) compared to patients with non-saddle PE. No statistical differences were observed in the rate of arterial hypotension, oxygen saturation < 90% and 30-day PE-related outcomes in patients with saddle vs. non-saddle PE.

Conclusions: Patients with saddle PE were more likely to be symptomatic at PE diagnosis although patients with non-saddle PE showed similar PE-related outcomes. These findings suggest that PE burden and location (specifically saddle PE) are not potentially predictive variables of adverse outcomes in this setting.

TABLE 1 Baseline characteristics of patients according to saddle vs. non-saddle PE

	Saddle PE N=36 (%)	Non-saddle PE N=253 (%)	*p-value*
Age (mean age ± SD)	65 ± 10.8	64 ± 11.8	p>0.5
Radiological test			**p=0.025**
CT scan angiography (acute suspected PE)	21 (58%)	98 (39%)	
Conventional CT scan (UPE)	15 (42%)	155 (61%)	
PE symptoms	25 (69%)	130 (51%)	**p=0.042**
Dyspnea	24 (71%)	112 (45%)	**p=0.005**
Blood pressure < 100mmHg	1 (3%)	20 (8%)	p=0.49
Heart rate > 100 per minute	16 (44%)	61 (24%)	**p=0.01**
Oxygen saturation < 95%	20 (56%)	79 (31%)	**p=0.004**

TABLE 2 Outcomes of patients according to saddle vs. non-saddle PE

	Saddle PE N=36 (%)	Non-saddle PE N=253 (%)	*p-value*
Outcomes within 30 days			
Overall mortality	5 (14%)	37 (15%)	p>0.5
PE-related mortality	2 (6%)	3 (1%)	p=0.12
Major bleeding	1 (3%)	5 (2%)	p>0.5
VTE recurrence	1 (3%)	13 (5%)	p>0.5

PB2437 | Early Physiotherapy Intervention Reduces the Long Term Effects of Venous Thromboembolism and Promotes an Improved Quality of Life: Results from a Pilot Study

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Background: Evidence suggests that early intervention in the form of physical activity improves the exercise capacity of patients suffering from PE; possibly reduces chance of recurrence; reduces bleeding complications inpatients on anticoagulants and reduces post thrombotic symptoms.

Aims: To offer physiotherapy intervention as appropriate to patients based on an exercise programme, structured group support to reduce the physical and psychological symptoms of VTE.

Methods: Patients with a recent VTE diagnosis were invited for an early physiotherapy discussion. Additionally, patients were assessed and if suitable were offered a 10-week, hour long rolling exercise programme consisting of supervised group exercise with a combination of strengthening and cardiovascular exercises. Programme evaluation consisted of outcome measures: EQ5D5L, PEmbQoL/HADS, GPPAQ. fatigue and confidence to exercise visual analogue scales (VAS), Incremental Shuttle Walking Test (ISWT), modified BORG rate of perceived exertion scale and grip strength.

Results: Twenty-one patients were enrolled. Ten attended a post-programme assessment with an average of 8.6 sessions completed. No adverse events were recorded. The 11 non-completers attended between 1-7 sessions. Average shuttle walking distance went from 375m to 532.5m, an improvement of 157.5m on mean average which is over three times more than the MCID of 48m. The modified BORG scores remained static. Average grip strength increased from 33.4kg to 37.4kg. Confidence to exercise scores went from 6.8 to 8.8 on average. Fatigue severity decreased from 4.8 to 4.2.

The PEmbQoL questionnaire the average score went down from 19.2 to 13.4. In the EQ5D5L, combined scores improved for nine out of ten patients.The rating of overall health improved from 52.5 to 67.8.

Conclusions: Early intervention in the form of a structured physiotherapy program has the potential to reduce the physical and psychological comorbidity associated with VTE. Further work is planned to apply this approach amongst a larger population.

PB2438 | Retrospective Study of Patients with Upper Extremity Clots Presenting to Emergency Departments in Tertiary Care Hospitals in a Major Canadian City in the Last Five Years

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Background: Upper extremity (UE) clots result in an incidence of approximately 0.4-1 per 10,000 cases, with no randomized controlled trials on the choice of agent or the duration of anticoagulation, resulting in a wide variability in daily practice.
Aims: 5 year retrospective study of patients with UE DVT to determine practice patterns and management of UE clots.
Methods: All adult patients with suspected UE DVT or superficial vein thrombosis (SVT) presenting to any of the tertiary care centers in the city, who had undergone diagnostic imaging were included in the study, from January 2014 to December 2018. Exclusion criteria included any known cancer. Data collected included basic demographics, duration and choice of anticoagulation, disposition from emergency, workup for thrombophilia and thoracic outlet obstruction (TOO).
Results: 1236 patient records were reviewed, of which 211 were malignancy associated. Of the remaining 1025 patients, 14.7% were positive for UE DVT and 11.1% for SVT. Mean age was 47 years and 50.2% were males. Duration of treatment for both DVT and SVT ranged anywhere from no therapy to lifelong treatment, with an average of 5.01 months for DVT and 1.15 months for SVT (Table 1).
Anticoagulant agents for DVT included low molecular weight heparin (7.9%), direct oral anticoagulants (39.1%) and warfarin (42.4%) (Figure 1). Followup was quite variable from the emergency department. 30.5% of the patients with DVT were referred to General Internal Medicine (GIM) outpatient, compared to 24.5% to Hematology and 33.7% to their family doctor. 39.7% of the people with DVT were tested for thrombophilia, of which 10% tested positive for any type of thrombophilia. 29.8% were tested for TOO, of which 48.9% were positive and 11.1% received surgery for same.
Conclusions: Our study demonstrates significant variability in the management of UE clots, highlighting the lack of literature in this field and the need for a structured, standardized approach.

TABLE 1 Duration of Treatment in Patients with DVT and SVT

DVT n=151 (14.7%)	
Mean UE DVT treatment duration (months)	5.01
Lifelong therapy (%)	34 (22.5)
No treatment (%)	13 (8.6)
SVT n=114 (11.1%)	
Mean UE SVT treatment duration (months)	1.15
Treated greater than 45 days (%)	21 (18.4)
Lifelong therapy (%)	8 (7.0)

Figure 1. Choice of anticoagulant used in patients with confirmed upper extremity DVT.

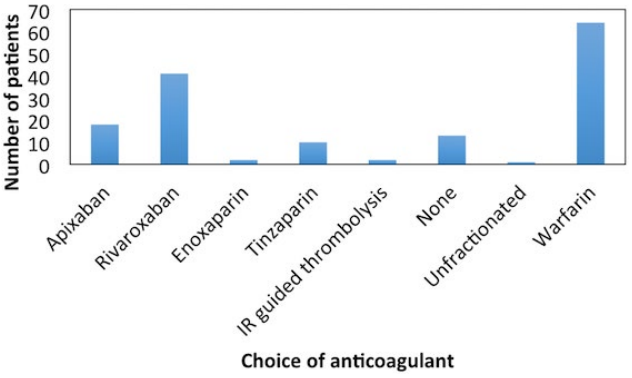

FIGURE 1

PB2439 | How Often Measurements of Direct Oral Anticoagulant Levels Have Consequences in Anticoagulation Management

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Background: Systematic monitoring is not required in direct oral anticoagulant (DOAC) treatment. However, DOAC level measurement is frequently performed and it is yet to be established when it is valuable.
Aims: Our aims were to investigate why DOAC levels were measured and if this had any clinical consequences. Consequently, we aimed to reveal determinants associated with changes in anticoagulation therapy.
Methods: In the Amsterdam UMC location AMC all available DOAC levels measured in clinical practice from 2013 until 2019 were included. We retrospectively collected DOAC level related information. Logistic regression analyses were performed to associate patient characteristics with changes in anticoagulation therapy. Patients followed an opt out procedure for the use of their data.

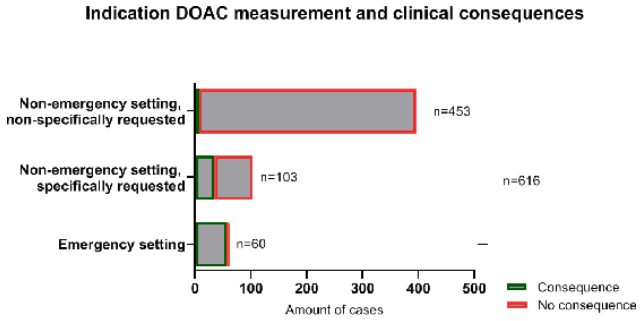

FIGURE 1 Overview of the proportion of reasons for DOAC level measurement and their clinical consequences in anticoagulant management

TABLE 1 Median (IQR) random DOAC levels in patients using standardized DOAC doses and proportion of patients above the reference ranges of the expected peak p

Measurements cases with standard DOAC dosing (n=490)	Median (IQR) drug level in ng/mL	Proportion **above** range according to Dutch national guidelines[1-3], n (%)	Proportion **above** range according Douxfils et al for AF patients[4], n (%)	Proportion **above** range according Douxfils et al for VTE patients[4], n (%)
Apixaban (n=106)	108 (68-182)	3	10 (9)	11 (10)
Dabigatran (n=39)	89 (45-163)	2 (5)	5 (13)	5 (13)
Edoxaban (n=36)	101 (62-254)	N. A.	10 (28)	3 (8)
Rivaroxaban (n=309)	90 (47-215)	25 (8)	28 (9)	17 (5)

AF = atrial fibrillation, VTE = venous thromboembolism; N.A. = not applicable.

1. Mueck W, Stampfuss J, Kubitza D, Becka M. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. *Clinical pharmacokinetics*. 2014;53(1):1-16.
2. van Ryn J, Stangier J, Haertter S, et al. Dabigatran etexilate--a novel, reversible, oral direct thrombin inhibitor: interpretation of coagulation assays and reversal of anticoagulant activity. *Thrombosis and haemostasis*. 2010;103(6):1116-1127.
3. European Medicines Agency (EMA). Summary of Product Characteristics: Eliquis 2.5 mg film-coated tablets. In.
4. Douxfils J, Ageno W, Samama C-M, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians *Journal of Thrombosis and Haemostasis*. 2018;16(2):209-219.

Results: Preliminary analysis of 616 randomly measured DOAC levels in 363 patients showed that DOAC levels were mainly requested by the vascular medicine (46%) and cardiology (23%) department. Of all DOAC levels, 60 (10%) were measured in 1) an emergency setting, 103 (17%) in 2) a non-emergency setting with a specific reason (e.g. renal insufficiency or drug interaction) and 453 (74%) in 3) a non-emergency setting without a specific reason. Changes in anticoagulation management followed in 93%, 34% and 8%, according to setting 1, 2 and 3, respectively (Figure 1). Median drug levels and the proportion above the reference ranges are presented in Table 1 for all patients on standard DOAC dosing (n=490). DOAC levels were not associated (OR 0.99; 95%CI 0.97-1.01).

Conclusions: The majority of DOAC levels was requested without a specific reason by the vascular department with only a fraction leading to changes in anticoagulant therapy. The proportion of changes in anticoagulation therapy after DOAC level measurement was substantial in emergency settings. However, there is no indication that the assessment of the DOAC level had any influence on decisions to adjust DOAC treatment.

PB2440 | Survey on the Prescription and Biological Monitoring of Direct Oral Anticoagulants in Algeria

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Background: Direct oral anticoagulants (AOD) have known great success since their arrival on the market more than 10 years ago. Unlike Vitamin K antagonist (VKA), their use does not require regular monitoring of INR, however they are not without risks.

Aims: The objective of our survey is to establish an inventory on the prescription of AOD in Algeria. Our study aims to assess the use of AOD and to understand the possible obstacles to their prescription.

Methods: We carried out a declarative cross-sectional study with a sample of liberal and hospital specialists selected randomly, during the period from January to April 2019. The questionnaire included 29 questions divided into 3 items: professional characteristics, prescription of AOD and biological monitoring.

Results: According to the results of our study, the prescription of AODs is modest compared to the VKA which remain the most prescribed in Algeria.

The major obstacle that holds prescription in Algeria is firstly the unavailability of all AODs on the market, only rivaroxaban is marketed. Besides these economic brakes, the risk of hemorrhage, the lack of biological monitoring of the anticoagulant activity which makes it possible to appreciate the tolerance and the harmlessness of these molecules as well as the absence of antidote constitute the main factors influencing the therapeutic decision of our doctors in terms of approval and prescription of AOD. Regarding the evaluation of anticoagulant activity by measuring anti-FIIa and anti-Xa activity, our study has shown that all doctors want to have it available in analysis laboratories.

Conclusions: Faced with the multiplication of anticoagulants, and their own operating mode, it becomes complicated for prescribers to find their way around but over time, new anticoagulants will certainly find their place. The availability of specific anticoagulation assessment tests and the marketing of antidotes should reassure them and strengthen their use in everyday practice.

PB2441 | Outcomes of the First Australian Pulmonary Embolism Response Team (PERT) Model of Care for Intermediate to High Risk Pulmonary Embolism

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Background: In 2018, we formed the first Australian PERT to provide rapid multidisciplinary co-ordination of diagnosis, risk stratification

and management of high/intermediate-risk PE. 2019 European Society of Cardiology (ESC) Pulmonary Embolism (PE) Guidelines have recommended formation of PERTs for management of high and intermediate-risk PE.

Aims: Review clinical characteristics, management and outcome data for intermediate/high-risk PE patients managed during the first 12 months of PERT implementation. Compare treatment with patients managed at our centre in the 12 months immediately prior to PERT introduction (pre-PERT).

Methods: We performed a retrospective review of 28 consecutive PERT-managed patients from 2018-July 2019. We stratified patients into high/intermediate-risk according to 2019 ESC guidelines. Data collected included 7 and 30 day mortality, reperfusion modality [systemic or catheter-directed thrombolysis (CDT)], troponin, RV:LV ratio before and after CDT and treatment complications. We compared the PERT managed cohort with 28 consecutive high/intermediate-risk PE patients identified by ICD-10 codes presenting between August 2017 and July 2018 (pre-PERT). Statistical analysis included Mann-Whitney and paired/unpaired t tests.

Results: Clinical characteristics of PERT patients are tabulated below. Twenty PERT patients underwent CDT, 3 had systemic thrombolysis. RV:LV ratio improved in 16 of 17 patients following CDT from 1.41 (0.25) to 1.07 (0.14); p< 0.0001. Four deaths occurred within 7 days, with 1 major bleeding complication. Compared to pre-PERT patients, more PERT patients had saddle/main pulmonary artery PE (82% vs 29%; p< 0.01), intermediate-high risk PE (64% vs 14%, p,0.01) and underwent CDT (74% vs 4%, p< 0.01). Risk stratification was incomplete in pre-PERT patients, with fewer echocardiographic assessment for RV dysfunction (64% vs 100%; p < 0.01).

Conclusions: Our PERT model of care 12 month data is consistent with international published patient outcomes for mortality, complications and successful reperfusion. It resulted in improved PE risk stratification, assessment for RV dysfunction and increased CDT use in intermediate/high risk patients.

TABLE 1 Clinical characteristics of PERT patients

	n (%)or mean	Standard deviation (SD)
Age (mean)	63 years	15.3 years
Male Sex	19 (68%)	
High risk for mortality	6 (21%)	
Intermediate-high risk	17 (61%)	
Intermediate-low risk	5 (18%)	
PESI index	1.6	1.1
Elevated troponin	25 (89%)	
ICU/HDU stay	2 days	range 0-29 days

PB2442 | Anticoagulant Therapy for Splanchnic Vein Thrombosis in Patients with Liver Cirrhosis: A Systematic Review and Meta-Analysis

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Background: The clinical course of patients with liver cirrhosis may be complicated by the occurrence of splanchnic vein thrombosis (SVT). Anticoagulant treatment for these patients is challenging due to the increased risk of gastrointestinal bleeding of cirrhotic patients.

Aims: In this systematic review and meta-analysis, we evaluated the efficacy and safety of anticoagulation in cirrhotic patients with SVT.

Methods: Studies reporting the rate of SVT recanalization and progression, recurrent VTE, major bleeding, and overall mortality were searched through MEDLINE, EMBASE, and clinicaltrial.gov up to December 2019. Pooled proportion and risk ratio (RR) with corresponding 95% confidence intervals (CI) were calculated using a random-effects model.

Results: A total of 1,609 patients were included in 27 studies (24 observational studies and 3 randomized controlled trials). In patients receiving anticoagulant therapy, the rate of partial or complete SVT recanalization was 71% (95% CI, 64-77), the rate of SVT progression was 6% (95% CI, 4-8), and the rate of recurrent VTE was 11% (95% CI, 5-22). Major bleeding occurred in 8% (95% CI, 5-11) and overall mortality in 9% (95% CI, 5-14) of patients.

In patients who remained untreated, the rates of SVT recanalization, SVT progression, recurrent VTE, major bleeding, and overall mortality were 26% (95% CI, 9-54), 24% (95% CI, 9-53), 15% (95% CI, 8-26), 16% (95% CI, 12-21), and 22% (95% CI, 15-33), respectively. The rate of SVT recanalization (RR 3.06; 95% CI, 1.28-7.32) was significantly higher while the rates of thrombosis progression (RR 0.24; 95% CI, 0.12-0.51) and major bleeding (RR 0.66; 95% CI, 0.45-0.96) were significantly lower in patients receiving anticoagulation compared with untreated patients.

Conclusions: Anticoagulant therapy seems to improve SVT recanalization and to reduce SVT progression without increasing the risk of major bleeding. The incidence of recurrent VTE during anticoagulation remains substantial.

PB2443 | Analysis of Outcomes Following Inferior Vena Cava (IVC) Filter Placement in a Single Center Population

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Background: IVC placement have increased in the last years despite uncertainty about their efficacy and safety. They are usually indicated for patients with diagnosis of proximal VTE with contraindication for therapeutic anticoagulation.

Aims: To retrospectively evaluate the use of IVC filter in a single center from Buenos Aires, Argentina.

Methods: We retrospectively reviewed data from 56 patients from June 2013 to October 2018. Analysis included: risk factor for vte, reason for filter placement, removal, overall retrieval rates, causes for no removal, complications and mortality.

Results: Overall, 56 IVC filters were placed. Mean age was 61 years old (21-87), 58.9% patients were male. 29 patients (51.7%) had diagnosis of VTE at admission, 10 (17.8%) PE and 17 (30.3%) VTE/PE. Risk factors for VTE were as follows: 27 patients (48%) had active cancer, 14 (25%) immobilization, 9 (16%) recent surgery (within 4 weeks), 2 (3.5%) known thrombophilia and 4 (7.4%) had no risk factors for VTE. Retrievable filter were used in 55 patients (98.21%) and permanent in 1. Indication for placement were: 41 cases (74%) contraindication for anticoagulation, 13 (23.21%) bleeding after initial anticoagulation and 2 (1.82%) anticoagulation failure. 13 filters (23.21%) were successfully retrieved with a mean implantation time of 22 days (10-68days). Retrieval was not attempted in 43 (76.7%) patients; in 25 (58.1%) as a result of ongoing contraindication to anticoagulation, lack of material for retrieval in 1 (2.3%) and unknown reason in 17 patients (39%). Bleeding complications were reported in 2 patients. 10 patients (17.8%) died during admission, 2 because of filter placement or retrieval complications respectively.

Conclusions: We observed a low retrieval, complications and death rate. IVC filters are useful in certain situations. This study has a small number of patients, lager studies are needed to determine their impact in health care.

PB2444 | Single Centre Experience of DOAC Use in Patients with Antiphospholipid Syndrome (APS) with Venous Thromboembolism

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Background: Current guidelines for management of antiphospholipid syndrome (APS) recommend patients with unprovoked thrombosis have lifelong anticoagulation to prevent recurrence. This has been mainly with vitamin K antagonists (VKAs), but recently, direct oral anticoagulants (DOACs) have been used for management of venous thromboembolism (VTE)

There is limited data regarding efficacy and safety of DOACs in APS. A randomised trial comparing VKAs with rivaroxaban in 'triple positive' APS was stopped early due to high rates of arterial thrombosis and bleeding with rivaroxaban. Subsequently, the EMA issued recommendations to avoid DOACs in APS.

Aims: To evaluate use of DOACs in patients with APS with VTE at a tertiary level specialist centre.

Methods: We reviewed outcomes of patients at our institution during 2019, who had APS according to International Consensus statement criteria, and had taken a DOAC for prevention of recurrence following a diagnosis of VTE.

Results: 47 patients were included; 35 male, 12 female, median age 53 (range 26-81) years. 24 (51%) had single, 11 (23%) double, and 12 (25%) triple antiphospholipid positivity.

Median time of use with DOAC was 42 months (range 3-72). 29 (62%) had initial treatment with warfarin, 1 with LMWH (2%) and 17 with DOACs (36%). Patients took warfarin for a median time of 12 months (range 6 - 324), prior to switching to DOACs (rivaroxaban n=41, apixaban n=5, dabigatran n=1). All patients were counselled regarding DOACs in APS, 5 switched back to warfarin (3 with triple positive APS).

1 patient experienced VTE recurrence (upper limb DVT) but had thoracic outlet syndrome and one had an episode of thrombophlebitis. There were no major bleeding events.

Conclusions: We have demonstrated DOAC use in a patient cohort with predominantly single or dual aPL positivity and previous VTE that their use appears to be efficacious and safe. Further study is however required to confirm these findings.

PB2445 | Anticoagulation in Cirrhosis: Thrombin Generation Assay vs Anti FXa Activity

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Background: Anticoagulation in cirrhotic patients is a complex issue since different variables must be considered, such as the decrease in factors and inhibitors of coagulation, as well as alterations in the fibrinolytic system and platelet function. Basic coagulation tests do not interpret the hemostatic rebalancing in these patients, but thrombin generation assay (TGA) allows to assess this hemostatic condition. When cirrhotic patients are treated with enoxaparin, dose adjustment is made with anti FXa activity test (AXa). However, this technique modifies "ex vivo" the particular hemostatic condition of cirrhosis and may underestimate the level of enoxaparin.

Aims: To determine the usefulness of TGA and AXa in monitoring an obese cirrhotic patient treated with enoxaparin

TABLE 1 Thrombin generation in cirrhotic patient

	LT min	ETP nM/min	Peak nM	TTP min
Basaline	2.0	1160.4	165.6	4.0
Enoxaparin 100 mg/12h	4.0	519.8	55.6	8.0
Enoxaparin 120 mg/12h	17.7	14.4	0.9	24.5

Methods: A female patient (55 y); BMI 41.6 kg/m^2 with diagnosis of cirrhosis Child Pugh B score and portal vein thrombosis treated with enoxaparin 100 mg/12h and then with 120 mg/12 h.

A complete hemostatic screening including AXa was determined. TGA was measured by fluorometric method evaluating: lag time (LT), peak time (TTP), endogenous thrombin potential (ETP) and thrombin peak (peak).

Results: AXa at 100 and 120 mg of enoxaparin were 0.37 and 0.46 IU/ml, respectively, without reaching the desired therapeutic range (0.7-1.2 IU/ml). A significant decrease in ETP (45%) and peak (34%), as well as prolongation in LT and TTP (200% for both) were achieved at enoxaparin 100 mg. At 120 mg enoxaparin dose, a greatly decrease in thrombin generation was observed. Results are summarized in the table.

Conclusions: TG proved to be an effective functional test to assess the enoxaparin treatment, while aFXa activity underestimated anticoagulation, which led to the unnecessary dose increase in an obese cirrhotic patient.

PB2446 | Investigation of the Optimal Dose of aPCC in Reversing the Effect of Factor Xa Inhibitors Rivaroxaban and Apixaban- an in vitro Study

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Background: Direct acting oral anticoagulants (DOACs) including Factor Xa inhibitors, are safe and effective alternatives to warfarin and the prescribing rate is increasing. There have been concerns about how to reverse their effect in case of serious bleeds or need for emergency surgery. The antidote andexanet alfa was introduced in 2018, but is still not available in all parts of the world. There are few clinical studies evaluating the reversing effect of activated prothrombin complex concentrate (aPCC) and the optimal dose is not known. Because of the feared thrombogenicity of aPCC, administration of a low dose is desireable.

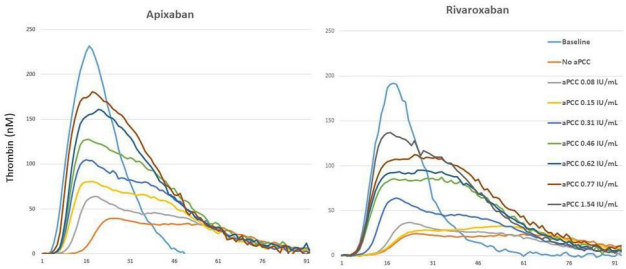

FIGURE 1 Thrombin generation in apixaban- and rivaroxaban-spiked samples. aPCC causes a concentration-dependent increase in thrombin generation

Aims: To find the lowest concentration of aPCC sufficient to reverse the effect of rivaroxaban and apixaban.

Methods: Whole blood from healthy volunteers was spiked with apixaban or rivaroxaban to mimic peak concentration, and then aPCC in different concentrations ranging from 0.08-0.77 IU/mL (corresponding to doses of 5-50 IU/kg) was added. For rivaroxaban an additional dose of 1.54 IU/ml was tested. One aliquot was left untreated (baseline). We used thromboelastometry with minimal tissue factor activation and thrombin generation assay to assess the reversal effect of the different concentrations of aPCC.

Results: Apixaban and rivaroxaban increased thromboelastometry clotting time (CT) and reduced thrombin generation. aPCC improved these parameters in a concentration-dependent manner. For apixaban, aPCC at a dose of 0.15 IU/mL shortened CT to baseline level and for rivaroxaban the dose shortening CT to baseline-level was 0.46 IU/mL. The thrombin generation parameter peak thrombin concentration was not fully restored by aPCC at any dose, but both time to peak and endogenous thrombin potential (ETP) were back at baseline level at a dose of 0.46 IU/mL for both apixaban and rivaroxaban (Figure 1).

Conclusions: aPCC seems to reverse the effect of apixaban and rivaroxban at doses of 0.46 IU/mL or below.

PB2447 | Time in Therapeutic Range in Venous Thromboembolic Diseases in a North African Geriatric Population

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Background: Despite the rise of direct oral anti-coagulants, vitamin K antagonists (VKA) still dominate clinician prescriptions in venous thromboembolic disease (VTED), mainly in low- and middle-income countries. Maintaining INR within the therapeutic range is a real challenge especially for elderly patients who are often polymedicated and non-autonomous. The lower risk efficacy of VKA treatment appears to be favorable when the time spent in the therapeutic range (TTR) is ≥ 65%.

Aims: The aim of this study was to evaluate the stability of the INR of patients over 65 years old followed for VTED through the TTR.
Methods: This was a prospective, longitudinal and descriptive study involving patients over 65 years old, admitted for VTED treated by VKA in the internal medicine department between January 2015 and December 2017. The TTR was calculated by the Rosendaal method.
Results: Twenty-two patients were included. The average age was 75 years old [65-96]. The gender ratio was 0.69 with 13 (59%) men and 9 (41%) women. Twenty-one (96%) patients had deep vein thrombosis and one patient (4%) had pulmonary embolism. The mean TTR of the studied population was 40.5% [2.2%-77.6%]. Four (18%) cases of hemorrhagic complications and 4 (18%) cases of thrombotic complications were noted with mean TTRs of 19.5% and 41.5%, respectively. At the control imaging, 36% (8 patients) of the total cases had totally recanalized and 64% (14 patients) had partially recanalized with mean TTRs respectively of 33.8% and 44.3%.
Conclusions: TTR is a pertinent therapeutic safety factor that could be a marker of cognitive decline in the elderly population.

PB2448 | Clinical Characteristics and Outcomes of Non-cancer, Non-CVC Associated upper Extremity DVT

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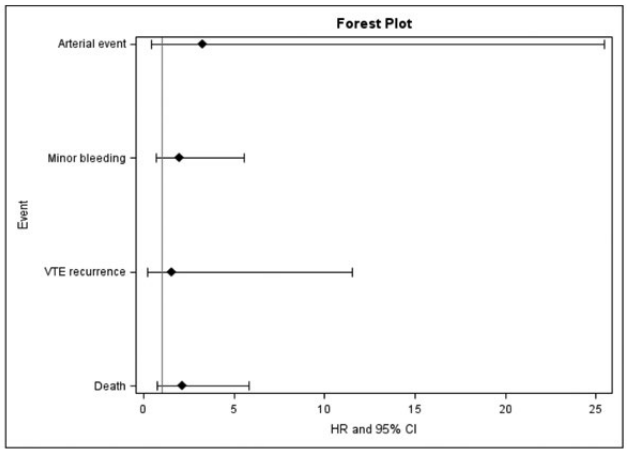
Background: Upper extremity deep venous thrombosis (UEDVT) is rare, and typically associated with cancer and central venous catheters (CVCs). Risk factors, management and outcomes of UEDVT outside the context of cancer and CVCs are not well characterized.
Aims: Compare the clinical characteristics, risk factors, management and outcomes of patients with UEDVT not associated with cancer or CVC, with those of patients with non-cancer, non-CVC associated lower extremity DVT (LEDVT) enrolled in the Survey on anticoagulaTed pAtients RegisTer (START2-Register).



	LEDVT			UEDVT		
	n	Incidence (per 100 PY)	HR (95%CI)*	n	Incidence (per 100 PY)	HR (95%CI)*
Death	76	2.5 (2.0; 3.1)	1.0 (ref.)	4	4.6 (1.7; 12.2)	2.1 (0.8; 5.8)
Recurrence VTE	21	7.0 (4.5; 10.7)	1.0 (ref.)	1	1.1 (1.6; 8.1)	1.5 (0.2; 11.5)
Major Bleeding	30	0.9 (0.7;1.4)	1.0 (ref.)	0	NE	NE
Minor Bleeding	58	2.0 (1.5; 2.6)	1.0 (ref.)	4	4.8 (1.8; 12.7)	2.0 (0.7; 5.5)
Arterial events	11	0.4 (0.2; 0.7)	1.0 (ref.)	1	1.1 (0.2; 8.1)	3.3 (0.4; 25.5)

* Incidence rate and Hazard ratio with 95% confidence interval by Cox regression model,
NE = Not estimable,

FIGURE 1 Clinical outcomes in non-cancer, non-CVC patients with UEDVT vs. LEDVT enrolled in the START2-Register

Methods: The START2-Register (ClinicalTrials.gov Identifier: NCT02219984) is an observational, multicenter, prospective cohort study that includes adult patients (>18 years) who start anticoagulation therapy for various indications. Patients with cancer, history of cancer and CVCs were excluded from analysis. Of the 6,835 patients enrolled in the register, for the purpose of this analysis 2,174 patients were included for whom data were collected between September 2010 and November 2019.
Results: Eighty nine patients with UEDVT were compared with 2,085 patients with LEDVT. Patients with UEDVT were significantly younger (51.7 vs. 63.3 years, p-value< 0.0001) and were more likely to receive extended parenteral anticoagulant treatment with LMWH

TABLE 1 Baseline characteristics of non-cancer, non-CVC patients with UEDVT vs. LEDVT enrolled in the START2-Register

		All patients		LEDVT		UEDVT		
N		2174		2085		89		
Follow-up (months)	Median (range)	12.2	(0-75)	12.3	(0-75)	7.3	(0.2-59.3)	P-value
Age (years)	Mean (SD)	62.8	±18.1	63.3	±17.9	51.7	±20.1	<0.0001
Treatment								
VKAs	n (%)	1135	52.2	1084	52	51	57.3	0.33
LMWH	n (%)	45	2.1	39	1.9	6	6.7	0.002
DOACs	n (%)	994	45.7	962	46.1	32	36	0.06
Thrombophilia	n (%)	279	12.8	267	12.8	12	13.5	0.85
Previous VTE	n (%)	503	23.1	487	23.4	16	18	0.24

(6.7% vs. 1.9%, p-value=0.002). There was a trend towards preferring oral treatment with VKAs (57.3% vs. 52%, p-value=0.33) rather than with DOACs in patients with UEDVT as compared to patients with LEDVT (36% vs. 46.1%, p-value=0.06) (Table 1).

After a median follow-up of 12.2 months (IQR 5.9-25.1 months), all-cause mortality rates (incidence rate 4.6 vs. 2.5 per 100 person-years, HR 2.1, 95% CI 0.8-5.8) and arterial events rates (incidence rate 1.1 vs. 0.4 per 100 person-years, HR 3.3, 95% CI 0.4-25.5) were non-significantly higher in UEDVT patients than in LEDVT patients (Figure 1).

Conclusions: Outside the context of cancer and CVCs, patients with UEDVT are younger, and show a tendency towards higher all-cause mortality and arterial event rates than LEDVT patients.

PB2449 | Venous Recanalization after a Thromboembolic Event in a North Afriacan Population

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Background: Venous thromboembolic disease (VTED) is a common and multifactorial disease. The determination of the optimal duration of anticoagulation is a challenge for clinicians.

Aims: The aim of this study was to assess the prevalence of recanalization in control imaging of patients followed for VTE at the end of treatment with vitamin K antagonists (VKA).

Methods: A prospective, longitudinal and descriptive study involving patients with VTE treated by VKA was conducted in an internal medicine department between January 2015 and December 2017. Control imaging was performed for each patient at the end of the agreed period of anticoagulation. The studied population was subsequently divided into two groups according to the absence or presence of venous recanalization in the control imaging in order to investigate its influencing factors.

Results: Sixty patients were included whose average age was 56 years. Twenty-nine patients were male. Eighty percent (n=53) of patients lived in urban areas and 12% (n=7) lived in rural areas. Fifty (83%) patients had deep vein thrombosis, 9 (15%) had pulmonary embolism, and one had superficial thrombophlebitis. Twenty one (35%) were smokers and 19 (32%) were obese. Two patients had a family history of VTED and 3 had a personal history of VTED. 17% of patients had varicose veins in the lower limbs. Hypothyroidism was noted in 4 (7%) patients and active cancer in 2 (3%) patients. 22% (n=13) of patients had recent surgery and 3 (5%) patients had an orthopedic cast. At the control imaging, 65% (n=39) of the patients had totally recanalized and 33% (n=20) had partially recanalized. A complete absence of recanalization was noted in one case. Only the urban / rural living environment had a statistically significant effect on venous recanalization.

Conclusions: Control imaging is not common during therapeutic follow-up of VTE cases. However, it may help to optimize the duration of anticoagulation.

PB2450 | Antithrombin III Levels Immediately Following Acute Venous Thromboembolism

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Background: Up to 40% of patients treated for acute venous thromboembolism (VTE) with a standard dose of unfractionated heparin (UFH) never reach therapeutic anticoagulation within the first 48 hours of treatment. Heparin binds and activates the enzyme inhibitor antithrombin III (AT), which then inactivates thrombin, factor Xa and other proteases. Low AT activity in the setting of VTE could explain the difficulty in achieving therapeutic UFH anticoagulation for some patients. However, the proportion of patients with low AT levels immediately after VTE diagnosis is not known.

Aims: Quantify the proportion of patients with VTE who have low AT activity immediately after diagnosis.

Methods: We performed a secondary analysis of a multicenter study of patients with VTE diagnosed in emergency departments of two university hospitals between 2016-2018. The study was approved by the Human Research Committee of Partners Healthcare. All patients had hypercoagulability testing performed < 24h after VTE diagnosis to avoid confounding by heparin treatment. We used descriptive statistics to calculate proportions of patients with AT activity < 80U/dL.

Results: We enrolled 378 patients. The mean age was 54 (SD=17) years and 223 (59%) were male. Overall, 46/378 (12%) VTE, including 17/161 (10%) deep vein thrombosis and 29/210 (14%) pulmonary embolism patients had AT levels below < 80U/dL.

Conclusions: AT deficiency is relatively common in patients with acute VTE; and >200 times more prevalent than hereditary AT deficiency. Low AT levels associated with acute VTE may contribute to the difficulty of therapeutic anticoagulating VTE patients using heparin.

PB2451 | Concentration Levels of Direct Oral Anticoagulants and Clinical Relevance in a Tertiary Hospital

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Background: Availability of tests for measuring levels of direct oral anticoagulant (DOAC) is increasing in hemostasis laboratories and may be useful for patient management in several clinical situations, namely bleedings, thrombosis or before urgent invasive procedures, as some studies point out that DOAC levels can correlate with thrombotic or hemorrhagic events.

Aims: We aim to assess the usefulness of the DOAC concentration levels and actions taken after the concentration results.

Methods: We performed a retrospective analysis including all the DOAC level measurements during 2019 at our hospital. We recorded age, gender, DOAC level, indication for anticoagulation and reasons for checking DOAC levels and actions taken upon.

Results: A total of 514 tests were performed in 434 patients. Patient characteristics according to their anticoagulant therapy are shown in table 1. The main reason for anticoagulation was atrial fibrillation (76.5%). The main reasons for level monitoring were bleeding, urgent invasive procedures or surgery and thrombosis (40.6%,33.6%,12.0%)..

As Figure 1 shows, there are significant differences in drug concentrations when comparing different clinical decisions. Patients who needed DOAC reversal agents had higher concentration levels.

When analyzing each DOAC, no significant differences were found in the therapeutical approach after assessment of DOAC level. Patients without clear indication for DOAC levels evaluation had fewer changes in their therapy (dose or change).

Patients with history of chronic kidney disease (CKD) showed similar drug concentration levels as those without CKD. Patients with acute kidney injury (AKI), when compared with those without AKI, had higher DOAC concentrations (median level 123*vs.*73ng/mL), were more frequently given reversal agents (26.8*vs.*17.0%), and resume anticoagulation less frequently (2.4*vs.*12.4%).

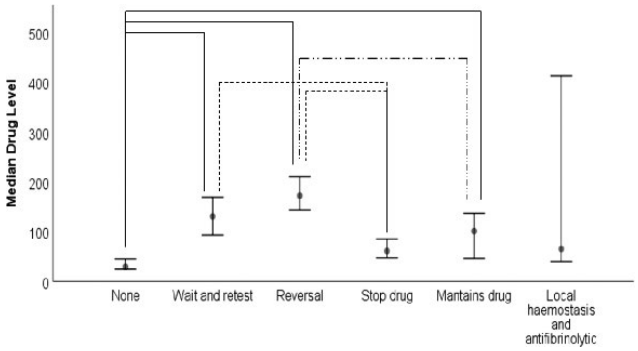

FIGURE 1 Median and 95% CI of the DOAC level for each therapeutic decision. Connecting lines represent p<0.05 between two groups

Conclusions: The evaluation of DOAC levels, integrated into the patient's clinical situation, can be useful in the decision to manage anticoagulation, in different clinical scenarios. CKD does not interfere with DOAC levels, unlike AKI which greatly increases DOAC levels.

PB2452 | Antithrombotic Stewardship: A Novel Approach to Appropriate Anticoagulant Prescription

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Background: Anticoagulants are a leading cause of morbidity among hospitalized patients. The advent of direct oral anticoagulants (DOACs) has led to further occasions for prescribing errors, thus adverse events. Literature describing the effect of antithrombotic

TABLE 1 Characteristics of the population according to the prescribed DOAC

Variable	Apixaban n=186	Dabigatran n=86	Edoxaban n=44	Rivaroxaban n=118
Gender, male, n (%)	75 (40.3)	45 (52.3)	27 (61.4)	59 (28.6)
Age, years, median [IQR]	81 [77.0;86.0]	81 [74.0;87.0]	74 [67.3;83.7]	79 [71.5;84.0]
Drug level, median [IQR]	122.5 [52.8; 225.8]	117 [38.8; 236.3]	61 [20.0; 122.8]	38 [25.0; 129.0]
Motive - bleeding n (%)	71 (38.2)	34 (39.5)	23 (52.3)	48 (40.7)
Motive - thrombosis, n (%)	16 (8.6)	14 (16.3)	4 (9.1)	18 (15.3)
Motive - urgent Invasive procedures and surgery n (%)	72 (38.7)	23 (26.7)	10 (22.7)	41 (34.7)
Reversal of DOAC, n (%)	43 (23.2)	13 (15.7)	6 (14.3)	17 (14.7)
Chronic kidney disease, n (%)	37 (19.9)	12 (14.0)	5 (11.4)	18 (15.3)
Acute kidney injury, n (%)	38 (20.4)	16 (18.6)	8 (18.1)	21 (17.8)

stewardship programs (ASP) is scarce, despite the vast breadth of data demonstrating effectiveness the antibiotic realm. We hypothesize that the implementation of a multidisciplinary ASP leads to more appropriate anticoagulant prescriptions.

Aims: To determine the effect of a multidisciplinary ASP on anticoagulant prescription among inpatients in a Canadian tertiary care center. Secondary outcomes include the incidence of bleeding, thromboembolism, mortality during hospital stay, and length of stay.

Methods: We performed a retrospective cohort study of all patients prescribed therapeutic anticoagulants on select hospital units between September 1st, 2019 and January 31st 2020, at the Jewish General Hospital in Montreal, Canada. Our ASP was implemented on June 1st, 2019. Patient-, anticoagulant-, admission- related characteristics were collected. Effect of ASP was measured as the proportion of adjusted prescriptions following a recommendation by the team. Recommendations in compliance with Canadian best practice guidelines were made if prescriptions were suboptimal; they were grouped into descriptive categories.

Results: A total of 447 inpatients were prescribed therapeutic anticoagulation during the study period. Among them, 301/447 (67%) required a recommendation; 247/301 (82.1%) led to an adjustment. The total proportion of adjusted prescriptions was 247/447 (55%). The most frequent types of recommendations were warfarin dose adjustment (18%), laboratory test orders (13.3%), and DOAC dose adjustment (9%).

Conclusions: The implementation of an antithrombotic stewardship program leads to optimization of anticoagulant prescriptions. Further research measuring the effect of such programs on clinical outcomes such as bleeding and thromboembolism is needed.

PB2453 | Management Strategy of Venous Thrombosis in a Dialysis Patient with History of Heparin Induced Thrombocytopenia

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Background: In patients with end stage renal disease, the risk of thrombotic events increases. Anticoagulant treatment for these patients are also challenging due to altered pharmacokinetics, increase risk of bleeding and limited evidence based data due to exclusion of these patiens in clinical trials. In patients with history of HIT the challenge is higher.

Aims: To describe a case report of a patient on hemodialysis, history of HIT with emergency access with subclavian vein and treatment of venous thrombosis in subclavian vein.

Methods: A case report of 56 years old female with Diabetic Kidney Disease came with dyspnea due to metabolic acidosis. Hemodialysis performed with access through right subclavian vein. Two days post hemodyalisis showed swelling in both arms, the right one more severe. The D Dimer increased and Doppler US showed thrombosis in subclavian vein. Active cancer and arrhythmia were rule out. Fondaparinux 2.5 mg started for 7 days. The swelling subsides and the subclavian cathether were replaced with AV shunt. No subsequent bleeding and the patient were discharged on day 9 with scheduled regular HD with Fondaparinux as anticoagulation.

Results: Fondaparinux safety in renal failure is limited, however the constraint of anticoagulant drugs availability force the use of it. Case reports showed fondaparinux can be used in hemodyalisis patient with heparin induced thrombocytopenia. Fondaparinux adjust dose doses ranged from 2.5 mg every 48-72 hours to 0.03mg/kg daily, with several studies adjusting doses with anti-Xa levels.

Conclusions: Fondaparinux may be a reasonable treatment option for patients with VTE on dialysis.

PB2454 | A Case Report of Deep Vein Thrombosis after Kidney Transplantation

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Background: One of the serious surgical complication after kidney transplantation is Deep Vein Thrombosis (DVT). In kidney transplant recipient there is an imbalance of hemostatic system, leading to persistent hypercoagulable state and increased risk of thrombotic disease.

Aims: Purpose of this case is to discuss about the DVT as a complication of kidney transplantation.

Methods: We report a case of 43 years old male patient with end stage of kidney disease underwent kidney transplantation from living donor. He received Basiliximab as an induction therapy and maintained on tacrolimus, mycophenolate mofetil and steroid. On the third day of transplantation, the patient suffered from pain and edema on the right lower limb. Doppler examination showed partial thrombus of the right external iliac vein and total thrombus of proximal profunda and superficial femoral vein. The laboratory results showed high D Dimmer level (8310 ng/mL). He was treated with unfractionated heparin and underwent catheter-directed thrombolysis (CDT). The patient′s renal function recover after five days of therapy. The creatinine level was getting lower. At one year post kidney transplantation, the patient′s condition remain stable with no limb edema and normal level of serum creatinine.

Results: More than 90% of the DVT in general surgical patients occur within the 1st week after surgery. Most of the early thrombotic events that occur in this setting can be attributed to technical failures, external compression, preexisting asymptomatic DVT, or thrombophilia. The cause of hypercoagulable state in kidney transplant recipients is multifactorial. They include the use of

immunosuppressive agents, presence of antiphospholipid antibody, native kidney disease and infection. The high result of D-Dimer supports the diagnosis of Deep vein thrombosis.

Conclusions: This case report presented a DVT after kidney transplantation diagnosed using Doppler and supported with D-Dimer examination. Catheter-Directed Thrombolysis can fix this condition and recover the kidney function.

PB2455 | Extended Treatment in Unprovoked Venous Thromboembolism: What Is Important to Patients?

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Background: After the initial three months of anticoagulation for unprovoked venous thromboembolism (VTE), a decision must be made to stop or continue indefinitely. Patient's wishes are ideally taken into account during a risk-benefit analysis through shared decision-making (SDM). However, it is unknown how to do this since patients' perspectives on treatment duration and SDM have not been studied in unprovoked VTE.

Aims: To explore patients' perspectives on VTE, patient education and their understanding of the risk-benefit consideration of extended anticoagulation; to improve SDM in practice.

Methods: Semi-structured interviews were conducted between May 2019 and February 2020 with adults with unprovoked VTE in one university hospital and one general hospital. Interviews were audio-recorded and transcribed verbatim. Data were analyzed using conventional content analysis. Data collection is ongoing until data saturation is reached.

Results: Interviews of nine patients aged 44-83 years have been analyzed thus far. Three major themes were identified (Figure 1). Coping strategies and opinion on medical care suffused throughout the major themes. The narratives demonstrated a need for individualized care, clear information about treatment options and associated (dis)advantages and insight into physician's considerations.

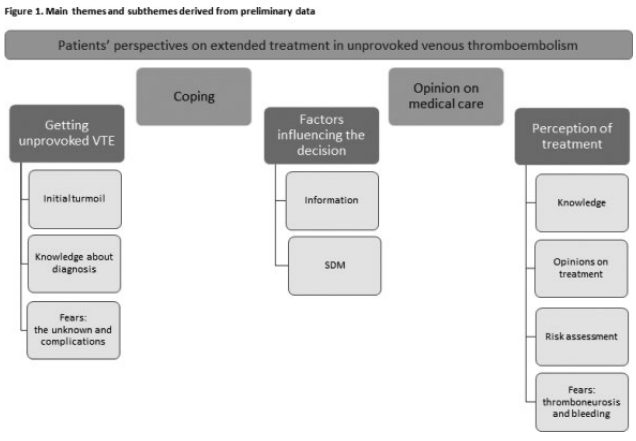

FIGURE 1 Main themes and subthemes derived from preliminary data

Some experienced a lack of information and SDM. They were unaware of the choice at hand as physicians only proposed a treatment plan without explaining other options. This often led to frustration, anxiety, dissatisfaction with received care and distrust in treatment. Patients who experienced SDM and found the received information sufficient were more content with received care and trusted the efficacy of anticoagulation. Perceived disadvantages of extended treatment were heterogeneous and sometimes based on misconceptions.

Conclusions: Preliminary findings show that sufficient information and adequate SDM are of crucial importance for patients when deciding on treatment duration after unprovoked VTE.

Further analyses are ongoing to identify additional themes and verify findings to provide practical tools to improve SDM in practice.

PB2456 | The Increasing Bleeding Risk from Drug-drug Interactions in Patients with Direct Oral Anticoagulants

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Background: Drug-drug interactions (DDIs) are a concern for patients and providers, as polypharmacy is becoming more common in managing complex diseases or comorbidities. Direct oral anticoagulants (DOACs) have the potential for DDIs linked to increased bleeding.

Aims: This study evaluated the bleeding risk when the drugs of potential DDIs were simultaneously prescribed with DOACs.

Methods: From January 2014 to December 2016, We included new users of DOACs in patients with non-valvular atrial fibrillation (AF) and venous thromboembolism (VTE) and analyzed the actual rate of bleeding according to the number of drugs with potential DDIs. CHA_2DS_2-VASc score and Charlson Comorbidity Index (CCI) were assessed as covariates.

Results: Among 115,362 patient with AF or VTE who were newly administered DOACs (median age, 73 years [range: 19-108]; men, 53.0%; AF, 81.9%; DOAC exposure: rivaroxaban, 48.7%; dabigatran 24.5%; apixaban 21.2%, and edoxaban,5.6%). A total of 7001 any bleeding (16.5%) and 2283 major bleeding (2.0%) events occurred with DOAC prescriptions. In multivariable logistic regression analysis showed that CHA_2DS_2-VASc score, CCI, and number of DDIs were significantly associated with bleeding events: as the score increased. In major bleeding events, CHA_2DS_2-VASc scores 4 or more (OR =2.35; 95% CI, 1.86-2.97, $P < 0.0001$], and number of DDIs with 2 or more prescribed medicines (OR = 3.73; 95% CI, 3.36-4.15, $P < 0.000$) were associated with significant increase in risk of major bleeding. The rates of exposure to DDI drugs in any bleeding and major bleeding were 56.7% and 66.1%, respectively. The most common drugs of DDIs showed the similar distributions; non-steroidal anti-inflammatory drugs,

antiplatelet agents, diltiazem, amiodarone, and selective serotonin reuptake inhibitors were frequently prescribed.

Conclusions: Physicians prescribing DOAC medications for AF or VTE need to know the increasing bleeding risks associated with the number of DDIs drugs regardless of comorbidities.

PB2457 | Improvements in Short- and Long-term Outcomes in Patients with Pulmonary Embolism after Outpatient Pulmonary Rehabilitation

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Background: Patients with pulmonary embolism (PE) may suffer from long-term consequences, including decreased functional capacity with chronic exercise limitations. Data on pulmonary rehabilitation (PR) in patients with PE are scarce, and no data about effects of outpatient PR in PE patients are available so far.

TABLE 1 PE characteristics and Comorbidities

Variable	No. with data (n=22)	Count (%)
PE characteristics		
DVT present at index event	21	12 (57.1%)
Unprovoked PE	22	13 (59.1%)
Provoked PE	22	9 (40.9%)
Comorbidities		
History of venous thromboembolism	22	7 (31.2%)
Arterial hypertension	22	10 (45.5%)
Chronic obstructive pulmonary disease	22	2 (9%)
History of acute lymphatic leukemia	22	1 (4.5%)

Aims: To investigate the effect of outpatient PR in PE patients with persisting symptoms.

Methods: We analyzed data of 22 PE patients, who attended outpatient PR between 2012 and 2019. The reason for admission to PR was PE with exertional dyspnea in all patients. Patients underwent a multi-professional and individualized 6-week PR program. Three times a week for 3-4 hours participants completed exercise training, physiotherapy, and patient education seminars. The primary outcome was change in 6-minute walk test (6MWT) after completion of PR. Secondary outcomes included assessments of cycle ergometer exercises (peak performance, constant work rate test), upper and lower extremity strength and inspiratory muscle strength performed at admission and discharge of PR. To assess long-term benefits, follow-up was performed in median 39 months after PR.

Results: Patients started PR in median 19 weeks after the acute PE event. Their median age was 47.5 (interquartile range 42.5-54.3) years, 7 (32.8%) were women and all presented with NYHA class II and higher. After PR, patients showed significant and clinically relevant improvements in 6MWT (mean difference: 49.4m [95% confidence interval 32.0, 66.8]). Similarly, patients increased performance of cycle ergometer exercises and inspiratory muscle strength (data shown in *table 2*). At long-term follow-up, 78% of patients reported improved health status when asked to compare it to their status before admission to PR.

Conclusions: We observed significant improvements in exercise capacity in PE patients undergoing outpatient PR. The majority of patients reported also a long-term improvement in health status. Prospective studies are needed to identify patients who would benefit most from a structured PR.

PB2458 | Efficacy of Reversible Factor Xa Inhibitors is Related to the Level of Factor VIII: Best Practice is Adjustment of the Dose for Factor VIII Level?

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Background: Reversible factor Xa inhibitors (Xa-DOACs) inhibit in clot growth specifically the factor Xa generated by factor IXa /VIII.

TABLE 2 Exercise parameters at admission to outpatient pulmonary rehabilitation and reassessment at completion of pulmonary rehabilitation

Assessment (Measurement)	No. with data (n=22)	Admission	Discharge	Difference (Percent)	p-value
6-minute walk test	20	556.1 (±104.8)	605.5 (±96.0)	+ 49.4 (9%)	< .001
1-minute sit-to-stand test	14	35.1 (±12.6)	39.0(±14.3)	+ 3.9 (11%)	.034
Maximal inspiratory pressure	22	94.7 (±30.4)	125.2 (±27.0)	+ 30.5 (32%)	< .001
Maximal Workload (Watt)	18	156.8 (±63.6)	188.5 (±57.1)	+ 31.7 (20%)	< .001
Constant work rate test (min)	14	12.7 (±6.7)	21.2 (±7.7)	+ 8.5 (67%)	.002
Lower extremity strength (kg)	11	117 (±17.9)	146.9 (±16.5)	+ 29.9 (26%)	< .001
Upper extremity strength (kg)	16	34.9 (±12.6)	44.5 (±11.7)	+ 9.6 (28%)	< .001

Aims: We evaluated the influence of levels of factor VIII on clot growth and inhibition by Xa-DOACs.

Methods: Clot growth analysis with the thrombodynamics methods, spiking with Xa-DOACs, apixaban, rivaroxaban and edoxaban. Variation in factor VIII by spiking with Advate.

Results: Inhibition of clot growth and clot size with Xa-DOACs was biphasic with a fixed plateau for all three Xa-DOACs. The inhibition in factor IX or VIII deficient plasma was nihil. This identifies the residual activity from tissue factor/factor VIIa activation and inhibition of specifically the activity of factor Xa generated by factor IXa / VIII. Increase in factor VIII up to 300% showed a dose dependent increase in the inhibitable portion only.

Inhibition by Xa-DOACs was different for high and low factor VIII. For instance, with apixaban the inhibition was optimal for 0.1 μM/l apixaban when factor VIII was lower than 100%; while 0.3-0.5 μM/l was needed for factor VIII > 100%.

Conclusions: The inhibition by Xa-DOACs is dependent upon the factor VIII level. The standard clinical dose is expected to inhibit to much lower residual activity at low factor VIII compared to higher residual activity with higher factor VIII. It suggests that the dose of Xa-DOACS may be fine-tuned to the factor VIII level. It should be tested whether this is best practice to reduce bleeding and thrombosis.

PB2459 | Drug-Drug Interactions between Direct Oral Anticoagulants and Hepatitis C Direct-Acting Antiviral Agents: A Systematic Review

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Background: Direct oral anticoagulants (DOACs) are widely used for treatment of venous thromboembolism and non-valvular atrial fibrillation. Due to predictable pharmacokinetics, they do not require routine coagulation monitoring. However, DOACs are substrates of CYP3A4 and/or P-glycoprotein, making them susceptible to drug-drug interactions (DDI). Hepatitis C direct-acting antiviral agents

TABLE 1 Characteristics of the included studies

	Kosloski P et al	Boyle A et al	Ouwerkerk-Mahadevan S et al	Garrison KL et al
Journal and Year	J Pharmacol Exp Ther 2019	Journal of Hepatology 2019	Clinical Pharmacology & Therapeutics (CPT) 2018	Journal of Hepatology 2017
Quality	Full text	Congress poster (EASL*)	Congress poster (ASCPT*)	Congress poster (EASL*)
Study Type	Phase I	Retrospective Observational	Phase I	Phase I
Population	Healthy volunteers (11)	UK DOACs* + DAAs* patients (54) over 1 year. DOACs: 37 rivaroxaban, 14 apixaban, 2 dabigatran, 1 edoxaban	Healthy volunteers (15)	Healthy volunteers
Intervention	Dabigatran + glecaprevir/ pibrentasvir	None. Review of management strategies for DDI between DOACs and DAAs	Dabigatran + odalasvir/ simeprevir Sequential Administration	Dabigatran + sofosbuvir/ velpatasvir/ voxilaprevir
Comparator	No comparator	No comparator	No comparator	No comparator

TABLE 2 Summary of outcome measures in the studies included

	Kosloski P et al	Boyle A et al	Ouwerkerk-Mahadevan S et al	Garrison KL et al
Outcome measures	Dabigatran Cmax and AUCinf were 105% and 138% higher during coadimistration with glecaprevir/pibrentasvir compared with dabigatran alone	Management strategies: 24 (44.4%) clinical monitoring, 13 (24.1%) anti-Xa activity blood monitoring, 9 (16.7%) switch to LMWH, 7 (13%) switch DAAs, 1 (1.9%) wait until DOACs completed for finite courses. No serious bleeding reported	Dabigatran Cmax, AUClast and AUCinf increased 1.48-, 1.76- and 1.68-fold upon coadministration with odalasvir (day17), and 1.70-, 2.14- and 2.03-fold upon coadministration with odalasvir/simeprevir (day26), versus dabigatran alone (day1). No deaths and serious adverse events reported	Total dabigatran AUC was ↑161% following administration of sofosbuvir/ velpatasvir/ voxilaprevir with dabigatran

(DAAs), *via* P-glycoprotein inhibition, may increase DOACs exposure with relevant bleeding risk.

Aims: We performed a systematic review on DDI between DOACs and DAAs.

Methods: Two reviewers independently identified studies through Pubmed and Embase database until August 2019. We supplemented our search by handsearching of conference abstracts and Clinicaltrial.gov. According to prespecified selection criteria, studies evaluating the possible DDI between DOACs and DAAs were eligible.

Results: We identified 1314 references through systematic database search; 17 studies were retrieved in full and 4 articles were finally included after applying exclusion criteria; one available in full text and 3 congress abstracts (Table1). Three were phase 1 clinical studies in healthy volunteers, each assessing interactions between dabigatran and glecaprevir/pibrentasvir or odalasvir/simeprevir or sofosbuvir/velpatasvir/voxilaprevir. All of them showed an increase of dabigatran concentration during the co-administration with DAAs, with the area under the concentration-time curve (AUC) increased respectively by 138%, 103% and 161%. One was a retrospective observational study in patient taking DOACs and DAAs, with the aim of reviewing management strategies adopted by clinicians to handle DDI. Clinical monitoring was the most common strategy; no serious bleeding event were reported (Table2).

Conclusions: DAAs increase dabigatran concentration, as predicted from pharmacokinetics. Real world studies are needed to assess clinical relevance of this interaction and describe actual spectrum of possible DDI between DAAs and other DOACs.

PB2460 | Anticoagulation Control with the Point-of-Care INR: A Retrospective Pre-/Post-analysis

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Background: An improvement in the time within the therapeutic range (TTR) was obtained by monitoring the international normalised ratio (INR) by patients themselves with point-of-care (POC) devices. However, the use of POC in anticoagulation clinics was recently reported to be associated with significantly lower TTR than the standard laboratory INR.

Aims: To compare the TTR between laboratory and POC INR monitoring, both performed by healthcare professionals.

Methods: Retrospective review of consecutive adult patients on warfarin treatment attending the POC INR Anticoagulation Clinics at 7 Health Centres in Malta in the years 2014-2015, and prescribed with INR target ranges 2.0-3.0 or 2.5-3.5. For each patient, 3 timeframes were considered: 1) the last year of standard laboratory INR monitoring (pre-POC); 2) the first year of POC INR monitoring (initial-POC); 3) the results of year 2017 (stable-POC).

Results: 700 patients were analysed (median age 70 years, 56.6% males). Most common indications for warfarin were atrial fibrillation (72.4%), mechanical heart valve (15.6%), venous thromboembolism (12.6%). The analysis of the three timeframes included 471 patients and showed a significant increase of the median TTR (70.0% in the pre-POC period, 74.2% in the initial-POC, 77.2% in the stable-POC, $p< 0.001$). The median INR difference was 5.1% (95% CI, 2.2-7.0%) for time 2 vs. time 1; 6.6% (95% CI, 3.7-8.9%) for time 3 vs. time 1; 2% (95% CI, 0-3.4%) for time 3 vs. time 2. There was also a significant increase in the number of days between two INR tests ($p< 0.001$).

Conclusions: The use of POC devices in anticoagulation clinics was associated with 5.1% increase of the TTR in the short term (initial-POC) and 6.6% increase in the long term (stable-POC), compared to the standard laboratory INR monitoring. Our findings suggest that the immediate availability of INR results and consequent dose adjustment is associated with better anticoagulation control.

PB2461 | Association Between Index VTE Event and 12-Months Outcomes for Patients in Routine Clinical Practice Enrolled in the ETNA-VTE-Europe Registry

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Background: Edoxaban, a direct FXa inhibitor, is approved for the treatment and secondary prevention of acute venous thromboembolism (VTE). The efficacy and safety of edoxaban was evaluated in the randomized phase III trial Hokusai-VTE. ETNA-VTE-Europe is a prospective, single-arm, non-interventional, post-authorization

safety study designed to confirm the real-world safety and effectiveness of edoxaban.

Aims: To assess patient characteristics and evaluate the rates and associations between the index VTE event (pulmonary embolism [PE] with or without deep vein thrombosis [DVT] versus DVT alone) and 12-month outcomes.

Methods: A total of 2407 patients with acute symptomatic VTE who were receiving edoxaban in routine practice were enrolled across eight European countries. Patients with an index event of PE with or without DVT (PE±DVT) were compared with those with an index event of DVT without PE.

Results: Patients with acute PE±DVT had a slightly higher age, a higher percentage of frailty, more hypertension and a more frequent history of PE than patients with DVT only (Table 1). The patients with DVT alone had more history of chronic venous insufficiency and an increased history of prior DVT. VTE recurrence was 2.83%, rates of any bleeding 13.00%, stroke rate 0.83% and all-cause mortality 3.37%. Patients with index PE±DVT had numerically higher rates of all-cause mortality (4.08% versus 2.85%), cardiovascular mortality (1.69% versus 1.21%) and major bleeding (2.39% versus 1.57%) rates than those with index DVT alone (Table 2). Table 1: Patien… Table 2. Clini…

TABLE 1 Patient baseline characteristics according to type of index event 1-year follow-up analysis set [N=2407]

	Overall (N=2407)	PE±DVT (n=1004)	DVT alone (n=1403)
Female, n (%)	1121 (46.6)	485 (48.3)	636 (45.3)
Age (years), mean ± SD	63.2 ± 15.87	64.4 ± 15.29	62.3 ± 16.23
Recalc. eGFR (Cockroft-Gault, mL/min), mean ± SD	94.2 ± 38.97	92.8 ± 38.30	95.3 ± 39.47
Frailty (physician judgement), n (%)	306 (12.7)	140 (14.0)	166 (11.8)
Medical history, n (%)	1041 (43.2)	476 (47.4)	565 (40.3)
Hypertension	264 (11.0)	75 (7.5)	189 (13.5)
Chronic venous insufficiency	162 (6.7)	86 (8.6)	76 (5.4)
COPD	234 (9.7)	110 (11.0)	124 (8.8)
Cancer	89 (38.2)	38 (34.9)	51 (41.1)
Cancer still active*			
Prior VTE, n (%)			
Prior PE±DVT	177 (7.4)	112 (11.2)	65 (4.6)
Prior DVT only	388 (16.1)	110 (11.0)	278 (19.8)
Edoxaban treatment at baseline, n (%)			
Edoxaban 60 mg	2100 (87.2)	891 (88.7)	1209 (86.2)
Edoxaban 30 mg	307 (12.8)	113 (11.3)	194 (13.8)

* Percentage based on patients with cancer history. COPD, chronic obstructive pulmonary disease; DVT, deep vein thrombosis; eGFR, estimated glomerular filtration rate; PE, pulmonary embolism; SD, standard deviation; VTE, venous thromboembolism.

TABLE 2 Clinical outcomes of patients enrolled in the ETNA-VTE-Europe study during 12-months follow-up according to type of index event (n=2407)

Outcomes during 12-months follow-up, n (%)	Overall (N=2407)	PE±DVT (n=1004)	DVT alone (n=1403)
Any VTE recurrences	68 (2.83)	29 (2.89)	39 (2.78)
PE with or w/o DVT recurrence	27 (1.12)	17 (1.69)	10 (0.71)
PE w/o DVT recurrence	21 (0.87)	13 (1.29)	8 (0.57)
PE with DVT recurrence	6 (0.25)	4 (0.40)	2 (0.14)
DVT only recurrence	47 (1.95)	16 (1.59)	31 (2.17)
Any bleeding	313 (13.00)	149 (14.84)	164 (11.69)
ICH	12 (0.50)	10 (1.00)	2 (0.14)
Major bleeding (ISTH)	46 (1.91)	24 (2.39)	22 (1.57)
CRNM bleeding	82 (3.41)	38 (3.78)	44 (3.14)
Major GI bleeding	10 (0.42)	4 (0.40)	6 (0.43)
Stroke or systemic embolism	24 (1.00)	11 (1.10)	13 (0.93)
All-cause mortality	81 (3.37)	41 (4.08)	40 (2.85)
CV mortality	34 (1.41)	17 (1.69)	17 (1.21)

CRNM, clinically relevant non-major; CV, cardiovascular; DVT, deep vein thrombosis; GI, gastrointestinal; ICH, intracranial hemorrhage; ISTH, International Society on Thrombosis and Haemostasis; PE, pulmonary embolism; VTE, venous thromboembolism.

Conclusions: Low rates of bleeding and VTE events were reported after 12 months of treatment with edoxaban in clinical practice. Patients with index PE with or without DVT reported a higher risk of mortality and bleeding events than those with index DVT alone.

PB2462 | Oral Anticoagulation Therapy for Venous Thromboembolism in Norway: Patient Characteristics, Treatment Trends and Patterns

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Background: Data on oral anticoagulants (OAC) use among Venous Thromboembolism (VTE) patients in Norway is limited.
Aims: Describe demographic and clinical characteristics, trends and treatment patterns of OACs in VTE patients in Norway between 2013 and 2017.
Methods: Cohort study using prospectively collected data from the national registries in Norway between 2013 and 2017. Patients were included if they had a VTE diagnosis followed by an OAC dispensation within 30 days in this time-frame. Patients were excluded if they had presence of atrial fibrillation or a prior OAC. Kaplan Meier methods were used to assess the duration of treatment. The study was approved by the Regional Ethics Committee (2017/1328-9).
Results: 3,557 warfarin, 3,088 apixaban and 5,828 rivaroxaban patients were identified. Counts for dabigatran and edoxaban were low and are not described further. Warfarin patients were younger than apixaban (median [IQR]: 64 [26] vs. 66 [24] years; p=0.0045), but older than rivaroxaban (61 [24]; p< 0.0001). Gender distributions were similar across all groups (%female 48.5 (warfarin), 46.4 (apixaban), 43.6 (rivaroxaban)). The most prevalent comorbidity in all groups was hypertension (44%). From 2013 to 2017, of total OAC use, apixaban increased 54.5%, rivaroxaban 5.7%, warfarin declined 60.2% (Figure 1). A higher proportion of apixaban patients were persistent after 12 months of follow-up compared with warfarin, (mostly apparent after 6 months; log-rank: p< 0.0001); no difference was observed between rivaroxaban and warfarin patients (p=0.36). More than three quarters of patients, irrespective of OAC, were persistent at 3 months. At 12 months, 30.8% of patients were still persistent on apixaban, followed by 21.9% on warfarin and 19.2% on rivaroxaban (Table 1).
Conclusions: Between 2013 and 2017, apixaban and rivaroxaban use in VTE patients increased in Norway, warfarin use declined. A higher proportion of apixaban patients were persistent on treatment over time followed by warfarin and rivaroxaban.

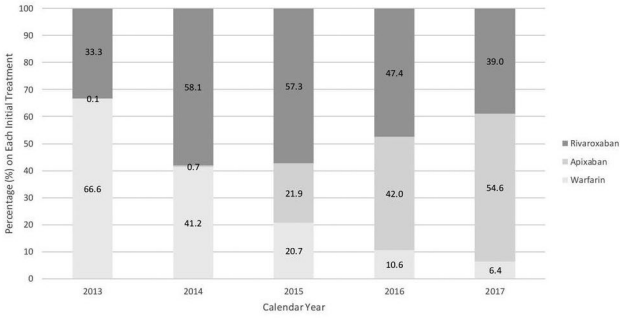

FIGURE 1 Distribution of OACs Used Among Newly Treated Incident VTE Population in Norway

PB2463 | Major Bleeding Complications from Direct Oral Anticoagulants in Thai Adult Patients

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Background: Direct oral anticoagulants (DOACs) have a better safety profile compared with warfarin. However, the real-world data specifically in Asian patients are lacking.

TABLE 1 Median Length of Time on Therapy and Percentage of Patients Persistent on Index Therapy at Certain Timepoints (Kaplan Meier estimates)

Kaplan-Meier estimates		Warfarin N=3,557	Apixaban N=3,088	Rivaroxaban N=5,828
Length of Time on Index Therapy, months:	Median (IQR)	6.3 (3.4-10.6)	6.4 (3.7-15.9)	6.0 (3.9-9.2)
Patients Persistent on Treatment at Certain Timepoints, months:				
0-3	% (95%CI)	79.7 (78.3-81.0)	83.7 (82.3-85.0)	87.4 (86.5-88.2)
0-6	% (95%CI)	53.1 (51.4-54.7)	53.9 (51.9-55.8)	49.7 (48.4-51.1)
0-9	% (95%CI)	30.3 (28.8-31.8)	36.5 (34.5-38.4)	25.4 (24.3-26.6)
0-12	% (95%CI)	21.9 (20.6-23.4)	30.8 (28.8-32.8)	19.2 (18.2-20.3)

TABLE 1 Sites of bleeding and case-fatality rate of warfarin and direct oral anticoagulants (DOACs)

	Warfarin (n=248)	DOACs (n=50)
Major bleeding, n	163	34
-Gastrointestinal bleeding, n (%)	114 (69.9%)	16 (47.1%)
-Intracranial bleeding, n (%)	39 (23.9%)	9 (26.5%)
-Retroperitoneal bleeding, n (%)	6 (3.7%)	3 (8.8%)
-Genitourinary tract bleeding, n (%)	0	2 (5.9%)
-Intraarticular bleeding	0	1 (2.9%)
-Bleeding in other sites, n (%)	4 (2.5%)	3 (8.8%)
Case-fatality rate (%) -Total bleeding cases	12.9%	8%
Case-fatality rate (%) -Major bleeding cases	19.6%	11.8%

Aims: To determine the rates, clinical outcomes and risk factors for mortality in patients who had major bleeding while receiving DOACs.
Methods: This is a retrospective cohort study from 2010 to 2019. The medical records of all patients with major bleeding from DOACs according to ISTH criteria were reviewed.
Results: During the study period, 13,541 patients received oral anticoagulants; warfarin (10,517), dabigatran (425), rivaroxaban (1,622), apixaban (903) and edoxaban (74). There were 298 bleeding events (2.2%); 248 cases (2.4%) from warfarin and 50 cases (1.7%) from DOACs. Major bleeding rates in warfarin-treated and DOAC-treated patients were 1.5% (163) and 1.1% (34), respectively. Major bleeding events occurred in patients receiving dabigatran (12), rivaroxaban (12), apixaban (9) and edoxaban (1). The mean age of patients with major bleeding was 79±9 years (range 49-95) and 55.9% were male. The sites of bleeding were shown in Table 1. The majority of DOAC bleeding (50%) were managed with surgical or endoscopic treatments for gastrointestinal bleeding (50%). Red blood cell transfusion was given in 19 cases (55.9%). Furthermore, 7 (20.6%) cases received prothrombin complex concentrate and 5 received (14.7%) idarucizumab. Compared with DOACs, warfarin was associated with both intracerebral hemorrhage (ICH) (Relative risk [RR] 2.80, 95% confidence interval [CI] 1.01-7.84) and gastrointestinal (GI) bleeding (RR 2.05, 95%CI, 1.22-3.45). The case-fatality rates were shown in Table 1. After adjusting for age, sex, and anticoagulant type, ICH (Odds ratio [OR] 2.8, 95% CI 1.01 -7.87) was associated with an increased risk of deaths.
Conclusions: In our study, DOACs showed lower rates of ICH and GI bleeding compared with warfarin. The case-fatality rate was also lower. ICH was associated with higher mortality.

PB2464 | Long-term Anticoagulant Treatment of Splanchnic Venous Thrombosis in High Risk Patients: Direct Oral Anticoagulants vs Vitamin K Antagonists

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Background: Splanchnic vein thrombosis (SVT) is an uncommon but potentially life-threatening disease. It can be related to different underlying clinical conditions (liver cirrhosis; solid and haematological cancer; intra-abdominal inflammatory conditions and surgery). Studies on the long-term clinical outcome of SVT patients reported how the risk of thrombotic recurrences is high over the time and nearly doubles the risk of bleeding, in high thrombotic risk patients. Vitamin K antagonists (VKA) represent the mainstay treatment for SVT. Data about the efficacy and safety of direct oral anticoagulants (DOACs) are reported in literature for the treatment of acute SVT, less is known about their application for the secondary prophylaxis of DVT.
Aims: Aim of the present study was to assess the efficacy and safety of long-term DOACs secondary prophylaxis in high thrombotic risk patients, compared to VKA.
Methods: Seventy patients with SVT candidate to long-term anticoagulant treatment were evaluated between 2017 and 2019. All the patients were at high thrombotic risk (table 1) and were on anticoagulant treatment with VKA. Patients were shifted to DOACs if:

TABLE 1 Risk factors

Patients	70
Mieloproliferative neoplasms	28/70 (40%)
Paroxysmal nocturnal haemoglobinuria	4/70 (5.7%)
Major Inherited Thrombophilia	6/70 (8.6%)
Antiphospholipid antibody Syndrome	4/70 (5.7%)
Idiopathic non cirrhotic portal hypertension	6/70 (8.5%)
Non-Hodgkin Lymphoma	5/70 (7.2%)
Solid tumor	9/70 (12.8%)
Cirrhosis Child-Pugh A e B	8/70 (11.5%)

TABLE 2 Characteristics of patients based on the therapy performed

Type of Therapy	VKA	DOACs	p
Patients	42	28	
Median age at diagnosis	48.1	56.1	0.223
M/F n°(%)	21/21 (50/40)	16/12 (57/43)	0.558
Thrombosis site			0.672
Portal vein	32 (76.2%)	22 (78.7%)	
Splenic vein	1 (2.4%)	2 (7.1%)	
Inferior mesenteric vein	3 (7.1%)	2 (7.1%)	
Budd-Chiari syndrome	6 (14.3%)	2 (7.1%)	

platelet count ≥50x10^9/L, renal and liver function in normal range, esophageal varices < F3, cirrhosis Child-Pugh A-B.

Results: Of the 70 patients enrolled in the study, 42 (60%) continued VKA therapy and 28 (40%) switched to DOACs. The 2 groups were homogeneous (table 2). The clinical observation was done for 2 years from the start of anticoagulant therapy in both groups: thrombotic complications occurred in 3 (7.1%) patients on VKA and in 2 (7.1%) patients on DOACs (p 0.608). No major haemorrhagic events occurred. Minor bleedings were reported in 11 (26%) patients on VKA and in none of the DOAC patients (p 0.09).

Conclusions: Our results highlight that DOACs could represent an alternative therapeutic regimen to the VKA, effective and safe in high risk SVT patients who require long-term anticoagulation.

PB2465 | Direct Oral Anticoagulants in Comparison with Vitamin K Antagonists and Antiplatelet Agents on Timing and Outcomes in Hip Fracture Surgery Patients Older than 75: The ORTHO-GER-DOAC Study

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Background: In case of high risk of bleeding surgery, such as hip fracture, guidelines recommend waiting at least 48 hours after stopping direct Xa inhibitors and up to 96 hours for dabigatran, without drug testing.

Aims: To assess:

1). whether direct oral anticoagulants (DOACs) measurement allows surgery within 48 hours when compared with vitamin K antagonists (VKA) or antiplatelet agents or no antithrombotic drug in hip fracture;

2). peri-operative total blood loss and major bleeding associated with DOACS.

Methods: Hip fracture subjects above 75 admitted to the Orthogeriatric Unit, S.Orsola Malpighi University Hospital, and to Orthopaedic Dpt of I.R.C.C.S. " Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy from 2015 to 2018 were retrospectively evaluated. Antithrombotic agents were stopped at admission. Patients on VKA received vitamin K to obtain an INR < 1.5. DOACS daily testing was conducted and surgery was performed only with near or below trough levels. Hours from admission to surgery, total blood loss and major bleeding during hospitalization were recorded.

Results: Among 256 patients (M/F: 29/120; mean age: 86;range: 75-100), 102 were on antiplatelet agents, 33 on VKA, 35 on DOACS. DOACs were associated with a statistically significant longer mean time to surgery (83.5 h) than VKA (59 h) or antiplatelet agents (45 h) or no antithrombotic drug (37 h) (p=0.001). All patients underwent neuraxial anesthesia. DOACS were associated with higher mean total blood loss (1532 ml) when compared with VKA (1343 ml) or antiplatelet agents (1287 ml) or no antithrombotic drug (1105 ml) (p=0.007). Major bleedings were observed in 13 patients (4.7%) with more bleeding (5/32 -15.6%) with DOACs when compared to all the other groups (8/224 3.5%).

Conclusions: DOAC testing can delay hip fracture surgery while DOACs are associated with significantly higher total blood loss even in subjects with pre-surgical DOAC low levels.

PB2466 | The Effect of rs4148738 Polymorphism of ABCB1 on the Plasma Concentrations of Direct Oral Anticoagulants and Bleeding an Thromboembolic Complications

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Background: Pharmacokinetic and pharmacodynamic characteristics indicate that clinical, demographic and pharmacogenetic factors can influence DOAC antithrombotic efficacy.

Aims: To establish the influence of ABCB1 rs4148738 polymorphism on DOAC plasma levels and on bleeding and thromboembolic complications.

Methods: Patients were enrolled in our Anticoagulation Clinic from Sept 2013 to April 2015 within the START Register (Survey on anTicoagulated pAtients RegisTer) (NCT 02219984). The study was approved by the local Ethics Committee and all enrolled patients provided informed consent to blood sampling and testing.

Patients taking DOACs for either venous thromboembolism or atrial fibrillation since at least 7 days were enrolled. Demographic and clinical characteristics were collected at enrollment on a standardized form. Diluted thrombin time was measured for dabigatran and anti-Xa activity for apixaban and rivaroxaban on plasmas obtained from blood samples collected in sodium citrate after one month at trough and at peak, e.g. at 2 hours after the morning dose. DNA was extracted from peripheral leukocytes for detection of rs4148738 ABCB1 - P-glycoprotein (P-gp) (intronic region G>A) polymorphism. Major and clinically relevant bleeding and thromboembolic complications were reported during follow-up.

Results: All DOACs showed a high inter-individual variability for both peak and trough concentrations in the 291 enrolled patients. The ABCB1 gene rs4148738 polymorphism was determined in 142 patients and it influenced peak levels of rivaroxaban 20 mg with lower levels in homozygotes for the minor A allele but not those of apixaban. A non significant effect was observed on dabigatran 150 mg bid peak and trough levels. In subjects on rivaroxaban, the cumulative rate of major bleeding was lower (8%) in subjects with AA allele than in subjects (17%) with GA/GG alleles, while thromboembolic complications were similar.

Conclusions: The rs4148738 polymorphism of ABCB1 gene of P-gp can influence rivaroxaban peak and trough levels and bleeding complications.

PB2467 | Direct Oral Anticoagulants in Patients with Severe Familial Thrombophilia

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Background: Patients affected by severe inherited thrombophilia - antithrombin, protein C and protein S deficiency, homozygous mutation of Factor V Leiden, homozygous mutation of Factor II G20210A, combined heterozygous mutation of factor V Leiden and Factor II G20210A -are exposed to a higher risk of venous thromboembolism (VTE). Direct oral anticoagulants (DOACs) are increasingly used in acute and long-term treatment of venous thromboembolism. However, their role in management of patients affected by severe inherited thrombophilia and associated VTE is controversial.

Aims: To evaluate whether DOACs are an appropriate treatment option in acute and long-term treatment of VTE in carriers of severe thrombophilia.

Methods: In this prospective study we enrolled a total of 35 subjects who were family members of a proband with objectively diagnosed VTE and documented severe inherited thrombophilia. Eighteen patients were treated with DOACs for an acute VTE, seventeen were switched from VKA to DOACs.

Results: The follow-up of DOACs treatment was 52 years in the first group and 54 in the second group. The patients were treated with the following DOACs: rivaroxaban, apixaban. During the study period no thromboembolic events occurred in the two groups.

The VKA treatment follow-up was 252 years: during VKA treatment, one thrombotic event occurred with an annual incidence of 0.4%(95% CI 0.01 to 2.2).

No hemorrhagic complications occurred during treatment with DOACs, while two hemorrhagic complications occurred during VKA treatment annual incidence 0.8% (95% CI 0.09 to 2.9).

Conclusions: The results of our observation suggest that DOACs are an appropriate treatment option in VTE treatment of patients with severe familial thrombophilia.

PB2468 | Cerebral Vein Thrombosis Treatment with Direct Oral Anticoagulants: A Case Series

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Background: Cerebral vein thrombosis (CVT) is a rare and potentially fatal disorder. Patients with CVT can require extended anticoagulation.

Aims: Data on direct oral anticoagulants (DOACs) for the treatment for CVT are available but information on long-term outcomes is scanty.

Methods: In this case series, we report data on CVT patients consecutively referred to our clinics and prescribed with DOACs after an initial course of conventional treatment (UFH/LMWH ± vitamin K-antagonist/VKA). Data on clinical and radiological follow-up were collected approximately every 6-12 months according to scheduled visits. DOACs prescription for CVT is currently off label in Italy. All patients were properly informed and signed a consent.

Results: We report data on 10 CVT patients treated with DOAC. The mean age at diagnosis was 49±13 years (range, 21-65); 7 patients (70%) were female. Conditions predisposing to CVT were identified in 2 patients (i.e. neurosurgery and hormonal treatment, respecitvely), while CVT was unprovoked in 8/10 patients. DOACs treatment was started after a medium period of 12±15 months of conventional treatment. Dabigatran at highest available dosage was the preferred drug (7/10 patients), while rivaroxaban and apixaban were used in a minority of patients. Four out of ten patients were concurrently treated with antiepileptic drugs (levetiracetam). The main reason for switching drugs (7/10) was the difficulty in achieving and maintaining an adequate time in therapeutic range while in VKA treatment; patients' preferences were considered as well. During a period of DOACs' treatment of 9±9 months (range 1-26 months), no CVT recurrences nor major/non-major clinically relevant bleedings were recorded.

Conclusions: CVT is often unprovoked and can require an extended anticoagulant treatment. While on VKA treatment, maintaining a

therapeutic INR is sometimes challenging. According to our experience, DOACs seem to be a promising option for extended treatment in patients with CVT. Further investigations on this topic are needed.

PB2469 | An Evaluation of the Basis for Anti-Xa Testing in Patients on Low Molecular Weight Heparin and its Impact on Subsequent Management

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Background: Low molecular weight heparins(LMWH) are used for the prophylaxis and treatment of venous thromboembolism and peri-procedure bridging of patients on anticoagulation. Anti-Xa activity is widely used for monitoring select groups of patients on LMWH despite the lack of consensus for its use.

Aims: To determine reasons for ordering anti-Xa test for patients on LMWH. Secondary objectives:To assess the appropriateness of anti-Xa testing and sample collection and their impact on patient care.

Methods: Retrospective,single-centre observational study which included hospitalised patients ≥21 years old,with anti-Xa testing from October 2018 to September 2019. Patients were identified using hospital electronic records with results and outcomes assessed through laboratory and clinical records.

Results: 292 anti-Xa tests were performed on 183 patients on enoxaparin. Of the tests performed, 115(62.8%), 27(14.8%), 92(50.3%) and 59(32.2%) samples were from patients with severe renal impairment, moderate renal impairment extreme body weight and bleeding concerns(Figure 1).

Trough anti-Xa tests were taken 21.6h(IQR=11.6-23.7) after the last enoxaparin dose for renally impaired patients.

56(30.6%) of the 183 patients had more than one anti-Xa taken. 14(25%) had their enoxaparin dose adjusted thereafter. 3 of 14 had major or clinically relevant non-major bleeding(CRNMB) event during the follow up period.

Patients on Enoxaparin (N = 183)	
Reasons for Checking Anti-Xa (n)	
Severe renal impairment (CrCl < 30)	115 (62.8%)
Severe renal and bleeding concerns	28 (15.3%)
Severe renal and extreme body weight	50 (27.3%)
Severe renal, extreme body weight and bleeding concerns	13 (7.1%)
Moderate renal impairment (CrCl 30-60)	27 (14.8%)
Moderate renal and bleeding concerns	15 (8.2%)
Moderate renal and extreme body weight	13 (7.1%)
Moderate renal, extreme body weight and bleeding concerns	6 (3.3%)
Extreme body weight (BMI < 18.5 or BMI > 27.5)	92 (50.3%)
Underweight (BMI < 18.5)	12 (6.6%)
Overweight (BMI > 27.5)	80 (43.7%)
Bleeding concerns	59 (32.2%)
Bleeding concerns and extreme body weight	29 (15.8%)
Others	11 (6.0%)

Figure 1: Reasons for checking anti-Xa activity

FIGURE 1 Reasons for checking anti-Xa activity

Patients who had a bleeding event during enoxaparin therapy (and up to 30 days of LMWH cessation, or till 1/1/2020; whichever is earlier) (n = 46)	
Major bleed	**24/46 (52.2%)**
Attributed directly to high anti-Xa level / inappropriate enoxaparin dosing due to lack of renal adjustment	4/24 (16.7%)
Enoxaparin is a contributing factor	12/24 (50.0%)
Not due to enoxaparin	8/24 (33.3%)
Clinically relevant non major bleed	**11/46 (23.9%)**
Attributed directly to high anti-Xa level / inappropriate enoxaparin dosing due to lack of renal adjustment	1/11 (9.1%)
Enoxaparin is a contributing factor	7/11 (63.6%)
Not due to enoxaparin	3/11 (27.3%)
Minor bleed	**11/46 (23.9%)**
Attributed directly to high anti-Xa level / inappropriate enoxaparin dosing due to lack of renal adjustment	0/11
Enoxaparin is a contributing factor	9/11 (81.2%)
Not due to enoxaparin	2/11 (18.2%)

Figure 2: Patients who had a bleeding event during enoxaparin therapy and during the follow up period

FIGURE 2 Patients who had a bleeding event during enoxaparin therapy and during the follow up period

26 patients(14.2%) had their enoxaparin doses adjusted after the first anti-Xa result. 14(53.8%) of them had a second anti-Xa performed, none of whom had their doses adjusted thereafter. 4 of the 14 patients had major or CRNMB during the follow up period.

29 of the 46 patients who had bleeding event(s) during enoxaparin therapy had anti-Xa monitoring performed before the bleeding event. 9 of the 29 patients had enoxaparin dose adjustments thereafter(Figure 2).

Conclusions: Anti-Xa monitoring is most commonly performed on patients with renal impairment followed by extremes of body weight. These patients had increased bleeding events despite monitoring, attesting to their higher risk. The value of monitoring remains uncertain.

PB2470 | May Thurner Syndrome, Experience on Argentinean Hospital

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Background: May-Thurner syndrome (MTS) results from compression of the left common iliac vein between the right common iliac artery and the vertebrae, causing venous stasis and predisposing to develop deep venous thrombosis (DVT) in the left lower extremity. Its prevalence is 20-30%. Treatment is based on endovascular interventions, surgery and/ or medical management. DVT recurrence is observed in 20-30% of the cases.

TABLE 1 Cohort characteristics

	N 36 (100%)
female sex, n (%)	28 (78)
Age at the thrombotic event, years (IQR)	41 (15-83)
DVT extension, n (%)	
Iliac	3 (8)
Iliac-femoral	12 (33)
cava-Iliac-femoral	2(6)
Iliac-femoral-popliteal	15 (42)
cava-Iliac-femoral-popliteal	4 (11)
Presence of prothrombotic risk factor, n (%)	29 (81)
Number of prothrombotic risk factor, n (%)	
1	11(38)
2	7(24)
3	8(27,5)
4	2(7)
5	1(3,5)
Anticoagulation, n(%)	32 (89)
Local Thrombolitic treatment, n(%)	8(22)
ECOSONIK + Local thrombolitic treatment, n(%)	3 (8)
Systemic thrombolitic treatment, n(%)	1 (3)
Thromboaspiration, n(%)	17 (47)
Angioplasty AND STENT colocation , n(%)	32 (89)

Table 1- Cohort characteristics

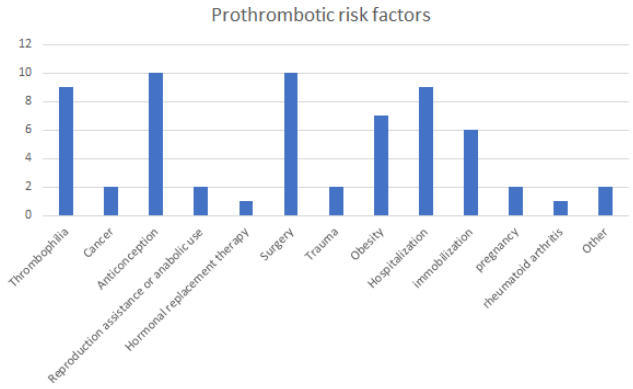

Figure 1

FIGURE 1 Prothrombotic risk factors

Aims: To describe clinical characteristics, treatment and evolution of patients with DVT and MTS that have been treated with endovascular interventions.

Methods: Descriptive, retrospective study of patients with DVT and MTS treated with endovascular interventions from January 2015 to December 2019 in the Hospital Italiano de Buenos Aires.

Results: Thirty-six patients have been included. Median age at the thrombotic event was 41 years (IQR 15-83) (table 1). Twenty- eight (78%) patients were women. Twenty DVT (56%) were acute. Fifteen DVT involved iliac-femoral-popliteal veins. The most frequent clinical presentation was pain and swelling (83%). Fourteen patients (39%) had pulmonary thromboembolism as well. In 29 cases (81%) additional prothrombotic factors were recognized, 50% of them had 2 or more factors associated (figure 1). Hormonal treatment and surgery were the most frequent prothrombotic factors found. Angioplasty and STENT colocation was performed in 32/36 of the cases. No post thrombotic syndrome was observed after the procedure. No complications were reported. Thirty- two patients (89%) received anticoagulation. Recurrence was observed in 8/32 of the cases, 6 of them had 1 or more prothrombotic factors associated.

Conclusions: In our cohort, MTS is more frequent in women, as reported in the literature. It is associated to extensive thrombosis. In most cases of DVT and MTS is possible to evidence additional prothrombotic factors. Recurrence is observed in 25% of the patients, being related to persistence of prothrombotic factors in most cases.

PB2471 | Systematic Review of the Risk of Bleeding in Patients with Chronic Kidney Disease Treated with Tinzaparin

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Background: The progression of chronic kidney disease (CKD) is associated with an increase in the risk of bleeding. The half-life of low molecular weight heparins (LMWH) increases as the clearance of creatinine (ClCr) decreases. Some LMWH recommend a dose adjustment for patients with a ClCr< 30 ml/min. But the available evidence indicates that tinzaparin does not accumulate above ClCr>20 ml/min.

Aims: To analyze the risk of bleeding in patients with venous thromboembolism and CKD treated with full doses of tinzaparin.

Methods: A PubMed, EMBASE and BIOSIS search was conducted. Articles and abstracts containing information on bleeding complications with tinzaparin in patients with CKD were selected. In the tinzaparin arm, groups with ClCr< 30 ml/min were compared to ClCr>30 ml/min or ClCr< 60 ml/min to ClCr> 60 ml/min. Overall, 8 prospective studies (4 clinical trial and 4 observational) with 1831 patients were included.

Systematic review registration: PROSPERO CRD42020139413.

Results: Major bleeding (MB): Six (3.7%) out of 161 patients with ClCr< 30 ml/min and 25(3.4%) out of 732 patients with ClCr>30 ml/min had MB [1.89 95%CI (0.76-4.7) p=0.17]. Among 1016 patients with ClCr< 60 ml/min vs ClCr>60 ml/min, the risk of MB was 2.1% and 3.5% respectively [0.98 95%CI (0.41-2.37) p=0.97].

Clinically relevant bleeding (CRB):: Eleven (8.5%) out of 130 patients with ClCr< 30 ml/min and 64(6.4%) out of 997 with ClCr>30 ml/min had CRB [1.75 (95%CI 0.85-3.61) p=0.13]. Among the 302 patients with ClCr< 60 ml/min and 978 with ClCr>60 ml/min, 25(8.3%) and 87(8.9%) had CRB respectively [1.17 (95% CI 0.72-1.9)p=0.52].

Conclusions: This systematic review does not show a significantly higher risk of MB nor CRB to tinzaparin in patients with moderate and/or severe CKD.

Tinzaparin is the largest LMWH and clearance is less dependent on the kidney function.

The risk/benefit balance should be individualized in these patients.

PB2472 | The Psychosocial Impact of Venous Thromboembolism: An Exploratory Systematic Review and Narrative Synthesis

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Background: VTE is a leading cause of morbidity and mortality and the personal experience of these events can be psychologically challenging. VTE events are known to have significant physical impact however less is known about their psychosocial impact.
Aims: To explore the experiences of individuals following a VTE diagnosis and the psychosocial impact of VTE events.
Methods: MEDLINE, EMBASE, Web of Science, Cochrane Database of Systematic Reviews, CINAHL and PsycINFO databases were systematically searched between 2009 and 2019. All studies identified for the systematic review met inclusion and exclusion criteria and were appraised using the Critical Appraisal Skills Programme (CASP) framework and a narrative synthesis was undertaken.
Results: 3493 records were identified, retrieved and screened for eligibility. Six full text studies were included in the systematic review. The narrative synthesis identified two themes with eight subthemes. The first theme 'Psychological impact of VTE' had the following subthemes: traumatic life event, misdiagnosis and loss of trust, fear of the future and benefit finding and growth. The second theme, 'Disruptive impact of VTE to daily activities and life goals' included the subthemes loss of self, anticoagulation therapy, concealment and isolation, and biographical disruption.
Conclusions: VTE events were identified as traumatic and life changing for individuals. The psychosocial impact of VTE was far reaching, impacting multiple aspects of participant's lives. Alongside clear evidence of psychosocial impairment there is also evidence that VTE events led to benefit finding and growth. Identifying patients who are at risk of psychosocial impairment and require clinical intervention at critical times may reduce the escalation of the negative psychosocial impact of VTE. Current practice and service provision has the potential for development to better integrate physical and mental health care.

PB2473 | Correlation of Reperfusion Therapy and In-hospital Mortality in Patients with Intermediate Risk Acute Pulmonary Embolism and Right Ventricular Dysfunction

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Background: Reperfusion therapy (RTh) in stable patients with intermediate risk acute pulmonary embolism (PE) and right ventricular (RV) dilatation is not recommended in guidelines. Patients with RV dilatation and reduced tricuspid annular plane systolic excursion (TAPSE) or RV wall motion abnormalities may carry a worse prognosis. Insufficient evidence exists about the benefit of RTh in this population.
Aims: Establish the benefit of RTh in terms of in-hospital mortality (IHM) in patients with intermediate risk PE with RV dysfunction (RVD) defined as RV dilatation and concomitant reduced TAPSE or RV wall motion abnormalities.
Methods: Single-center observational cohort registry performed at a University Hospital in Argentina. Patients hospitalized with a diagnosis of PE and RVD between 2008-2018 were eligible. RV diameter, TAPSE, and wall motion abnormalities were determined by echocardiography. We also collected information related to clinical presentation, PESI score, troponin levels and electrocardiographic pattern. We compared the IHM of patients who received RTh versus those treated with a conservative approach (no-RTh). Patients with surgical thrombectomy were excluded. All statistical analysis was carried out using the STATA version 13.1.
Results: 230 consecutive patients were hospitalized with a diagnosis of PE. 45 patients with RVD were included. Mean age 64±16 years; 54.6% female. Global IHM in patients with RVD was 10.7%. Univariate analysis showed that the RTh group had a higher proportion of males (57.6 vs 26.3%; p:0.03) and more dyspnea (100 vs 84.2%; p:0.03). Clinical characteristics of both groups are shown in the table. After multivariate logistic regression analysis, the IHM of patients who received RTh did not differ from those in the no-RTh group (11.5 vs 15.7%; p:ns).
Conclusions: In this cohort of patients with intermediate-risk PE and RVD, reperfusion therapy did not correlate with lower IHM.

TABLE 1 Reperfusion therapy (RTh) vs. conservative approach (no-RTh). Clinical characteristics and in-hospital mortality

VARIABLE	RTh	no-RTh	p value
Age (mean)	62 (±19)	68 (±13)	ns
Male	15 (57.6%)	5 (26.3%)	0.03
Dyspnea	26 (100%)	16 (84.2%)	0.03
Syncope	4 (15.3%)	1 (5.2%)	ns
Systolic blood pressure (mmHg)	119 (SD 24)	124 (SD 22)	ns
Sinus tachycardia (bpm)	19 (73%)	12 (63.1%)	ns
Right bundle branch block	3 (11.5%)	5 (26.3%)	ns
Elevated troponin levels	22 (84.6%)	11 (57.8%)	ns
in-hospital mortality	3 (11.5%)	3 (15.7%)	ns

PB2474 | Risk of Intracranial Bleeding in Patients with Primary Brain Cancer Receiving Therapeutic Anticoagulation for Venous Thromboembolism: A Meta-analysis

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Background: Venous thromboembolism (VTE) is common in glioma patients. Also spontaneous intracranial hemorrhage (ICH) is frequently observed in subjects with primary brain tumors. Thus, the management of anticoagulant therapy for VTE is challenging and controversial in these patients.

Aims: We performed a meta-analysis to clarify the risk of ICH in subjects with primary brain tumor who were diagnosed with VTE and treated with full dose anticoagulant therapy.

Methods: A systematic search of the literature was conducted using PubMed, Scopus, and EMBASE databases between January 1980 and January 2019 without language restrictions. Summary statistics for ICH were obtained by calculating the odds ratio (OR) using a random effects model and heterogeneity across studies was estimated by the I(2) statistic. The Newcastle-Ottawa Scale (NOS) was used to evaluate the quality of studies.

Results: A total of 368 studies were initially identified. Of these, 346 were excluded after title review. The remaining 22 studies were reviewed in detail. According to the PICO criteria, 15 studies were excluded. Finally, 7 studies were included in the meta-analysis [Table 1]. All the included studies were retrospective and the indication for anticoagulant use was always acute VTE. In a random effects model, the pooled odds ratio for ICH in patients with a primary malignant brain cancer receiving therapeutic anticoagulation for VTE vs those without anticoagulation was 3.66 (95% confidence interval [Figure 2]. Publication bias was evaluated by Funnel plot and asymmetry of rates of hemorrhage was not apparent.

Conclusions: This meta-analysis demonstrates that anticoagulation for VTE increases the risk of ICH in subjects with malignant brain

Figure 2

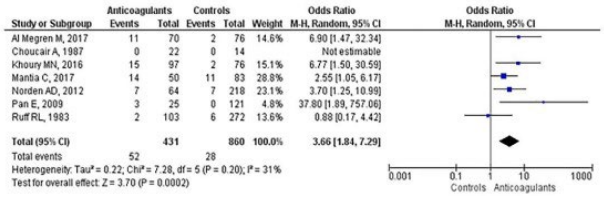

FIGURE 2

TABLE 1

Author, year	Study design	N° of patients	N° on AC therapy	N° without AC therapy	Type of AC	Total N° of ICH	N° of ICH in AC group	N° of ICH in control group	VTE in both groups
Mantia et al [24], 2017	Retrospective	133	50	83	Low-molecular-weight heparin	25	14	11	No
Pan et al [25], 2009	Retrospective	146	25	121	Low-molecular-weight heparin Vitamin K antagonists	3	3	0	No
Choucair et al [22], 1987	Retrospective	36	22	14	Unfractionated heparin Vitamin K antagonists	0	0	0	Yes
Ruff et al [28], 1983	Retrospective	375	103	272	Unfractionated heparin Vitamin K antagonists	8	2	6	No
Norden et al [26], 2012	Retrospective	282	64	218	Low-molecular-weight heparin Vitamin K antagonists	14	7	7	No
Khoury et al [27], 2016	Retrospective	173	97	76	Low-molecular-weight heparin Unfractionated heparin Vitamin K antagonists	17	15	2	Yes
Al Megren et al [23], 2017	Retrospective	146	70	76	LMWH UFH Fondaparinux Unknown AC	13	11	2	No

tumors. Future studies are warranted to fully understand the best medical treatment of VTE in glioma patients.

PB2475 | Correlation between Anti-Xa Activity and Plasmatic Levels of Apixaban

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Background: Apixaban, unlike Vitamin-K antagonists, has a predictable effect and does not require biological control routinely. However, estimation of plasmatic concentration could be useful in individual dose adjustment, certain clinical circumstances and selected patients.

Aims:

1). Evaluate biological effect of apixaban with chromogenic anti-Xa test with calibrators for apixaban and heparin
2). Analyze correlation between both techniques.
3). Study application of a correction factor to estimate plasmatic concentration of apixaban based on anti-Xa activity.

Methods: 100 patients anticoagulated with apixaban were included (1Jan2018 2018-31Dec2018). Anti Xa activity (HemosIL Liquid anti-Xa) and a chromogenic assay to estimate apixaban concentration with specific calibrator (TECHNOVIEW apixaban Cal High Set) in the ACL TOP coagulometer (Instrumentation Laboratory, MA, USA), were determined. Pearson's coefficient was calculated to assess the correlation between both techniques. A linear regression was performed to calculate the estimated levels of apixaban based on anti-Xa activity. P< 0.05 were considered significant.

The results were validated in a prospective cohort of patients anticoagulated with apixaban (n=35) (1Jan2019-30Apr2019). Estimated apixaban levels (linear regression) and apixaban levels obtained with specific calibrator were compared. Statistical analysis was performed with SPSS version 19.0 for Windows (SPSS, Chicago, IL, USA).

Results: An excellent correlation was observed between the anti-Xa activity and the estimated levels of apixaban (Pearson's test: r=0.930, p< 0.001). Using linear regression (y=140.54x + 1.3356) we estimated apixaban levels and compared them with those obtained with a specific calibrator. The median difference was 8.9 ng/ml. Figure 1.

Conclusions: Plasmatic concentration of apixaban can be estimated by applying a correction factor to the anti-Xa activity (calibrated for heparins). Anti Xa chromogenic assay with specific calibrators for apixaban is a quantitative method and easy to perform, allowing to determine estimated plasma concentration. This approach would reduce the cost of reagents, although a different correction factor needs to be calculated in each laboratory.

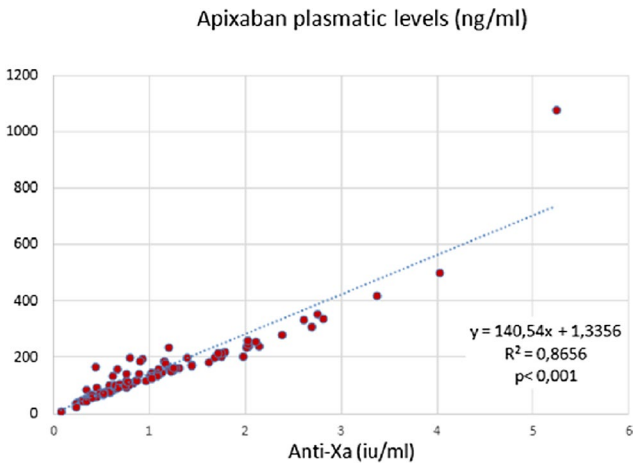

FIGURE 1

PB2476 | The Effect of Postoperative Analgesia on the Hemostatic System in Gerontological Patients with Multiple Trauma

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Background: In the process of aging, the body undergoes involutive changes that determine the peculiarities of analgesic methods in gerontological patients.

Aims: Goal. To study the effect of postoperative analgesia in gerontological patients with polytrauma on the state of the hemostatic system.

Methods: Materials and methods. A total of 94 patients over 70 years old with polytrauma after surgical correction were examined. Patients were divided into three groups: in the 1st group (n = 31), postoperative analgesia was performed with morphine, in the 2nd (n = 31) - nalbuphine, in the 3rd (n = 32) - with a combination of infulgan (COX inhibitor - 2) and rheumoxicam (COX-3 inhibitor). The hemostatic system parameters were studied: antithrombin-III (AT-III) and soluble fibrin-monomer complex (RFMC).

Results: On the 1st day after surgery in groups 1 and 2 there was a tendency to hypercoagulation: an increase in the level of RFMC and a decrease in the activity of AT III. In the 3rd group, the RFMC and AT III indicators did not change on the 1st day and amounted to 42.6 mg / L and 69.7% and began to differ from the 1st group. Until 3 days, these indicators did not change in group 1 and 2: the RFMC level decreased by 1.2% and 3.5% compared to the previous stage, while AT III increased by 3.8% and 3.2% compared with the previous stage 1 and 2, and in group 3, the concentration of RFMC continued to decrease by 11.1%, the activity of AT III - increased by 11.4% compared with the previous stage.

Conclusions: Conclusions. The use of a combination of COX-2 and COX-3 inhibitors for postoperative analgesia in gerontological patients with polytrauma has a positive effect on the hemostatic

system: the concentration of RFMC is significantly reduced and the concentration of AT-III is significantly increased.

PB2477 | VTE Treatment in Routine Clinical Practice: A Pre-specified Combined Evaluation of XALIA and XALIA-LEA with Additional Analyses of Special Patient Populations

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Background: XALIA and XALIA-LEA were prospective non-interventional studies observing treatment and secondary prevention of venous thromboembolism (VTE) across global regions. This pooled analysis combined the study datasets.

Aims: To explore the safety and effectiveness of rivaroxaban and standard anticoagulation (heparin/fondaparinux, usually overlapping with and followed by a vitamin K antagonist [VKA]) for VTE treatment in routine clinical practice, with further focus on quality control of treatment with VKAs and genitourinary bleeding patterns.

Methods: Adult patients with confirmed deep vein thrombosis or pulmonary embolism and an indication for ≥3 months' anticoagulation treatment received rivaroxaban or standard anticoagulation. Treatment type, dose and duration were at the physician's discretion. Primary outcomes were major bleeding, recurrent VTE and all-cause mortality. Propensity score-stratification and -matching designs were used to reduce confounding bias. Regulators and study centres' institutional review boards approved the protocol; written informed consent was obtained from all patients.

Results: Overall, 7129 patients were enrolled in 36 countries; the propensity score-stratified and -matched analyses included 6445 and 2714 patients, respectively. Major bleeding and recurrent VTE incidences were similar between treatment groups; all-cause mortality was lower with rivaroxaban than standard anticoagulation in both adjusted analyses (Table 1). Genitourinary bleeding incidences in the matched analysis (including prolonged menstrual bleeding) were higher with rivaroxaban than standard anticoagulation (46 events [4 major] versus 23 events [2 major]). VKA-treated patients showed marked variation in quality of international normalized ratio (INR) control (time in therapeutic range) (Table 2).

Conclusions: This supplemental pooled analysis provides the largest 'real-world' prospective dataset of rivaroxaban use for VTE treatment. Low major bleeding, recurrent VTE and all-cause mortality incidences supported the safety and effectiveness of rivaroxaban in a broad range of patients with VTE. However, the sites of bleeding were different between treatment groups and there was variation globally regarding quality of INR control.

TABLE 2 Time in therapeutic range patterns in patients in the safety population receiving VKAs

Treatment details	VKA (n=1944)
Initial heparin/fondaparinux	1586 (81.6)
No heparin/fondaparinux	358 (18.4)
Patients receiving VKA with available INR values	989 (50.9)
TTR, mean, % (SD)	
INR <2.0	33.5 (39.3)
INR 2.0-3.0	53.5 (39.0)
INR >3.0	13.0 (25.5)

Data are n (%) unless stated otherwise. INR, international normalized ratio; SD, standard deviation; TTR, time in therapeutic range; VKA, vitamin K antagonist.

TABLE 1 Treatment-emergent outcomes in the propensity score-stratified (top) and propensity score-matched (bottom) analysis sets

	Rivaroxaban (n=3902)		Standard anticoagulation (n=2543)			
Propensity score-stratified analysis Outcome	n (%)	Events per 100 patient-years (95% CI)	n (%)	Events per 100 patient-years (95% CI)	HR (95% CI)	*p-value*
Major bleeding	39 (1.00)	1.74 (1.24-2.38)	63 (2.48)	3.95 (3.03-5.05)	0.65 (0.39-1.08)	0.099
Recurrent VTE	55 (1.41)	2.47 (1.86-3.21)	71 (2.79)	4.49 (3.51-5.67)	0.85 (0.54-1.32)	0.460
All-cause mortality	41 (1.05)	1.83 (1.31-2.48)	117 (4.60)	7.28 (6.02-8.72)	0.55 (0.33-0.91)	0.021
Propensity score-matched analysis Outcome	Rivaroxaban (n=1357)		Standard anticoagulation (n=1357)		HR (95% CI)	*p-value*
Major bleeding	17 (1.25)	2.11 (1.23-3.38)	26 (1.92)	3.08 (2.01-4.52)	0.65 (0.35-1.20)	0.172
Recurrent VTE	21 (1.55)	2.62 (1.62-4.01)	27 (1.99)	3.23 (2.13-4.70)	0.79 (0.44-1.39)	0.412
All-cause mortality	19 (1.40)	2.36 (1.42-3.68)	34 (2.51)	4.00 (2.77-5.59)	0.55 (0.31-0.97)	0.039

CI, confidence interval; HR, hazard ratio; VTE, venous thromboembolism.

PB2478 | ETNA-VTE Global: Evaluation of Bleeding Risk Based on VTE-BLEED Score on 12-month Outcomes in a Population Treated with Edoxaban

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Background: Venous thromboembolism (VTE) is the third most common cardiovascular (CV) condition. Edoxaban has been approved for the treatment and secondary prevention of VTE. Relevant to clinical practice is the understanding of safety and effectiveness of oral anticoagulation treatment relative to the bleeding risk of the patient.

Aims: To investigate outcomes in VTE patients treated with edoxaban categorised into low or high bleeding risk using the VTE-BLEED Score (active cancer, anemia, age ≥60 years, male with SBP ≥140 mmHg, eGFR < 60 ml/min, history of CRNMB).

Methods: Patients with acute symptomatic VTE who received edoxaban were recruited across eight European and three Asian countries. Patients were divided into two bleeding risk categories low or high (VTE-BLEED Scores < 2 or ≥ 2) and followed for safety (bleeding) and effectiveness (VTE) events for 12 months.

Results: Of 3908 patients, 1994 (51%) had low, 1575 (40.3%) had high, and 339 (8.7%) had unknown bleeding risk (due to incomplete data). The proportion of high bleeding risk patients, who were generally older, had lower body weight, lower creatinine clearance and more comorbidities, was highest in Japan with 723/1245 (57.5%) followed by Korea/Taiwan 107/247 (43.3%) and Europe 745/2407 (30.9%) (Table 1). All categories of bleeding events, all-cause mortality and CV mortality were higher in the high-risk group compared with the low risk group, with approximately a 3-fold difference in major bleeding. The frequency of recurrent VTE and its components PE and DVT, were similar (Table 2).

Conclusions: In VTE patients on edoxaban, the results show that high vs low bleeding risk as identified by the VTE-BLEED score is associated with similar VTE recurrence risk but higher all-cause and

TABLE 1 Baseline characteristics by bleeding risk by VTE-BLEED score

	Overall	Low bleeding risk (n = 1994)	High bleeding risk (n = 1575)
Female, n (%)	2008 (51.4%)	1082 (54.3%)	811 (51.5%)
Age, years, mean ± SD	64.9 ± 15.50	58.4 ± 15.12	74.2 ± 10.58
Weight, kg, mean ± SD	73.6 ± 19.05	78.4 ± 18.93	66.5 ± 17.15
Creatinine clearance (CG), ml/min, mean ± SD	88.2 ± 38.44	104.7 ± 35.30	65.7 ± 30.69
Geographic region, n (%)			
Europe	2407 (61.6%)	1412 (70.8%)	745 (47.3%)
Japan	1254 (32.1%)	458 (23.0%)	723 (45.9%)
South Korea/Taiwan	247 (6.3%)	124 (6.2%)	107 (6.8%)
Type of index VTE event, n (%)			
PE w/o DVT	744 (19.0%)	423 (21.2%)	283 (18.0%)
PE with DVT	937 (24.0%)	503 (25.2%)	402 (25.5%)
DVT	2227 (57.0%)	1068 (53.6%)	890 (56.5%)
Medical history, n (%)			
Hypertension	1713 (43.8%)	727 (36.5%)	875 (55.6%)
Diabetes mellitus	506 (12.9%)	231 (11.6%)	246 (15.6%)
Dyslipidemia	849 (21.7%)	367 (18.4%)	427 (27.1%)
COPD	218 (5.6%)	87 (4.4%)	125 (7.9%)
Major surgery or trauma	559 (14.3%)	308 (19.0%)	206 (21.7%)
Malignancy[a]	449 (11.5%)	0 (0.0%)	449 (28.5%)
Bleeding history	175 (4.5%)	36 (1.8%)	137 (8.7%)
Major or CRNM bleeding	124 (3.2%)	13 (0.7%)	109 (6.9%)
Major bleeding	80 (2.0%)	6 (0.35%)	72 (4.6%)
Major GI bleeding	12 (0.3%)	2 (0.1%)	10 (0.6%)
Edoxaban 60 mg at baseline, n (%)	2716 (69.5%)	1659 (83.2%)	760 (48.3%)

[a] active at enrolment

Abbreviations: BMI, body mass index; CG, Cockcroft-Gault; COPD, chronic obstructive pulmonary disease; CRNM, clinically relevant nonmajor; DVT, deep vein thrombosis; GI, gastrointestinal; PE, pulmonary embolism; SD, standard deviation; VTE, venous thromboembolism.

TABLE 2 Clinical events during 12-month follow-up by VTE-BLEED score

Events, n (%)/year	Overall	Low bleeding risk (n = 1994)	High bleeding risk (n = 1575)
VTE recurrence	111 (3.45)	58 (3.26)	44 (3.85)
PE with or w/o DVT	44 (1.36)	23 (1.28)	18 (1.56)
PE w/o DVT	37 (1.14)	16 (0.89)	18 (1.56)
PE with DVT	7 (0.21)	7 (0.39)	0 (0.00)
DVT only	77 (2.38)	42 (2.35)	29 (2.52)
All–cause mortality	198 (6.07)	38 (2.11)	153 (13.19)
CV mortality[a]	37 (1.13)	12 (0.67)	23 (1.98)
Any bleeding	459 (15.42)	223 (13.56)	209 (19.86)
Major bleeding [ISTH]	83 (2.57)	27 (1.51)	51 (4.46)
ICH bleeding	20 (0.61)	6 (0.33)	10 (0.87)
Major GI bleeding	23 (0.71)	5 (0.28)	18 (1.56)
CRNM bleeding	123 (3.86)	56 (3.17)	58 (5.14)

[a] Sensitivity analysis.

Abbreviations: CRNM, clinically relevant nonmajor; CV, cardiovascular; DVT, deep vein thrombosis; GI, gastrointestinal; ICH, intracranial haemorrhage; ISTH, International Society on Thrombosis and Haemostasis; PE, pulmonary embolism; VTE, venous thromboembolism.

cardiovascular mortality and higher incidences of any bleeding type or category.

PB2479 | DOACs in Real Clinical Practice

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Background: DOACs are effective in preventing and treating VTE. However, in clinical practice, treatment failure occurs (recurrent VTE, post-thrombotic syndrome during and after anticoagulation). If patients are adequately anticoagulated, the occurrence of thromboembolism (whether primary or recurrent) constitutes anticoagulation failure.

Aims: We defined the failure of DOAC treatment in adequately anticoagulated patients. We identified etiological factors, the cause of VTE recurrence during and after the treatment with DOAC

Methods: We present a group of 15 adequately anticoagulated patients with recurrent VTE despite anticoagulation (USG) and the post-thrombotic syndromes. All participants were assessed for the presence of the inherited thrombophilia including factor V Leiden, prothrombin mutation, MTHFR mutation, PAI-1 mutation, protein S and C deficiency, antithrombin deficiency and presence of the thyroid autoimunity, celiac disease, cardiovascular disease, inflammatory bowel disease, cancer, antiphospholipid antibody.

Results: 10 patients experienced recurrence of VTE (USG verified) during the treatment DOAC. Five patients developed the post-thrombotic syndrome and recurrent VTE 4 to 8 weeks after the discontinuation of DOAC. We assume that the treatment failure was caused by clinical condition, clinical risk factors but not by DOAC.

Conclusions: The optimal management of complications in patients with DOAC treatment failure is unclear and the options include the following: 1. Dose escalation, 2. Switching over to an alternative anticoagulant, 3. Adding an antiplatelet agent. In clinical practice, it is important to decide whether treatment failure is due to drug (DOAC) or underlying disease. It is important to correctly diagnose recurrence of VTE, to distinguish recurrence from the residual thrombosis, and to correctly interpret the laboratory test results

PB2480 | Predicting Factors for Pulmonary Embolism Response Team (PERT) Activation in a General Pulmonary Embolism Population

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Background: Pulmonary Embolism Response Teams (PERT) aim to improve treatment of acute pulmonary embolism (PE). While the original intent of PERT was to focus on the challenging intermediate- and high-risk PE, recent multicenter studies show that low-risk PE patients compose one in five of all PERT cases. Conversely, not all intermediate- and high-risk PE patients trigger a PERT activation. The factors leading to PERT activations remain unknown.

Aims:

1). To describe the patient characteristics associated with a PERT activation for low-risk PE patients and

2). to describe the patient characteristics precluding a PERT activation for intermediate/high-risk PE patients.

Methods: We analysed data from all patients with confirmed PE diagnosed in the Massachusetts General Hospital (MGH) Emergency Department from August 2013 to February 2017 and cross-referred these data with patients who received a PERT activation and patients who did not. Patients were stratified according to the ESC guidelines into low-risk or intermediate/high-risk PE. Data were extracted on demographics, comorbidities, PE risk factors, medication use, presenting symptoms, imaging, and laboratory findings. Univariate analyses were performed within each risk group comparing patients with a PERT activation and patients without.

Results: Fifteen percent (56/374) of low-risk patients triggered a PERT activation. Patient characteristics associated with PERT activation (Table 1) were:

1). vascular disease,
2). pulmonary diseases,
3). thrombophilia,
4). current use of anticoagulants,
5). central PE and
6). concurrent DVT.

Thirty-five percent (110/283) of intermediate/high-risk patients did not trigger a PERT activation. Patient characteristics precluding a PERT activation (Table 2) were:

1). vascular disease,
2). malignancies and
3). asymptomatic at presentation.

Conclusions: In this retrospective analysis, we found that low-risk patients with PERT activations had more extensive clot burden or had failed anticoagulation treatment whereas intermediate/high-risk patients without PERT activations tended to have malignancies and were more often asymptomatic on presentation.

PB2481 | A Single Centre Experience on the Use of Direct Oral Anticoagulants in People with Human Immunodeficiency Virus (HIV)

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Background: Warfarin has been the mainstay anticoagulation option for people with human immunodeficiency virus (HIV). Direct oral

TABLE 1 Characteristics of low-risk patients

	All patients		No activation		PERT activation		P-value
	n	%	n	%	n	%	
	374	100	318	85	56	15	
Vascular disease (CAD, AF, CHF, Stroke)	64	17	49	15	15	27	0.037
Pulmonary disease (Asthma, COPD)	63	17	45	14	18	32	<0.001
Thrombophilia	19	5	13	4	6	10	0.049
Anticoagulants (currently using)	40	11	29	9	11	20	0.019
Central PE	152	41	109	37	43	78	<0.001
Present DVT	104	28	78	25	26	50	<0.001

TABLE 2 Characteristics of intermediate/high-risk patients

	All patients		No activation		PERT activation		P-value
	n	%	n	%	n	%	
	283	100	110	35	173	65	
Vascular disease (CAD, AF, CHF, Stroke)	74	26	40	36	34	20	0.002
Malignancies (active and in the past)	108	38	51	46	57	33	0.024
Asymptomatic on presentation	13	5	11	10	2	1	<0.001

anticoagulants (DOACs) offer fixed dosing, predictable effects and no regular anticoagulation monitoring but research is lacking on efficacy and safety of DOACs with concurrent antiretroviral medication (ARVM) use. Theoretical drug interactions between DOACs and antiretrovirals exist but the clinical signification of these interactions is unknown.

Aims:

- To assess the safety of DOACs in patients with HIV
- To assess effects of drug-drug interactions with DOACs and ARVM

Methods: Patients prescribed DOAC and ARVM between January 2018 and July 2019 were included via electronic prescribing records. Data collection included: patient demographics, co-morbidities, HIV status, indication for anticoagulation therapy, DOAC and ARVM details, and review of medication switches and adverse effects.

Results: 16 patients (mean age 66 years; male 81% and female 19%) were identified with well controlled HIV; of these patients DOAC indication was: AF (9), VTE treatment (4), AF and VTE treatment (1), thromboprophylaxis following orthopaedic surgery (1), and other (1-thrombus in descending abdominal aorta). 31% (n=5/16) patients were on DOAC, antiplatelet and ARVM. Patients were prescribed apixaban (4), rivaroxaban (8), and dabigatran (3). One patient switched from rivaroxaban to apixaban due to pulmonary embolism on rivaroxaban therapy.

In one patient on apixaban a potential interaction with Rezolsta (protease inhibitor) was identified with no clinical consequences. One patient had appropriate dose reduction of dabigatran due to co-administration with voriconazole. For the remainder of patients, there were no significant drug interactions.

There were two bleeding events. One patient stopped apixaban due to upper gastrointestinal bleeding. One patient on rivaroxaban and aspirin with ischaemic heart disease and peripheral vascular disease developed intracranial haemorrhage.

Conclusions: DOACs can be considered the anticoagulant of choice to most patients with HIV, although drug-drug interactions should be considered.

PB2482 | The Use of Thrombolytic Therapy in a Multidisciplinary Pulmonary Embolism Response Team

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Background: Previous research has shown that implementation of a pulmonary embolism response team (PERT) is associated with an increased use of catheter-directed thrombolysis (CDT).

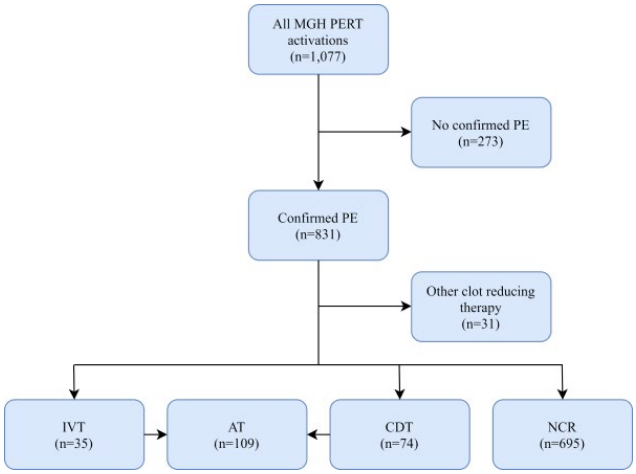

FIGURE 1 Flow chart describing patient selection and treatment stratification

However, it is unknown why clinicians in the PERT choose thrombolytic or other clot reducing therapies, nor is it known why patients are selected for CDT rather than systemic intravenous thrombolysis (IVT) or thrombectomy.

Aims: We aim to describe the patient- and operational characteristics associated with the use of CDT and IVT within the PERT program at Massachusetts General Hospital, Boston, USA (MGH).

Methods: This study was based on all confirmed PE patients within the MGH PERT database at January 1st, 2019. Patients were stratified according to treatment strategy, divided into patients having received CDT, IVT, any thrombolytic treatment (AT) or no clot-reducing therapy (NCR). Data were extracted on demographics, symptoms, medical history, clot burden, right heart strain, biochemistry and operational characteristics. Subsequently, univariate analyzes were performed for three different comparisons; ATvsNCR, CDTvsNCR and CDTvsIVT.

Results: Among 831 confirmed PE patients, 109 (13.1%) underwent thrombolytic therapy. Administration was divided between 74 (8.9%) CDT and 35 (4.2%) IVT. Four (0.5%) patients received both IVT and CDT and were excluded from analyzes. Several variables were significantly correlated to the general use of thrombolysis. Among others, central clot burden (OR 2.7, 95% CI 1.6;4.6), radiological evidence of right heart strain (OR 6.9, 95% CI 3.8;12.4), and syncope (OR 2.83, 95% CI 1.7;4.9) were strongly correlated to thrombolytic therapy. CDT was favored over IVT in patients with high central thrombus burden (OR 3.1, 95% CI 1.1;8.8) and during weekdays (OR 3.1, 95% CI 1.1;8.8).

Conclusions: In this retrospective analysis, we identified several factors associated with thrombolytic treatments within the MGH PERT program. This may help guide the design of future studies on thrombolytic therapy.

PB2483 | Impact Analysis of Prognostic Stratification for Pulmonary Embolism (iAPP): A Randomized Controlled Trial

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Background: Pulmonary Embolism Severity Index (PESI) is an extensively validated prognostic score on the risk of adverse events after acute pulmonary embolism (PE). However, impact analyses on management strategies, patient outcomes and health care costs are lacking

Aims: To assess whether the adoption of PESI helps physicians to correctly identify PE patients at low-risk of adverse events and to safely provide early discharge thus reducing the length of hospital stay (LOS) in internal medicine units

Methods: Multicenter randomized controlled trial, enrolling consecutive adult outpatients diagnosed with acute PE. Within 48 hours from diagnosis, treating physicians were centrally randomized, for every patient, to formally calculate PESI and reporting it in the clinical record form or to routine practice. Randomization was stratified by the intended treatment choice. The primary outcome was LOS.To find a statistically significant difference in the mean LOS of the two groups, 220 patients for each group were needed. ClinicalTrials.gov identifier NCT03002467.

Results: The study was prematurely interrupted due to slow recruitment rate. A total of 125 patients were enrolled at six internal medicine units, from 2016 to 2019. The mean age was 75.6 years (standard deviation, SD, 12.8). Treating physicians were randomized to the use of PESI for 61 patients or to perform routine practice for 64 patients. Overall, the mean PESI score was high (116.3, SD 47.5) and was similar between the two groups (115.5 and 117.1, respectively). The mean LOS was 10.1 days (SD 8.1) and was similar between the two groups (10 days, SD 8.7 and 10.3 days, SD 7.6, respectively).

Conclusions: The use of PESI in patients admitted to internal medicine divisions did not impact on the duration of hospital stay.

PB2484 | Direct Oral Anticoagulants vs. Warfarin for the Treatment of Venous Thromboembolism in Patients with Thrombophilia. A Single Center Experience

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Background: Direct oral anticoagulants (DOACs) are currently used in the acute and long-term treatment of venous thromboembolism (VTE). Because of their distinctive advantages, international guidelines recommend the use of DOACs over warfarin in patients without active cancer. However, the role of DOACs in the treatment of thrombophilia-associated VTE remains controversial.

Aims: To compare the efficacy and safety of DOACs vs. warfarin in thrombophilia patients using the following endpoints:

1. VTE recurrence rates,
2. residual symptoms at 12 months of index VTE, and
3. bleeding rates.

Methods: A retrospective study of thrombophilia patients who were started on anticoagulation, either DOAC or warfarin, by the hematology consult service at Emory University Hospitals, for the treatment of VTE in 2015.

Results: We investigated 49 thrombophilia patients. Average follow up duration was 10.2 months (range, 1-18 months). 20 (40.8%) patients were treated with DOACs, while 29 (59.2%) received warfarin. In the DOAC group, four patients (25%) were switched from warfarin to DOAC. *Table 1* illustrates the distribution of patient characteristics. *Table 2* illustrates the distribution of different thrombophilia. A total of four patients experienced VTE recurrence (three patients

TABLE 1 Distribution of patient characteristics

Characteristic	DOAC (N=20)	Warfarin (N=29)
Age at index venous thromboembolism, y (Mean, (SD))	42 (16)	46 (18)
Body mass index (Mean (SD))	28.27 (5.35)	30.33 (5.88)
Male, n (%)	11 (55)	18 (62.07)
Caucasian, n (%)	13 (65)	15 (51.72)
African American, n (%)	4 (20)	9 (31.03)
Congestive heart failure, n (%)	2 (10)	0 (0)
Inflammatory bowel disease, n (%)	1 (5)	3 (10.34)
Cancer, n (%)	0 (0)	2 (6.90)
History of venous thromboembolism, n (%)	10 (50)	11 (37.93)

TABLE 2 Distribution of different thrombophilia

Thrombophilia, n (%)	DOAC (N=20)	Warfarin (N=29)
Antiphospholipid syndrome	2 (10)	7 (24.14)
Heterozygous Factor V Leiden	7 (35)	5 (17.24)
Homozygous Factor V Leiden	1 (5)	0 (0)
Heterozygus Prothrombin gene mutation	2 (10)	3 (10.34)
Homozygous Prothrombin gene mutation	0 (0)	1 (3.45)
Protein C deficiency	3 (15)	1 (3.45)
Protein S deficiency	2 (10)	1 (3.45)
Antithrombin deficiency	1 (5)	3 (10.34)
Combined defects	1 (5)	6 (20.69)

in the DOAC group, and one patient in the warfarin group, p=0.179). Five patients (25%) in the DOAC group and five patients (17.2%) in the warfarin group complained of residual symptoms at 12 months of index VTE, p=0.32. Bleeding events occurred in four patients (20%) in DOAC group and four patients (13.8%) in warfarin group, p=0.42. Of these, five had gingival bleeding and three experienced gastrointestinal bleeding.

Conclusions: Rates of VTE recurrence and bleeding events were similar in DOACs and warfarin groups. DOACs may be a safe treatment option in thrombophilia patients. Further research is encouraged to explore whether these findings apply to specific patient subgroups such as high-risk Antiphospholipid syndrome and uncommon thrombophilia.

PB2485 | Appropriateness of Direct Oral Anticoagulant Prescriptions in Emergency Department Outpatients with Acute Deep Vein Thrombosis

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Background: Acute deep vein thrombosis (DVT) is a frequently encountered diagnosis with significant morbidity and mortality. Over the last decade, direct oral anticoagulants (DOACs) have gained favour as treatment. Though guidance statements have emerged to aid prescribing clinicians, significant rates of inappropriate prescriptions have been reported. There is however scarce data in patients with acute DVT discharged from the Emergency Department (ED) with a DOAC prescription.

Aims: To determine the proportion of inappropriate DOAC prescriptions in patients discharged from the ED with acute DVT at a large Canadian tertiary care center. Secondary outcomes were patient-, drug- and prescriber-specific characteristics associated with inappropriate prescriptions.

Methods: We conducted a retrospective cohort study of all patients with DOAC prescriptions discharged from the ED with acute DVT, between January 1st, 2012 and December 31st, 2016 at the Jewish General Hospital, Montreal, Canada. Data collected included drug-, prescriber- and patient-specific characteristics. Appropriateness was determined using the Medication Appropriateness Index (MAI). Prescriptions were categorized as A for appropriate, B for inappropriate with limited clinical significance, C for inappropriate based on specific criteria.

Results: A total of 44 patients with DOAC prescriptions for acute DVT were identified during the study period. Among the prescriptions, 10/44 (22.7%) were categorized as C and 24/44 (54%) as B; 5/44 (11.3%) had at least one C and one B criterion. The large majority 27/44 (61%) of inappropriate criteria pertained to cost-effectiveness; 16/44 (36%) were related to dosing, duration, and indication.

Conclusions: There is a large proportion of inappropriate DOAC prescriptions in patients discharged from the ED with acute DVT highlighting a care gap in this population. Further research aimed at developing targeted interventions to address this problem is needed.

PB2486 | E-selectin Inhibitor Is Superior to LMWH for Treatment of Experimental Venous Thrombosis

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Background: This study evaluated E-selectin inhibition with GMI-1271 alone and in combination with standard of care low-molecular-weight heparin (LMWH).

Aims: To improve vein recanalization, decrease vein wall inflammation and protect against adverse bleeding in a primate model.

Methods: Utilizing a well-documented primate animal model, Iliac vein thrombosis was induced by balloon occlusion of the iliac vein for 6 hours. Starting on post-thrombosis day 2, animals began treatment in two phases. In phase one, non-treated controls (CTR) received no treatment (n=5) versus animals treated with the E-selectin inhibitor GMI-1271 (GMI), 25 mg/kg, SC, once daily (n=4) for 21 days [results in-press JVS VL]. In Phase 2, animals were treated with GMI-1271 plus a combination of LMWH 1.5 mg/kg or 40 mg (GMI + LMWHc),

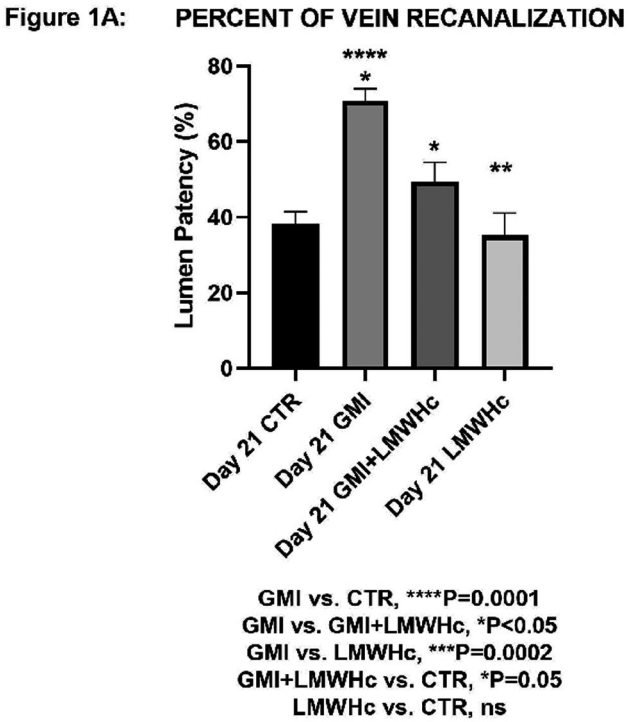

FIGURE 1 1A

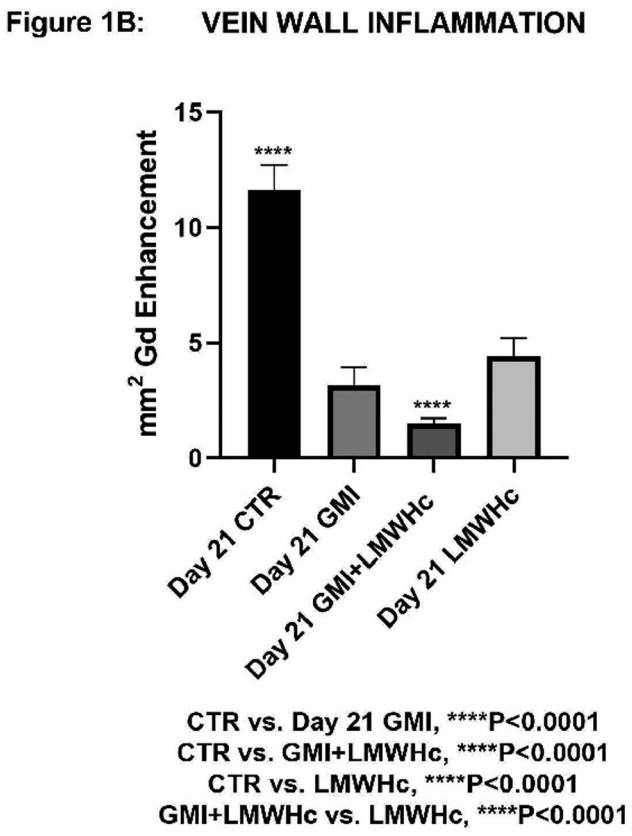

FIGURE 2 1B

SC, once daily (n=8) for 21 days; and animals treated with LMWH 1.5 mg/kg or 40 mg (LMWHc), SC once daily (n=6) for 21 days. Animals were evaluated by magnetic resonance venography (MRV) for vein recanalization and inflammation by gadolinium (Gd) extravasation, duplex ultrasound, coagulation tests (thromboelastography, bleeding time, PT, aPTT, fibrinogen) and CBC at baseline, days 2, 7, 14, and 21 at euthanasia.

Results: Percent vein recanalization by MRV was best in the GMI group followed by GMI + LMWHc (both p< 0.05 vs. CTR, Figure 1A). Vein wall inflammation decreased significantly in all groups (Figure 1B). By ultrasound, animals treated with GMI had no decreased vein opening by day 21, while groups GMI + LMWHc group decreased (-26%), LMWHc (-27%), and CTR (-80%). No clinically significant bleeding events were noted in any group. The LMWH groups trended to have prolonged coagulation test values while E-selectin inhibition with GMI did not cause clinically significant changes in coagulation measures.

Conclusions: E-selectin inhibition with GMI-1271 alone is superior to E-selectin inhibition combined with LMWH, LMWH alone and no treatment in this DVT model.

PB2487 | Heparinox, a Generic Version of Low Molecular Weight Heparin Enoxaparin, Is Bioequivalent to the Branded Version

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Background: Several generic versions of enoxaparin have also become available for clinical use in the indications for which this product is approved. The regulatory bodies require the generic products to conform with the product specifications in terms of biological and structural equivalence. Heparinox (Cristália, São Paulo, Brazil) represents a new generic version of enoxaparin which has become available in the South American continent recently.

Aims: The purpose of this investigation is to compare the branded enoxaparin (Sanofi Aventis, Paris, France) with heparinox using validated biologic and physico-chemical assays.

Methods: Heparinox brand of enoxaparin was obtained in commercial syringes containing 20 and 40 mg doses. Branded enoxaparin was commercially obtained in syringes containing 100 mg. The molecular weight profiling was carried out by gel permeation chromatography using a narrow range calibrator method. The anticoagulant activities were measured in whole blood and plasma based assays. The antiprotease activities were measured using anti-Xa and IIa chromogenic substrate methods. USP compliant assays were used to calculate potency relative to the NIBSC low molecular weight heparin standard (NIBSC, London, England).

Results: The molecular weight of 2 batches of heparinox was comparable to that of the branded enoxaparin (4.1 kDa vs. 4.1 kDa). In the ACT, APTT and thrombin time assays the concentration curves for

heparinox and branded enoxaparin were superimposable. Similarly, the antiprotease activities of the branded and generic products were comparable. There was no difference in the USP potency of the heparinox (96 anti-Xa units/mg, 32 anti-IIa units/mg) in comparison to the branded product (97 anti-Xa units/mg, 32 anti-IIa units/mg).

Conclusions: These studies demonstrate that the newly introduced generic low molecular weight heparinox exhibits similar molecular weight and functional profiles in comparison to the branded enoxaparin. Additional structural studies using NMR and oligosaccharide analysis, and pharmacological profiling in terms of antithrombotic, bleeding and PK/PD parameters are currently in progress.

PB2488 | Implementation of a Text-message Intervention to Warfarin Users in a Brazilian Primary Care Setting

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Background: There are lack of studies assessing the impact of short-text messages (SMS) to warfarin users.

Aims: To implement and analyze the feasibility of an intervention using SMS to primary care patients who use warfarin in a middle-sized city in Minas Gerais, Brazil, as well as patient satisfaction.

Methods: A bank of 75 messages was drafted and reviewed by an expert panel. During six months, three times a week, patients received semi-personalized messages about adherence and motivation, general information and diet and lifestyle about warfarin and anticoagulation. At the fourth month, participants answered a satisfaction questionnaire. The following tests were applied on baseline: Oral Anticoagulation Knowledge (OAK) Test; adapted MAT test and Short Assessment of Health Literacy For Portuguese-Speaking Adults. Patients signed informed consent. The study was approved by the *Universidade Federal de Minas Gerais* Research and Ethics committee.

Results: Thirty-eight patients were included, median age 60 (interquartile range 53-70) years-old, 51.6% women. The indication of anticoagulation was atrial fibrillation in 44.7%, venous thromboembolism in 34.2%, prosthetic heart valve in 23.7%. By the adapted MAT, 85.1% were adherent to therapy, the total number of correct answers in the OAK Test was 52.0% and 71.8% had insufficient health literacy. Thirty-one participants (84.2%) answered to the satisfaction questionnaire, 96.7% of them considered the messages were useful and easy to read. Patients perceived the messages helped them to better understand the treatment (93.5%), remember to take medication (87.0%) and change eating habits (74.2%). All participants reported to be willing to continure receiving the messages and were likely to recommend the program to others.

Conclusions: The intervention was well accepted by the local population and showed a positive impact on the perceived well-being, adherence and motivation of Warfarin users.

PB2489 | Optimal Duration and Dose Intensity of the Anticoagulation Therapy with Doacs in Venous Thromboembolism: Retrospective, Cohort Study "Real Choice"

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Background: Extended treatment for Venous Thromboembolism (VT) remain uncertain in terms of optimal duration and dose intensity even in the era of direct inhibitors (DOACs).

TABLE 1

Demographic and Clinical Characteristics	Group A: 180 patients	Group B: 180 patients	P Value
Age - yr Median (IQR)	69 (24-87)	68 (22-88)	NS
Male sex n° (%)	99 (55.0%)	112 (48.7%)	NS
Index VTE - no. (%) Proximal DVT Distal DVT Isolated PE Both DVT + PE Upper Extremity DVT Unusual Site VT	109 (60.6) 18 (10) 26 (14.4) 20 (11.1) 5 (2.8) 2 (1.2)	145 (80.6) 10 (5.6) 12 (2.2) 9 (9.4) 3 (1.7) 1 (0.6)	NS .01 .001 NS NS NS
Classification of index VT - no. (%) Unprovoked Provoked Previous VT	105 (58.3) 82 (45.6) 34 (18.9)	106 (58.9) 88 (48,8) 30 (15.0)	NS NS NS
Duration of previous anticoagulation no (%) 3 mo 6 mo ≥12 mo	9 (5.0) 67 (37.2) 104 (43.9)	57 (31.7) 95 (52.8) 28 (15.6)	.0001 .0001 .0001
No anticoagulation no (%) Anticoagulation no (%) Therapeutic dose Prophylactic dose	56 (31.1) 124 (68.9) 9 (5) 115 (63.9)	87 (48.3) 93 (51.7) -	.001 .001
Study Outcomes			
Primary Composite Outcome no (%) TV Events Major Bleeding	5 (2.8) 2(1.1) 3 (1.7)	16 (8.9) 14 (7.8) 2 (1.1)	.001 .001 NS

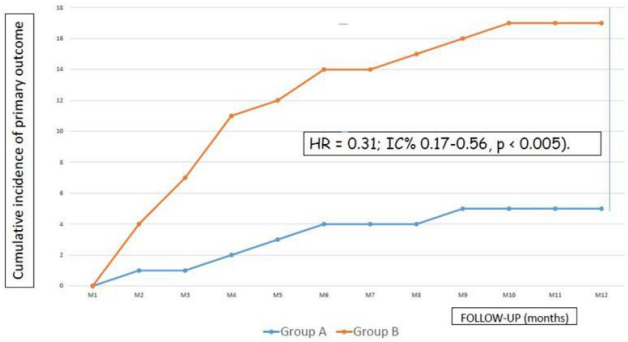

FIGURE 1

Aims: To assess the efficacy and safety of determining the duration and dose of the anticoagulation therapy in VT patients according to a clinical judgment risk stratification protocol focused on the thrombotic / hemorrhagic risk ratio.

Methods: Retrospective, cohort study. 180 patients with VT assessed at the Cardiovascular Medicine Unit of Reggio Emilia Hospital from January 2016 to December 2018 with minimum duration of DOACs therapy of three months were eligible (group A). Major exclusion criterion: active cancer. The decision to continue or not the therapy and dose intensity were modulated according to degrees of risk of recurrence and major bleeding. 180 patients as historical control group (group B) were considered in which the optimal duration of therapy with vitamin K antagonists (AVK) was established mainly on the basis of the D-dimer values. The primary composite outcome was symptomatic or fatal recurrent VT and major or fatal bleeding events.

Results: Table 1 shows demographic and clinical characteristics of patients and study outcomes. Figure 1 shows cumulative incidence of primary outcome in group A and B at one year of follow-up. The net benefit primary composite outcome is in favour of group A (HR = 0.31; CI% 0.17-0.56, p < 0.005).

Conclusions: Among patient with VT, 30% of the patients safely stopped anticoagulation therapy on clinical basis being exposed to risk of recurrence of 2.8% in the first year while 70% of the patients (90% in prophylactic dose) continued therapy being exposed to negligible major bleeding risk (less than 1%) in the first year. These real life results are aligned with data from Amplify Extension and Einstein Choice studies.

PB2490 | National Survey about Management of Venous Thromboembolism (VTE) Associated with Pregnancy / Puerperium or Oral Contraceptives in Uruguay

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Background: We focus on this VTE group since it comprises relatively frequent situations that affect young patients with events categorized as "associated with a weak risk factor" (1). There is controversy about the study of underlying thrombophilia and subsequent management in this group. The easy access to the study of thrombophilia and the lack of protocolization determines not only higher health costs but also inappropriate treatments.

Aims: To know the clinical practice in our country.

Methods: This is a descriptive study. We designed an online, anonymous survey, aimed at internists, hematologists and gynecologists. Based on defined clinical vignettes, the study of thrombophilia and length of treatment according to its presence or absence were assessed. We previously defined what we consider to be a completely correct study (tables 1-2).

Results: 178 specialists answered the survey: 86 internists, 46 hematologists and 46 gynecologists.

Regarding vignette 1: 100% of gynecologists and 89% of hematologists and internists decided to study thrombophilia. Of the internists and hematologists who studied thrombophilia, only 25.4% conducted a correct study (table 1). Length of anticoagulation: 5% of internists and hematologists would choose indefinite anticoagulation. The percentage increases to 24% if the patient was heterozygous for FV Leiden.

Regarding vignette 2: 93.5% of gynecologists and 75% of hematologists and internists decided to study thrombophilia. Only 19.2% of the internists and hematologists who studied thrombophilia, conducted a correct study (table 2). Length of anticoagulation: 5% of the internists and hematologists would prescribe indefinite anticoagulation. The percentage increases to 15% if the patient was heterozygous for GP20210A.

Conclusions: Although there are some recommendations (2-3), this group of patients is managed in a very heterogeneous way. This

TABLE 1 Vignette 1

Vignette 1
You assist a 25-year-old female patient with no personal or family history of VTE. She had started combined oral contraceptives 3 months ago. She developed an unprovoked proximal DVT.
The survey included questions about thrombophilia study and length of anticoagulation if Factor V Leiden was positive or negative.
Appropriate study: *AAF: at any time without warfarin *FVL and GP 20210A: at any time *Protein S and C: away from the acute event and without warfarin *Antithrombin: at any time without heparin

TABLE 2 Vignette 2

Vignette 2
You assist a 28-year-old female patient with no personal or family history of VTE. While 20 weeks pregnant, she is diagnosed with PE (pulmonary embolism)
The survey included questions about thrombophilia study and length of anticoagulation if GP20210A was positive or negative.
Appropriate study: *AAF: at any time without warfarin *FVL and GP 20210A: at any time *Protein S and C: 6 weeks after delivery and without warfarin *Antithrombin: at any time without heparin

leads to important implications for quality of life and health expenditure. This survey highlights the value given to low-risk thrombophilia to define the length of anticoagulation.

PB2491 | Biological and Pharmacological Profiling of Pentosan Polysulfate (PPS) in Comparison to Heparin and its Relative Neutralization by Protamine Sulfate

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[1]Loyola University Medical Center, Maywood, United States; [2]Global Thrombosis Forum, Suwanee, United States

Background: Pentosan Polysulfate (PPS) has traditionally been used to treat patients with Interstitial Cystitis and more recently osteoarthritis, cancer, HIV and prion disease.

Aims: The purpose of this study is to create a comprehensive profile and comparison of several batches of PPS with unfractionated heparin. Additionally, the feasibility of protamine sulfate as a neutralizing agent to reverse the anticoagulant effects of PPS is investigated.

Methods: Various batches of PPS at 1 mg/ml were prepared in saline. For whole blood clotting profile, thromboelastography (TEG) was carried from 2.5 - 5.0 ug/ml and activated clotting time (ACT) at 25ug/mL. Clotting profile in whole blood, retrieved plasma and supplementation in normal human pooled plasma (NHP) were carried out at 100 - 0.0 ug/ml by using anticoagulant assays. Amydolytic methods were used to measure anti-Xa/ IIa effects. Thrombin generation inhibition studies (TGA) were carried out in NHP from 100 - 0.0 ug/ml. Heparin supplementation and protamine sulfate neutralization in NHP from 10 -0 ug/mL were compared to PPS batches in a logarithmic scale.

Results: All drugs produced a concentration dependent effect. Similar effects in anticoagulation assays were noted except anti-Xa assay. In TEG, significant viscoelastic changes were observed at 5ug/mL. Prolongation of ACT was observed at 25ug/mL. In TGA, concentration dependent but no significant difference in between the batches were noted. Neutralization of protamine sulfate was observed up to 12.5 ug/mL in anticoagulant assays and marginal effect on anti-protease assays. Neutralization studies indicated that protamine sulfate is a weaker neutralizing agent of PPS compared to heparin.

Conclusions: These studies suggest that multiple batches of PPS have a similar anticoagulant profile. In comparison to heparin, PPS presents itself as a 10-fold weaker anticoagulant alternative for use in a variety of clinical applications.

PB2492 | Direct Oral Anticoagulants and Antiepileptic Drugs: Is There Room for Concurrent Treatment?

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Background: Current guidelines recommend caution when prescribing with direct oral anticoagulants (DOACs) patients concurrently taking antiepileptic drugs (AED), with particular regard to levetiracetam and valproic acid. Available data suggest a possible drug-drug interaction potentially leading to decreased DOACs plasma level and treatment failure.

Aims: We collected data on patients taking DOACs together with AEDs in order to evaluate the occurrence of DOACs failure during clinical follow-up.

Methods: In this case series, we report data on patients consecutively referred to our center receiving concurrent treatment with AED and DOACs (both for prevention of arterial embolism in atrial fibrillation [AF] and treatment of venous thromboembolism [VTE]). Data on clinical history and reasons for drug prescription, as well as plasma levels of DOACs and AEDs were collected. Patients were regularly followed as outpatient and information on cerebrovascular events and/or venous thrombosis was collected.

Results: We report data on 14 patients treated with DOACs + AEDs. The mean age at referral was 69±15 years; 43% (6/14) of patients were males. VTE treatment was the main reason for DOACs prescription (64.3%), being epilepsy prevention the indication for AED prescription. Five patients were taking apixaban, 4 rivaroxaban, 3 dabigatran and 2 edoxaban. Levetiracetam and valproic acid were the most prescribed AEDs (5 and 5 patients, respectively), while a minority of patients were treated with phenobarbital and phenytoin. At the laboratory tests, 13/14 patients had in-range values of DOACs; 1 patient on levetiracetam had dabigatran levels close to the lower limit. During a period of DOAC+AED treatment of 10±9 months (range 1-27 months), no adverse events were reported.

Conclusions: In our experience, concurrent treatment with AED and DOAC usually did not lead to a reduction in drug concentration. In a clinical follow-up, in both AF and VTE patients, no adverse events due to reduced DOACs effect have been registered at present.

WOMEN'S HEALTH

ESTROGENS AND PROGESTINICS

PB2494 | Oral Contraceptives and Venous Thromboembolism: Focus on Testing that May Enable Prediction and Assessment of the Risk

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Background: Combined oral contraceptives (COC) induce several changes in the levels of coagulation factors. These effects on hemostasis are more pronounced with third or fourth generation COC compared to second-generation COC and risk minimization measures are required to reduce the risk.
Aims: To review the strategies aiming at assessing the thrombotic risk in women taking COC in order to identify inherited or acquired hemostasis abnormalities associated with thrombotic events.
Methods: An literature review was performed on PubMed from inception to January 2020.
Results: COC use causes changes in coagulation that modify the prothrombotic state induced by pre-existing hemostatic alterations in a supra-additive manner. Therefore, testing appears to be of importance not only before implementing COC but also in order to monitor potential thrombogenicity induced by COC. Namely, inherited genetic factors like FV Leiden, G20210A prothrombin mutation, antithrombin, protein C or protein S deficiencies, non-O blood group, as well as the CYP2C9*2 and rs4379368 mutations, have all been identified as genetic predictive risk factors of venous thromboembolism in these women. Nevertheless, the screening of all these genetic biomarkers is costly and does not allow a global estimation of the prothrombotic risk.The "ideal" testing should be able to assess the additive expression of inherited or acquired thrombophilia with hormonal-induced coagulopathy. The ETP-based APC resistance is sensitive towards all the coagulation defects that are frequently recognized individually as prothrombotic factors and fit particularly well with the relative risk increase observed in epidemiological studies of vulnerable populations.
Conclusions: Thus, while further works are still needed to confirm its status as a non-expensive, widely implementable and generic coagulation screening test for the assessment of the prothrombotic state in women taking contraceptive or other hormonal therapies, the ETP-based APC resistance already demonstrated the capacity of detecting prothrombotic state which is the Holy Grail of preventive medicine in the field of thrombosis.

PB2495 | Hematologic Alterations in Patients Undergoing Controlled Ovarian Stimulation for in vitro Fertilization: A Prospective Observational Study

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Background: Pregnancy, hormonal contraception and hormone replacement therapy is associated with increased risk of venous thromboembolism (VTE). However, the pathogenesis of these hormone-induced changes is unclear. In vitro fertilization (IVF) offers a unique model to study hematologic alterations, due to rapid changes in endogenous estradiol (E_2) levels during controlled ovarian stimulation (COS).
Aims: To evaluate changes in coagulation profiles of patients undergoing COS for IVF.
Methods: Prospective, observational study of women < 40 years old undergoing their first IVF cycle. Women with known hypercoagulability, diminished ovarian reserve, or whose partners required surgical sperm retrieval were excluded. COS parameters included total COS days, total gonadotropins administered (IU), peak E_2 level, total number of oocytes retrieved and fertilization rates. Hematologic parameters were recorded on Day 1 of COS and on the day post-hCG

TABLE 1 Comparison of hematologic parameters (n=24) Data presented as (+/-SD), mean (SD)

Parameter	Day 1 of COS	Day post hCG trigger	P
CBC	Normal	Normal	–
PT (sec)	10.3 (0.7)	10.6 (0.8)	0.17
aPTT (sec)	29.2 (2.2)	28.3 (2.0)	0.14
PFA collagen-EPI (sec) PFA collagen-ADP (sec)	101.5 (30.3) 147.8 (48.3)	83.5 (14.2) 112.8 (43.4)	0.01 0.01
Fibrinogen (mg/dL)	284.5 (77.6)	274.7 (54.7)	0.62
Factor VIII	109.1 (44.1)	125.5 (47.4)	0.22
VWF antigen	125.5 (43.7)	119.5 (49.6)	0.66
APC resistance	5.3 (1.4)	5.6 (1.5)	0.48
D-dimer	Neg	Neg	–

TABLE 2 Comparison of hematologic parameters (n=24). Data presented as mean (+/-SD)

Parameter	Day 1 of COS	Day post hCG trigger	P
Protein S activity	91.7 (18.9)	85.9 (15.2)	0.25
Protein S antigen free	110.7 (23.3)	103.6 (14.6)	0.21
Lupus anticoagulant (DRVVT)	0.97 (0.1)	0.98 (0.1)	0.73
Lupus anticoagulant (Silica Clotting Time)	1.08 (0.7)	0.99 (0.1)	0.54
Anticardiolipin antibodies	Neg	Neg	–
Anti-β2 glycoprotein 1 antibodies	Neg	Neg	–
C-Reactive protein	<0.5	<0.5	–
Ferritin	41.1 (32.2)	44.7 (29.2)	0.69
Factor II G20210A	Neg	Neg	–

trigger, including prothrombin time, activated partial thromboplastin time, platelet function assay (PFA), fibrinogen, protein S activity, protein S antigen free, factor VIII, activated protein C resistance, lupus anticoagulant, anticardiolipin and anti-β2 glycoprotein 1 antibodies, and ferritin. Paired *t*-tests were used for statistical analysis.

Results: 37 women were screened; 24 met inclusion criteria. Median [IQR] age was 33 (28-37) years. Women underwent COS for 11 (8-15) days and required 2525 (975-3950) IU of gonadotropins. E_2 level on Day 1 of COS was 2719 (1956.5-2995.5) pg/mL. Total oocytes retrieved were 17 (15-23), of which 14 (11-19) were mature. Compared to levels on Day 1 of COS, PFA Collagen-ADP (101.5 vs. 83.5 seconds; *P*=0.01) and PFA Collagen-Epi (147.8 vs. 112.8 seconds; *P*=0.01) closure times were shortened by18% and 24%, respectively post-hCG trigger (Table 1). There were no significant differences in the other hematologic parameters (Table 2).

Conclusions: Our findings suggest that COS is associated with a mild hypercoagulable state demonstrated by a shortening closure time in PFAs. A larger sample size is necessary to validate our findings.

PB2496 | Development and External Validation of a Risk Assessment Model for First Venous Thromboembolism in Women Starting Combined Oral Contraceptives

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Background: The risk of venous thromboembolism (VTE) is increased in women who use combined oral contraceptives (COC). Therefore, a risk assessment model identifying women at low/high risk before starting COC would be clinically valuable in guiding the choice of whether or not to prescribe COC.

Aims: The aim of this study was to develop and externally validate a risk assessment model for VTE in women within one year of starting COC.

Methods: Data from the MEGA study, a large population-based case-control study was used. Candidate predictor were chosen based on literature. Multiple imputation was used for missing data, with five imputed datasets and pooling according to Rubin's rules. Using logistic regression, a backward selection procedure was performed to obtain the most optimal model (clinical+genetic and clinical only model). The discriminative value of the models was assessed by calculating the area under the curve (AUC) of the ROC curve.

Results: 179 Cases and 577 controls were included within 1 year after starting COC. Tables 1 and 2 show preliminary results regarding the AUC values for each model.

TABLE 1 Candidate predictors in each model

	Full	Clinical+Genetic	Clinical
Age Plastercast	x x	x	x
Hospital admission Surgery	x x	x	x
Bedridden Injury	x x	x x	x x
Inflammation Varicosis	x x		
Comorbidity Tromboflebitis	x x	x	x x
Family history of VTE Body mass index	x x	x x	x x
Smoking Factor V Leiden	x x	x	
Prothrombin	x	x	
Non-O bloodtype	x	x	

TABLE 2 AUC values of each model

	AUC	95% CI
Full	0.814	0.776 - 0.852
Clinical+Genetic	0.813	0.776 - 0.851
Clinical	0.771	0.729 - 0.812

The clinical model contained predictors which are not (commonly) used in the clinic, e.g., injury, co-morbidity, but also expected variables, e.g., family history of VT.

The clinical+genetic model had a higher AUC value: 0.813 (95%CI: 0.776-0.851) than the clinical only model: 0.771 (95%CI: 0.729-0.812).

Conclusions: Current results show that adding genetic risk factors improved the performance of the clinical model and may suggest that screening for genetic variants may be important in women starting COC. Future steps will be to explore other methods of variable selection, i.e., Lasso, to perform internal and external validation of the models, and to develop a scoring system based on regression coefficients to simplify the model and to make its usage optimal in the clinic.

PB2497 | Trends in Combined Oral Contraceptive Use in the Netherlands: 2002-2016

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Background: The type of progestogen in combined oral contraceptives (COC) became a focus of discussion in the mid-1990s as studies showed that progestogens gestodene and desogestrel were associated with an increased risk of venous thrombosis (VT) compared with the progestogen levonorgestrel. This led to a change in Dutch GP guidelines in 1998 which stated that physicians should preferably prescribe COC containing levonorgestrel.

Aims: The aim of this study was to investigate the trends in COC use after these guidelines were implemented. Trends in the use of other high risk progestogens drospirenone and cyproterone acetate (CPA) as well as the more recently developed progestogens dienogest and nomegestrol were also assessed.

Methods: Data from Dutch Foundation for Pharmaceutical Statistics (SFK) were used. We defined starters of COC as women who did not use hormonal contraceptives in the two years before starting COC.

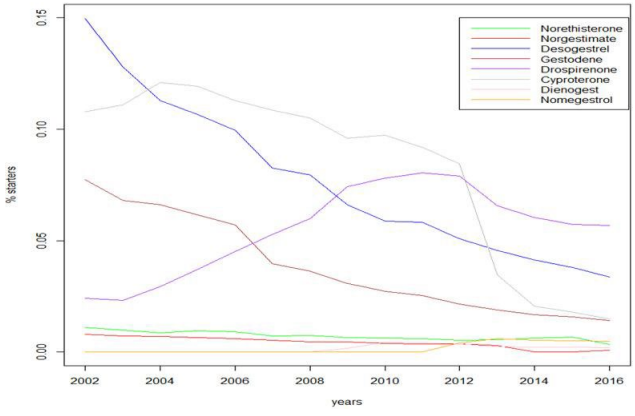

FIGURE 1 1a

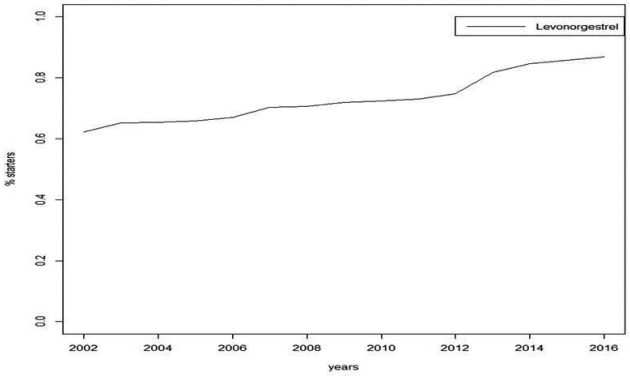

FIGURE 2 1b

We assessed the proportion of starters of each progestogen from 2002-2016.

Results: The largest proportion of starters started with levonorgestrel (>60%) and this proportion increased throughout the years (Fig. 1a). Simultaneously, gestodene and desogestrel starters showed a continuous decrease. Drospirenone and CPA starters showed a large decrease in 2012 onwards (Fig. 1b). At the same time, there was a large increase in levonorgestrel starters. The smallest proportion of starters started with COC containing northisterone, norgestimate, dienogest or nomegestrol.

Conclusions: Following a change in Dutch GP guidelines, we observed a steady increase in levonorgestrel starters and a decrease in gestodene/desogestrel starters. In 2012-2013, France banned the use of CPA following 4 deaths from VT in women using this high risk COC. The decrease in CPA in Netherlands was most likely a consequence of this pill-scare. At the same time drospirenone was shown to increase VT risk and its usage dropped from 2012 onwards. Use of other progestogens, including newer ones, remained low.

PB2498 | Knowledge vs Actual Thrombotic Risks Associated with Hormonal Contraception in Young Women

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Background: Hormonal contraceptives (HC) are widely prescribed in young women for contraception and other reasons. The relative risk of Venous Thromboembolism (VTE) is 3-5 folds compared to nonusers. This risk is related to estrogens and is influenced by dose, type, duration, family history of VTE or thrombophilia and obesity. Conventional haemostasis testing would not detect hypercoagulability or predict thrombosis risk. Thromboelastography (TEG) is a sensitive global haemostatic test that analyzes clot kinetics. Studies show many young women are unable to make informed decision about HC

due to poor knowledge. Around 300 healthy young women are dying yearly in the US due to HC.

Aims: To determine the level of knowledge of young females concerning the risks/benefits of HC and to evaluate the coagulability of blood of HC users.

Methods: 123 participants 18-30 years in various academic programs completed an anonymous online 32- items survey in three sections: demographics, use/type/duration of hormonal contraception and knowledge of risks and benefits. Of these, 43% high-school diploma, 23% college diploma, 22% bachelor, 11% post-graduate. 70 females consented to blood samples for testing using TEG.

Results: Of the 123 participants, 76 HC users (36 >1 year) and 47 non-users. Top reported benefits: not getting pregnant (84%), clearing acne (59%), regulating menses (84.5%), reducing cancer risk (31%). Common side effects were: weight gain (99%), mood swings (94%) and changes in sexual desire (85%). 92% believe HC increases risks for blood clots. 80% understand the terms: thrombosis, DVT and PE and 70% recognize their symptoms. TEG data for 4 out of the 20 tested female (20%) showed hypercoagulable traces as evidenced by increased MA and CI parameters above normal ranges.

Conclusions: Misconceptions remain among young women concerning benefits and risks of HC. Larger clinical studies using TEG have the potential to predict VTE risk and individualize or modify prescribed regimens.

PB2499 | Low Dose Warfarin (LDW) for the Prevention of Hypercoagulation of Blood and Venous Thromboembolism (VTE) in Women Receiving Hormone Replacement Therapy (HRT)

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Background: VTE is a rare but serious complication associated with HRT.

Aims: To assess the anticoagulant effect (AE) of LDW by well-known method - blood recalcification technique (BRT).

Methods: From 2016 to 2019 we studied 225 women aged 35-62 receiving HRT (estrogen). Method BRT: The recovery of ionized Ca++ initial concentration in citrated blood. In our method, we used the diminished volume of added 1.29% CaCl2 to blood than usually. We have found that this method increases the sensitivity of BRT in detection of the blood hypercoagulation (Fig. 1) (patent of the RF #2015515) and helps us to see better the AE. BRT was determined by thromboelastometry (TEM) (ROTEM, Germany): time of reaction "R" - normal range = 640-960 s. Blood for first analysis was taken before HRT.

Results: The results of blood analysis demonstrated that all tested women could be divided into two groups. The 1st group of women (# 185, 82%) had normocoagulation (R=886±115 s). This group began to receive HRT immediately. We repeated the analysis after 1-1.5 months of receiving HRT. If there was normocoagulation, we continued the HRT. If we found hypercoagulation, we began treatment with warfarin (the dose was estimated individually by TEM: target range 640-960 s). The 2nd group of women (# 40, 18%) had hypercoagulation (R = 591±145 s). This group received their treatment with warfarin as preliminarily step before HRT. After getting of target range by TEM, we began HRT. There was no one case of VTE in both of groups.

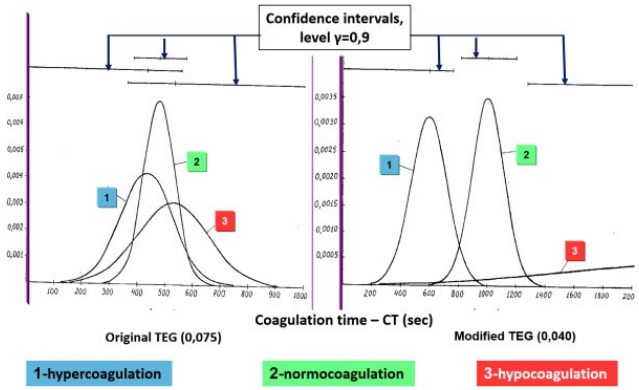

FIGURE 1 The difference of original and modified TEGs. Probability density function of coagulation time

Conclusions: The HRT can create the hypercoagulation and this may lead to the risk of VTE. Using the modified BRT and TEM we can not only diagnose this risk but also control the adequacy of anticoagulant treatment. LDW could be used rather effectively and at the same time with safety for elimination of hypercoagulation created by HRT.

PB2500 | Use of Oral Contraceptives (OCs) and Thrombin Generation: Cross-sectional Analysis of the Longitudinal Study of Adult Health (ELSA-Brazil)

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Background: Use of oral contraceptives (OCs) has been associated with increased thrombin generation (TG) and acquired resistance to the anticoagulant action of the activated Protein C (aPC) contributing to a hypercoagulability state.

Aims: To evaluate the association between current and past use of OCs and TG and resistance to aPC in a sample of Brazilian female civil servants.

Methods: Participants of this cross-sectional study were women of childbearing age enrolled in the baseline wave of the ELSA-Brasil cohort (N = 641). Thrombin generation test (TGT) was performed with low and high tissue factor (TF), with and without aPC and normalized aPC sensitivity ratio (nAPCsr) were calculated for the determination

TABLE 1 Crude and adjusted beta coefficients for the association between GT parameters in the Low FT condition and the categories of CO use. *P<0.001

Parameters	Former users	Current users	Former users (after adjustments for age and total cholesterol/HDL cholesterol ratio)	Current users (after adjustments for age and total cholesterol/HDL cholesterol ratio)
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
Lagtime (min)	-0.13 (-0.45-0.18)	-1.21 (-1.75 - -0.68)*	-0.08 (-0.40-0.23)	-1.06 (-1.60 - -0.52)*
Time to peak (min)	-0.13 (-0.60-0.35)	-1.97 (-2.77 - -1.16)*	-0.06 (-0.54-0.41)	-1.77 (-2.59 - -0.95)*
Peak (nM)	0.77 (-14.64-16.19)	82.44 (56.21-108.66)*	1.92 (-13.56-17.40)	85.96 (59.16-112.77)*
ETP (nM.min)	6.51(-62.32-75.35)	352.79 (235.67- 469.90)*	19.87 (-48.24-87.97)	388.85 (270.93-506.76)*
normETP	0.00 (-0.03-0.03)	0.14 (0.09-0.20)*	0.01 (-0.02-0.04)	0.16 (0.11-0.21)*

TABLE 2 Crude and adjusted beta coefficients for the association between GT parameters in the High FT condition, nAPACs and the categories of CO use. *P<0.001

Parameters	Former users	Current users	Former users	Current users
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
Lagtime (min)	-0.13 (-0.28-0.03)	-0.41 (-0.67 - -0.15)*	-0.11 (-0.27-0.04)	-0.35 (-0.62 - -0.09)*
Time to peak (min)	-0.01 (-0.16-0.13)	-0.49 (-0.74–0.25)*	0.01 (-0.13-0.15)	-0.42 (-0.67-0.17)*
Peak (nM)	-3.14 (-13.15-6.88)	43.57 (26.57-60.57)*	-1.06 (-10.92-8.80)	49.03 (32.00-66.06)*
ETP (nM.min)	1.16 (-63.9-66.25)	263.35 (152.86-373.84)*	15.48 (-48.48-79.44)	301.76 (191.29-412.23)*
normETP	-0.01 (-0.04-0.03)	0.12 (0.07-0.18)*	.001(-0.03-0.03)	0.15 (0.09-0.20)*
nAPCsr	0.03 (-0.15-0.21)	1.17 (0.87-1.47)*	0.03 (-0.15-0.21)	1.16 (0.86-1.47)*

of acquired resistance to aPC. The participants were questioned about the use of OCs and categorized in current users, former users and nonusers. Association between OCs and TG was investigated by multivariable linear regression, using the non-users as a reference category.

Results: Most current users of OCs used 30-40μg of estrogen and 3rd generation progestogen for more than one year. TGT in the low TF condition showed that current users of COs had significantly lower means of lag time and time-to-peak and significantly higher means of peak and ETP when comparing to non-users, even after adjustments for age and total cholesterol/HDL cholesterol ratio. There was no association between former use of COs and TGT. We found same results for all TGT parameters in the high TF condition as well as for nAPCsr. No difference in TGT parameters was observed between ex-users and nonusers suggesting that the effect of OCs on blood clotting is reversible.

Conclusions: Current use of COs, but not former use, showed to be associated with both higher TG and nAPCsr confirming resistance to aPC in women by OCs use.

PB2501 | Levonorgestrel Releasing Intrauterine System (LNG-IUS) in a Patient with Deep Endometriosis, Adenomyosis and Behcet's Disease: Case Report

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Background: Behçet's disease (BD) is a multisystemic inflammatory disease of unknown etiology that involves inflammatory processes in vessels of different calibers. Its clinical manifestations include cutaneous, oral, genital and ocular lesions, as well as neurological symptoms and vascular impairment, which is the main cause of death due to the risk of thrombosis.

Aims: Record case of a patient with BD, deep endometriosis and adenomyosis after using LNG-IUS for symptom control and lower risk of thromboembolic events.

Methods: The information was obtained by reviewing the medical record and discussed based on the review of the literature found in PubMed and Scielo sources.

Results: BD is a thrombophilic disease characterized by chronic systemic vasculitis that can involve both the venous system (88%) and the arterial system (12%). According to the 2015 World Health Organization eligibility criteria for the use of contraceptive methods in patients with a history of thrombosis, combined hormones are

contraindicated due to the estrogenic component, being an option, hormonal methods containing only progesterone, such as LNG-IUS. In this specific case, it was also used to control the symptoms of deep endometriosis and adenomyosis.

Conclusions: The use of LNG-IUS proved to be an effective option in the control of symptoms related to deep endometriosis / adenomyosis in a patient at high risk of thrombosis, and further studies are needed to demonstrate this efficacy.

PB2502 | Lack of Association between the Use of Female Hormones and Platelet Volume Indexes: Evidence from the Longitudinal Study of Adult Health (ELSA-Brasil)

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Background: Platelet volume indices (PVI), including mean platelet volume (MPV), platelet distribution width (PDW) and proportion of large platelets (P-CSF), are obtained using low-cost hematological analyzers. It is suggested that these indices can be used in clinical practice as markers of platelet function in women using oral contraceptives (OC) or hormonal therapy (HT).

Aims: To evaluate the association of PVI and the use (current or past) of OC and TH in a sample of Brazilian women of childbearing age or of menopause.

Methods: Cross-sectional study, carried out with 1,249 women, of childbearing age or of menopause, from the baseline (2008-2010) of the Longitudinal Study on Adult Health (ELSA-Brasil), from Minas Gerais and Rio Grande do Sul. PVI were performed on a specialized hematology analyzer, including MPV, PDW and P-LCR. All participants were questioned about the use of OC and of HT and were categorized in current users, former users and nonusers. The ANOVA test and Bonferroni post-test were used to compare means according to the categories of use of COs and HT and the value of $p < 0.05$ was considered significant.

Results: Participants of childbearing age had an average age of 45.1 ± 5.0 years, while those in menopause, 58.3 ± 6.5 years. No significant differences were observed in the mean values of VPM, PDW and P-LCR between non, formers and current users of OC and neither of HT.

Conclusions: No association was found neither between use of OC nor of HT (current and past) and platelet volume indexes including MPV, PDW and P-LCR. Longitudinal studies are needed to confirm our findings.

PREGNANCY AND PREGNANCY COMPLICATIONS

PB2503 | Management of a Pregnancy in a Woman with Hypodysfibrinogenemia: An Experiment in Strasbourg

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Background: Fibrinogen is an essential factor for pregnancy, being actually involved in trophoblastic adhesion and migration, and in placental integration. In order to fulfill its role, fibrinogen has to be in sufficient quantity and quality, with a cut-off rate estimated at 1 g/L.

Aims: The authors report the management of a pregnancy in a 26 year-old Syrian woman with an important obstetrical history owing to a hypodysfibrinogenemia. In fact, she underwent a fetal loss at 6 months, several early miscarriages, and a miscarriage at the 17th week secondary to a placental abruption associated with a severe bleeding estimated at 4000 mL. However, two full-term deliveries successfully occurred in Syria, each ended in a uterine embolization on account of a severe postpartum bleeding.

Methods: Her hypodysfibrinogenemia is characterized by a basal fibrinogen rate at 0.6 g/L and 1.2 g/L, respectively with the Clauss activity and the immunologic assays. The others hemostatic parameters are strictly normal. She carries two heterozygote mutations on the *FGG* gene, namely c.1086T>C on exon 8 responsible for hypofibrinogenemia, and c.124G>A on exon 3 whose impact remains unknown. The familial investigation revealed that her two children and her sister carry the same hypodysfibrinogenemia.

Results: A later pregnancy was recently managed in France. This reached successfully full term thanks to a therapeutic protocol used until the 34th week, consisting on infusions of fibrinogen concentrates two times per week, a daily injection of 4000 IU of enoxaparin, and a daily dose of 75 mg of aspirin. A caesarean section was made at the 40th week under fibrinogen concentrates in order to prevent bleedings. The postpartum follow-up was very good, without any complications.

Conclusions: This case illustrates the success of a prophylaxis regimen, associating fibrinogen concentrates and antithrombotic drugs, in a young woman with hypodysfibrinogenemia. These very rare situations should be collected in an international register.

PB2504 | Study of the State of the Hemostatic System in Women with Various Pregnancy Complications

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Background: One of the problems of present medicine is the risk of thrombosis during pregnancy. The relationship between thrombosis and pregnancy complications is shown, and the high effectiveness of anticoagulant prophylaxis is proved. However, there are practically no studies comparing the state of the hemostatic system in women with various pregnancy complications.
Aims: To compare the status of the hemostatic system in pregnant women with various pregnancy complications.
Methods: We examined 34 pregnant women. Informed consent was obtained from all patients in accordance with the Declaration of Helsinki. All women were divided into groups with various complications of pregnancy: venous insufficiency (n=7), thrombophilia (n=11), preeclampsia, placental insufficiency (n=4). The control group included women with a physiological course of pregnancy (n=12). In the study group pregnant women received low-molecular-weight heparin (LMWH) at a prophylactic dose. Women from the control group did not receive any anticoagulant therapy. A study of the status of the hemostasis system was performed using standard coagulation tests and the thrombin generation test (TGT).
Results: The parameters of the standard coagulation tests for each group were within the reference values. Statistically significant changes in the parameters of TGT were shown in the preeclampsia group compared with the control group (paired student t-test, ANOVA, $p< 0.05$).
Conclusions: The parameters of the state of the hemostatic system in pregnant women with venous insufficiency and thrombophilia while taking a prophylactic dose of LMWH do not differ from each other and from the control group. TGT parameters of the preeclampsia group significantly differ from the control group and other study groups, showing a greater tendency to increased thrombosis. Therefore, more careful monitoring of the state of the hemostatic system in this group of patients is necessary. Since the preeclampsia group contained only 4 people in this work, further studies are needed for final conclusions.

PB2505 | Analysis of Population Values in Pregnant Women

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Background: physiological changes that occur during pregnancy may affect biochemical parameters in women.
Aims: we assessed the analysis of population values for coagulation test prothrombin time (PT); INR, and Activated Partial Tromboplastin Time (APTT); according gestational trimester.
Methods: blood samples from 513 pregnant women (1-36 week) were collected in 0.32% trisodium citrate tubes (BD Medical Systems), centrifuged at 3.000 g for 10 minutes at room temperature .The women included, had uncomplicated pregnancies. Performed test were analyzed in an ACL Top 300 (IL) coagulometer, using: PT-Fibrinogen Hs Plus (IL), APTT-SP (liquid) (IL). The population was segmented in different trimesters of pregnancy. The data distribution was non parametric, the median and the 2.5 and 97.5 percentiles were used to describe the population. Regarding the values without pregnancy, the laboratory reference values were taken into account. One way Anova test was used to see if any quarter was significantly different.
Results: Median and percentiles 2.5 and 97.5 are expressed in each trimester. When advancing in the weeks of pregnancy, there is a shortening of the APTT in seconds and an increase in the PT %, due to haemostatic change before delivery.
Conclusions: haemostatic reference intervals are generally based on samples from non-pregnant women. Gestational specific reference values are essential for the accurate interpretation of a subset of haemostatic test during pregnancy. The results depend on the double reagent/coagulometer; therefore it is recommended that each laboratory obtains its own reference values for pregnant women.

TABLE 1 Results of reference values and different trimesters

Test	Values without pregnancy	1° trimester	2° trimester	3° trimester	p
n	120	187	103	223	
TP(%)	70-120	88.5(73-110)	100 (77-120)	105 (81-131)	<0.001
TP(Seg)	13.8-18.3	14.6 (13.1-16.1)	13.7(12.5-15.6)	13.5 (12.1-15.2)	<0.001
INR	0.91-1.26	1.07 (0.95-1.2)	1 (0.92-1.16)	0.98 (0.88-1.12)	<0.001
APTT(seg)	24-35	30 (24.6-35)	28 (25-35)	28 (25-33)	<0.001

PB2506 | Comparative Study of the Clinical Application of Two Bleeding Grading Systems for Pregnant Women with Immune Thrombocytopenia (ITP)

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Background: The severity of bleeding is usually not consistent with the platelet count in pregnancy with ITP. If the only criteria depend on platelet count, it can either cause overtreatment or delay the optimal treatment. The ITP International Working Group (IWG) recommended the use of ITP-specific bleeding assessment tool (ITP-BAT) in evaluating the risk of bleeding, but its clinical application was limited due to the long-term data collection. In order to shorten the evaluation time and improve the clinical operability, the 2016 version of ITP Bleeding Score Scale (ITP-2016) was recommended by Chinese specialists to be used in adult ITP. But it is unclear whether they are useful in pregnant women with ITP or not.

Aims: To compare the clinical practicability of two bleeding grading systems (BGS) in pregnancy with ITP.

Methods: 154 pregnancy with ITP were retrospectively analyzed with the two BGS. The correlation between the two BGS, and the relation among platelet counts, patients' age and disease phase were respectively analyzed.

Results: There was no significant difference between different patients' ages but statistically significant between the different phases (P=0.001; P=0.001). There was no significant difference between newly diagnosed and persistent group or chronic group. Statistical difference was found between persistent and chronic group (P=0.001, P=0.001). There was a negative correlation between bleeding grade and platelet counts (r=-0.436, P<0.001; r=-0.390, P<0.001), while the correlation between the two BGS was positive(r=0.921, P<0.001). The proportions of identical scores using the two BGS by two different physicians at the same time were 94.8% (P<0.001) and 93.5% (P<0.001) respectively. It takes less time to score with the ITP-2016(P=0.011)

Conclusions: Both BGS can be used as an effective tool for disease judgment, risk assessment and efficacy evaluation in pregnancy with ITP. The ITP-2016 takes less time and is more suitable for Chinese pregnancy with ITP.

PB2507 | Maternal Thrombocytopenia in Women Initiating Antenatal Care: A Hospital-Based Study

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Background: Thrombocytopenia is a reduction in the platelet count below the reference range of 150 x 10^9/L. It is the second commonest hematologic disorder, following anaemia in pregnancy. Women with thrombocytopenia in pregnancy have a higher tendency to bleed; postpartum haemorrhage is a major cause of maternal mortality in our setting.

Aims: The aim of this study was to determine the prevalence of thrombocytopenia in healthy mothers initiating antenatal care in our centre, assess the platelet indices and assess the severity of thrombocytopenia in women booking at different gestational ages.

Methods: This was a cross-sectional study, over a 6-month period. Women initiating antenatal care were assessed to know their age, parity and gestational age at booking and 3ml of venous blood was collected in EDTA vacutainer. The platelet counts and assessment of platelet indices was done with Sysmex® Haematology analyser KX-21N.

Results: A total of 1147 pregnant women were included in the study, however 1130 had complete data for analysis. They had a mean age of 31.6 ± 4.6 years. Their modal parity was para-1 (range 0-6), mean gestational age was 21.1 ± 7.6 weeks (range 4 - 40) weeks. The mean Platelet count was 214.8 x10^9/L (range 19 - 873), average Mean Platelet Volume was 10.5 ± 2.7fL, Mean Platelet Distribution Width was 13.5 ± 3.1fL. There was no significant difference in the mean platelet count for women booking in the first, second or third trimester, P= 0.297.

Conclusions: The prevalence of thrombocytopenia in this group of women is 11.7%. It is important to screen pregnant women routinely for thrombocytopenia to identify those that will need support through pregnancy and avoid bleeding during parturition or after; post-partum haemorrhage remains a significant cause of maternal mortality in our setting.

TABLE 1 Classification of Thrombocytopenia

Classification of platelet values	Frequency	Percentage %
Normal (>150 x 10Λ9/L)	996	88.1
Mild thrombocytopaenia (100-149 x 10Λ9/L)	112	9.9
Moderate thrombocytopaenia (50-99 x 10Λ9/L)	18	1.6
Severe thrombocytopaenia (< 50 x 10Λ9/L)	4	0.4
Total	1130	100

PB2508 | Pregnancy Complications among Filipino Women

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Background: There is an increasing trend of pregnancy complications in the Philippines. However, these are not well documented due to the lack of a national database.

Aims: This study describes the prevalence of hypertension and lipid abnormalities among Filipino pregnant women, which result to complications during pregnancy.

Methods: Multi-stage stratified random sampling was employed in the National Nutrition Survey and 6,166 pregnant women were included in the study. Demographic data was gathered through an interview questionnaire. Blood samples were drawn to determine fasting blood sugar (FBS), cholesterol, HDL and LDL, and triglycerides levels.

Results: One in ten pregnant women (n=661) showed signs of pregnancy complications. High risk pregnancies were identified, ranging from heart disease (2.9%), gestational diabetes (3.2%), asthma (3.51%) and goiter (4.12%). Prevalence of pregnancy-induced hypertension is 18.26%. Dyslipidemia is high among sample population with a prevalence of 39.54% (n=376). Results showed that patients with hypertension are twice more likely to have pregnancy complications than those with normal BP (OR:1.95, CI 0.47, 6.08).

Conclusions: Based on the data, there is a high prevalence of pregnancy complications in the Philippines which is associated with having hypertension and lipid abnormalities. Health policies should be made to cater to the early detection of disease in pregnant women and widespread campaign for affordable prenatal check-ups.

PB2509 | Haematologic and Haemorheologic Parameters of Pre-Eclamptic Patients in a Nigerian Tertiary Hospital

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Background: Pre-eclampsia is a gestational complication with immense outcomes on fetal and maternal health.

Aims: This study assessed haematologic and haemorheologic parameters of pre-eclamptic patients at the University of Calabar Teaching Hospital, Calabar.

Methods: Following ethical approval/consent, 90 subjects aged 18-45 years were enrolled. They comprised 30 pre-eclamptic patients admitted in antenatal ward, 30 pregnant women with no medical condition attending antenatal clinic and 30 apparently healthy non-pregnant women as control. Weight, height and blood pressures were measured while body mass index was calculated; a pre-tested structured questionnaire was used to obtain demographic data. Packed cell volume (PCV), haemoglobin (HB), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC) with differentials, platelet count and mean platelet volume (MPV) were determined using Sysmex Haematology autoanalyser. Erythrocyte sedimentation rate, relative plasma viscosity, total protein, albumin, globulin and fibrinogen concentration were determined by standard techniques. Data analysis was by one-way analysis of variance on statistical package for social sciences version 20 with significance set at $P< 0.05$.

Results: Significantly lower ($p< 0.05$) PCV, HB, MCV and platelet count were observed among pre-eclamptic patients compared to the pregnant and non-pregnant controls. Conversely, body mass index, blood pressures, MCHC, WBC and MPV of pre-eclamptic patients were significantly higher ($p< 0.05$) versus pregnant and non-pregnant controls. There were also significant differences ($p< 0.05$) in RBC and differential white cell count among the three groups. The erythrocyte sedimentation rate, relative plasma viscosity, fibrinogen concentration, total protein, albumin and globulin of pre-eclamptic patients were significantly higher ($p< 0.05$) compared to normal pregnant and non-pregnant controls.

Conclusions: Haematologic and rheologic changes occur in pre-eclampsia reflecting the body's response to systemic inflammation induced by pregnancy and amplified by the presence of hypertension with resultant pre-disposition to complications of hypercoagulation.

PB2510 | Systematic Review of Viscoelastic Testing (TEG/ROTEM) in Obstetrics and Recommendations for Future Studies

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Background: Thromboelastography (TEG) and rotational thromboelastometry (ROTEM) are point-of-care viscoelastic devices that use whole blood samples to assess coagulation and fibrinolysis. These devices have been studied extensively and are recommended in cardiac surgery, but there is limited robust evidence supporting its use in obstetrics. The hesitancy toward its routine use in obstetrics may be due to the current lack of randomized controlled trials and large observational studies.

Aims: The study aims to systematically review published studies that investigated TEG/ROTEM use in pregnancy or peripartum, and to provide recommendations for future studies to fill current research gaps.

Methods: We performed a systematic review of all published studies on viscoelastic testing in obstetrics by searching Ovid Medline and utilizing the reference list of relevant articles. Included studies were original research, utilizing TEG or ROTEM during the peripartum period or during pregnancy, and published in English. Studies that did not mention TEG/ROTEM or did not use it in women during pregnancy or the peripartum period were excluded.

Results: A total of 63 studies, spanning 24 years from 1995 to 2019 and with a total of 59,272 participants, were included. 42 (67%) of

TABLE 1 Recommendations for future studies in obstetrics

Topic Area	Background	Objectives of Future Studies	Ideal Method
Neuraxial anesthesia in pregnant women with thrombocytopenia or other bleeding disorders	There is no consensus on a safe platelet threshold for neuraxial anesthesia. There is hesitancy to perform neuraxial anesthesia in patients with thrombocytopenia due to the risk of neuraxial hematomas.	To monitor TEG/ROTEM parameters in women with thrombocytopenia, particularly less than 100K platelet count, and bleeding disorders (i.e. vWD, Factor XI deficiency, hemophilia) prior to neuraxial anesthesia and subsequent clinical outcomes.	Large multi-center prospective observational study
Thrombosis risk stratification and prophylaxis	TEG/ROTEM can detect hypercoagulability in the context of cancer and pregnancy. They have also detected hypercoagulability in obstetric complications such as obesity, pre-eclampsia/eclampsia, etc. A few studies have also used TEG/ROTEM to detect the anticoagulant effect of heparin and its derivatives for thromboprophylaxis.	To assess if TEG/ROTEM can be used to guide dosing of anticoagulants for thromboprophylaxis in women with active VTE or history of VTE and thrombophilia with other comorbidities (i.e. morbid obesity). To determine if TEG/ROTEM can predict risk for thromboembolic complications in the peripartum or postpartum period and determine when to discontinue therapy.	Large multi-center prospective observational study
Hemorrhage risk stratification and guided treatment	PPH is one of the leading causes of maternal morbidity and mortality. FIBTEM can independently predict the severity of PPH. A large RCT by Collins et al. helped confirm that a FIBTEM A5 cut-off value of <12 mm should be used to initiate fibrinogen concentrate in women with PPH. The WOMAN trial showed that TXA reduces mortality in women with PPH, but there is so far no evidence that TEG/ROTEM can be used to guide TXA treatment.	PPH risk assessment: To assess if pre-partum TEG/ROTEM parameters can predict intraoperative or delayed postpartum risk of PPH.	Large multi-center prospective observational study to look at PPH predictive capability of TEG/ROTEM
		PPH treatment studies: To expand upon the OBS2 trial and compare clinical outcomes as well as cost-effectiveness of women given placebo versus fibrinogen replacement triggered by FIBTEM <12 mm or fibrinogen <2.5 g/L. To assess the effects of TXA on coagulation and fibrinolysis by obtaining D-dimer levels and TEG/ROTEM parameters, and determine if TEG/ROTEM can be used to guide TXA in women with PPH.	Large multi-center randomized controlled trials to assess effectiveness of TEG/ROTEM guided treatment approaches for PPH

the studies used TEG and 21 (33%) used ROTEM. To date, there are a total of two randomized controlled trials on TEG/ROTEM use in obstetrics.

Conclusions: TEG and ROTEM can detect the hypercoagulable changes associated with pregnancy and can be used to guide therapy for PPH. Variability between study protocols and results suggests the need for future large prospective high-quality studies with standardized protocols to investigate the utility of TEG/ROTEM in assessing risk for thrombosis and hemorrhage as well as in guiding prophylaxis and treatment in obstetric patients. We provide concrete recommendations for future studies in this regard.

PB2511 | Reassessment of Venous Thromboembolism Risk and Prophylaxis in Post-Delivery Period of Healthy Women

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Background: The guidelines for venous thromboembolism (VTE) prophylaxis (VTEP) during delivery hospitalization for healthy women are inconsistent.

Vaginal delivery (VD) was proven as an independent risk factor for thromboembolic events. Caesarian section (CS) can also be a trigger for VTE.

Aims: The primary aim of our study is to find out if CS is a stronger triggering factor for thromboembolic events than VD and the second is to asses the need for anticoagulant prophylaxis.

Methods: In this prospective clinical study we compared two groups of women (30 in each group), in the first group parturients after normal VD and in the second group parturients after elective CS. The thromboelastography [TEG] profile was assessed at different time points: 10 minutes after VD or CS and 24 and 48 hour later. Statistically significant differences (p<0.01) were observed in several TEG parameters: fibrinogen levels. angle (α) and maximal amplitude (MA).

Results: Statistically significant differences (p<0.01) were observed in several TEG parameters: fibrinogen levels. angle (α) and maximal amplitude (MA). Our study shows clearly that the activation of the coagulation system after CS is enhanced compared with VD. The values of MA, which indicates the platelets function, were an unexpected finding of our study, increasing during the first 24 hours in all parturients but decreasing after 24 hours in VD group. The MA value continue to increase significantly after 24 hours in CS group. The angle (α) shows the speed of the clot formation and is closely related to the hypercoagulation. Like MA values, also angle α values decrease in VD group after 24 hours. None of the parturient women (from the two groups) developed a VTE event.

Conclusions: The suggested strong hypercoagulability tendency in CS group, compared to VD group, can justify routine anticoagulant treatment after surgical delivery.

PB2514 | Safety of Withholding Antithrombotic Treatment in Pregnancies after Antiphospholipid Antibodies Disappearance in Women with Obstetric Antiphospholipid Syndrome, a Cohort Study

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Background: Antiphospholipid antibodies (aPLs) in women with obstetric antiphospholipid syndrome (OAPS) may disappear. It is not clear whether antithrombotic treatment is needed during pregnancies while the aPLs are negative.

Aims: To compare proportion of miscarriages, placenta-mediated complications or fetal loss before and after aPLs negativization in patients who received treatment only during pregnancies while their aPLs were positive.

Methods: A retrospective cohort study was performed. Pregnancies of women with diagnosis of OAPS (according to Sapporo criteria) who had disappearance of aPLs and were attended at a single community center from January 2003 to March 2018 were included. Patients were identified through the database of Hematology Department and electronic medical records.

Negative aPL markers at least during two consecutive laboratory assessment at least 12 weeks apart was defined as disappearance of aLPs.

We compared the percentage of miscarriages, placenta-mediated complications or fetal loss before and after negativization among pregnancies of women who had disappearance of their aPL.

For the statistical analysis, STATA, version 13 was used. A p-value of < 0.05 was considered statistically significant.

Results: Out of 150 women with diagnosis of OAPS, 49 had disappearance of aPLs. 80 pregnancies from these women were included. Before aPL negativization, there were 51 pregnancies in 33 women. After the disappearance of aPL, there were 29 pregnancies in 21 women.

The mean time of follow up was 81.91 months (SD 51.5 0.1-305.3).

The rate of miscarriages, placenta-mediated complications or fetal loss was 7 out of 51 pregnancies before aPL negativization and 1 out of 29 after (OR 4.4 95% CI 0.52-38.17).

Conclusions: Only one miscarriage occurred out of 29 pregnancies after aPL disappearance, despite not receiving antithrombotic treatment. These results suggest that it is safe to withhold treatment in patients with diagnosis of OAPS whose aPL disappeared.

PB2515 | Microvesicles, Fibrinolytic and Coagulation Biomarkers in Blood from Pregnant Women

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Background: Multiple changes in the haemostatic system occur during pregnancy. The increase in the concentration of coagulation factors characterizes pregnancy as a hypercoagulable state, fuelling an increase in D-dimers, which suggests that fibrinolysis remains an active process. Recently, increased attention has also been paid to the relationship between circulating microvesicles and hypercoagulability in normal and complicated pregnancies.

Aims: The aim of our pilot study was to monitor procoagulant- and fibrinolytic-related parameters during pregnancy; namely D-dimers (DDI), fibrin monomers (FM) and pro-coagulant/pro-fibrinolytic microvesicles (MV).

Methods: DDI, FM and MV were assayed using two commercially available techniques for DDI and FM (Vidas D-dimer Exclusion™, Biomérieux, and STA®-Liatest® FM, Stago, France, respectively) and functional procoagulant and fibrinolytic activities for MV. Quantifications were performed from 50 normal pregnancies and 35 placenta-mediated counterpart complications (clinicaltrials.gov identifier: NCT01736826). Among the 50 normal pregnant women, 17 were randomly sampled for testing every 4 weeks for 6 months from the 3rd month of pregnancy to delivery.

Results: Kinetics of DDI, FM and MV showed that DDI and pro-fibrinolytic microvesicles increased over time during pregnancy ($p < 0.001$ for both), whereas there was no significant variation in FM or pro-coagulant MV ($p=0.128$ and $p=0.202$ respectively). However, the global aspect of the distribution of the FM and procoagulant microvesicles levels was not monotonous throughout pregnancy with higher values before delivery. Moreover, women with complicated pregnancies had a clear tendency towards increased DDI, FM, pro-coagulant and pro-fibrinolytic MV values overlapping those found in normal pregnancies, indicating a significant biological behavioral heterogeneity within the population termed "placenta-mediated complications".

Conclusions: Our work shows that fibrinolytic activity is maintained throughout pregnancy suggesting that it may be essential for the progress of pregnancy. It also suggests a biological heterogeneity in women with placenta-mediated complications, which deserves further investigation.

PB2516 | D-Dimer Variability in the Postpartum Period

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Background: D-Dimers are products of fibrin degradatio.

Aims: We examinated D-dimer levels early and late postpartum to assess whether D-dimer levels have role to play in predicting adverse events postpartum.

Methods: Only healthy pregnanat woman were included in the study.

Results: 35 women participated in this study and 27 women returned for the late postpartum period. In the immediate postpartum period 34 0f35 (97%) women had D-dimer levels higher than the normal nonpregnant range (< 500 ng/ml). By 4-6 weeks postpartum, D-dimer had returned to non-pregnant normal levels in 70% of women. There was a high variability in D-dimer levels in the early postpartum period: level ranged between 550-5700 ng/ml. In later postpartum period D-dimer level had decreased between 100- 900 ng/ml. D-dimers levels were significantly elevated among women who had cesarean section when comparated to women who had normal vaginal delivery (2500 ng/ml -1350 ng/ml).

Maternal age, BMI, maternal blood type, were not associated with D-Dimer levels.

None of the women who participated in our study have had any adverse events postpartum.

Conclusions: D-Dimer levels are elevated and highly variable in the immediate postpartum period. D-Dimer levels decrease subsequently and toward normal (< 500 ng/ml) by 4-5 weeks postpartum. C- section delivery is a major factor increasing D-Dimer levels postpartum.Higher levels od d-Dimer may be considered normal for the postpartum period ranges and ranges may need to be adjusted for delivery method.

More studies are indicated to help assessif normal ranges for D-dimers in the postpartum period can be determined if the test is to serve a useful diagnostic purpose.

PB2517 | Anticoagulation for Women with Mechanical Heart Valves during Pregnancy: A Survey

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Background: The optimal anticoagulation management strategy for pregnant women with mechanical heart valves has not yet been determined. Assessing current anticoagulation practice is a necessary step for planning future prospective studies.

Aims: To determine practice patterns for anticoagulation therapy for pregnant women with mechanical heart valves (MHV).

Methods: We conducted a cross-sectional study using a self-administered online questionnaire. Hematologists, Cardiologists, Obstetric Internists, and Maternal Fetal Medicine specialists were surveyed. Participants included members of professional associations and individuals with pertinent research expertise. The questionnaire consisted of case scenarios and partially closed ended questions. Type and dose of anticoagulant, use of antiplatelet agents, delivery management strategies, and physician demographics were assessed. The project was conducted under the auspice of ISTH'sWomen's SSC.

Results: 81/117 respondents managed anticoagulation during pregnancy and thus responded to the survey; 62% were Hematologists, 10% Cardiologists, 17% Obstetric Internists, and 4% Maternal Fetal Medicine specialists. Respondents were predominantly from Europe (41%) and North America (37%). In total, 32% of respondents reported using vitamin K antagonists (VKAs) during pregnancy. VKAs with

low molecular weight heparin (LMWH) during 6-12 weeks gestation (35%) and therapeutic LMWH antepartum (33%) were the two most common strategies for women with mechanical mitral valves. LMWH was predominantly selected for women with mechanical aortic valves (57%). Location and type of mechanical valve, previous embolic event, and patient preference were among the most common factors guiding decision-making by over 50% of respondents. Low dose Aspirin was considered by 31% of physicians. Delivery was predominantly (55%) selected based on obstetrical indications, and 56% of respondents indicated VKAs are restarted within 72 hours of delivery.

Conclusions: This survey data can be used to inform physician practice and is an initial step for planning research for antepartum anticoagulation in women with mechanical heart valves.

PB2518 | Multidisciplinary Care of Pregnancies with VWD Improves Rates of Primary Postpartum Hemorrhage

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Background: Von Willebrand disease (VWD) is the commonest inherited bleeding disorder and poses potential hemostatic challenges at delivery.

Aims: To determine whether multidisciplinary care improves the rate of postpartum haemorrhage (PPH) in pregnancies with VWD.

Methods: We conducted a retrospective review of all VWD pregnancies at two high-risk pregnancy units, Sinai Health System and Sunnybrook Health Sciences Centre in Toronto from 2009 to 2018. Primary PPH was defined as estimated blood loss (EBL) of >1000 mL for vaginal deliveries (VD) and caesarean sections (CS), within the first 24 hours of delivery. EBL >500 mL after 24 hours postpartum and up to 6 weeks after delivery was considered delayed PPH.

Results: 63 women with 80 pregnancies were included. Three women had twin pregnancies. Delivery data were missing for three pregnancies. 65 (84.6%) pregnancies were Type 1 VWD, Type 2 (unclear subtype) in four (5.1%), Type 2A VWD in three (3.6%), Type 2B VWD in three (3.6%), Type 2M in two (2.6%), and Type 1C in one (1.2%). The median age was 33 years (range: 19-43), most pregnancies were blood type O (65%), and 33 (41.3%) pregnancies were in nulliparas. The mean baseline bleeding assessment score was 8.00 [Range (4-10)]. Table 1 describes VW factor levels (VWF) and Table 2 depicts characteristics of women with PPH. VD occurred in 57 (73.3%) pregnancies and CS in 20 (25.6%). Peripartum hemostatic intervention was provided in 37 (48%) pregnancies, and epidural anaesthesia in 65 (84.4%) pregnancies. PPH occurred in 4 (5.2%) and delayed PPH in 6 (7.7%) pregnancies. One woman developed pulmonary embolism during the postpartum period.

Conclusions: Our rates of primary (5.2%) and delayed PPH (7.7%) appear improved compared to those previously reported for immediate (18-22%) and delayed PPH (20-28%) in patients with of VWD. Multidisciplinary care of pregnancies with VWD appears to improve their outcomes.

TABLE 1 Median and Range of VWF: Ag, VWF: Act, and FVIII levels at baseline, first, and third trimester

Time	Number of pregnancies	VWF: Ag (IU/ml) (Range)	VWF: Ac (IU/ml) (Range)	FVIII (IU/ml) (Range)
Baseline	51	0.48 (0.10-0.87)	0.44 (0.05- 1.02)	0.56 (0.20-1.43)
First Trimester	36	0.88 (0.11-2.24)	0.78 (0.09-2.00)	1.11 (0.09- 2.31)
Third Trimester	63	1.38 (0.11-3.17)	1.23 (0.48-2.50)	1.65 (0.09-3.02)

TABLE 2 Characteristic of women with PPH

No	Age	Bleeding assessment score	Third Trimester VWF: Ag (IU/ml)	Third Trimester VWF :Ac (IU/ml)	Third Trimester FVIII (IU/ml)	Type of PPH	Mode of delivery
1	35	9	1.69	1.58	1.7	Primary and Delayed	Emergency CS
2	33	4	1.63	1.03	1.84	Primary	Elective CS
3	38	6	1.75	1.17	2.0	Primary	Vaginal Delivery
4	36	13	1.83	1.3	1.34	Primary	Elective CS
5	27	4	1.64	1.45	1.62	Delayed	Elective CS
6	31	10	1.02	0.48	1.06	Delayed	Vaginal Delivery
7	31	13	0.99	0.88	1.02	Delayed	Vaginal Delivery
8	32	9	0.5	<0.25	0.055	Delayed	Vaginal Delivery
9	34	9	1.52	N/A	2.0	Delayed	Vaginal Delivery

PB2519 | Systemic Evaluation of Neuroserpin, Interleukin-17 and Hemostatic Factors in Association with Clinical Parameters in Preeclamptic Women: A Principal Component Analysis Approach

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Background: Preeclampsia (PE) is a serious hypertensive and multisystem disease of pregnancy that has a challenging diagnosis. Neuroserpin (NS) is a protein involved in neurological, hemostatic and immune (e.g. Th17 cell response) processes that has not been evaluated in PE to date.

Aims: Investigate NS and interleukin (IL)-17 plasma levels and their association with hemostatic factors (PAI-1, D-dimer, von Willebrand factor, ADAMTS-13, FVII, FVIII) and clinical parameters of preeclamptic, normotensive pregnant and non-pregnant women.

Methods: All enrolled women signed an informed consent and the study was approved by the participant institutions. ELISA assays were performed for all the measurements in the plasma obtained from 25 non-pregnant, 26 normotensive pregnant and 21 PE women. These data were analyzed by ANOVA with post-hoc Tukey (parametric) or Kruskal-Wallis with post-hoc Dunn (nonparametric) tests. The correlations were analyzed by Spearman coefficients (Rs). Principal component analysis was performed combining clinical and laboratory data of the participants.

Results: NS plasma level was increased in normotensive pregnant women. However, its level in PE was comparable to the non-pregnant state (Figure 1A). Plasma IL-17 was up-regulated during pregnancy, but it was similar between normotensive and PE women (Figure 1B). IL-17 and NS were strongly and positively correlated when all the participants (Rs=0.820, P< 0.001) or only PE women (Rs=0.725, P< 0.001) were included in the analysis. In PE women, IL-17 and PAI-1 levels were strongly and negatively correlated (Rs=-0.714, P=0.047). Other weaker correlations were detected among NS, IL-17 and hemostatic factors. NS, IL-17 and FVII, combined with clinical parameters, were able to differentiate PE women (upper quadrants) from normotensive pregnant (lower quadrants) and non-pregnant (left lower quadrant) women (Figure 2).

Conclusions: NS and IL-17 may participate in PE pathogenesis and could integrate a new screening tool for PE diagnosis.

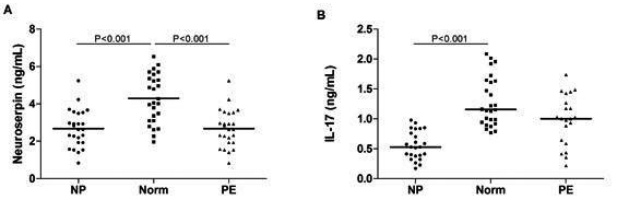

Figure 1- Plasma levels of neuroserpin and IL-17 in non-pregnant (NP), normotensive pregnant (Norm) and preeclamptic (PE) women.

FIGURE 1

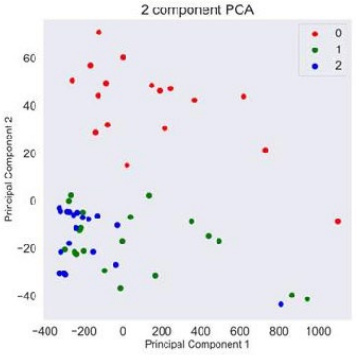

Figure 2- Principal component analysis (PCA) including the plasma levels of neuroserpin, IL-17, FVII and clinical parameters of preeclamptic women (group 0), normotensive pregnant (group 1) and non-pregnant (group 2).

FIGURE 2

PB2520 | Efficacy and Safety of Low-Molecular-Weight Heparin for the Prevention of Pregnancy-Related Recurrent Venous Thromboembolism and Obstetrical Complications

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Background: Pregnancy is associated with an increased risk of venous thromboembolism (VTE) and inherited thrombophilia also increases the risk of obstetrical complications (OC), but data on the efficacy of low- molecular-weight heparin (LMWH) for their prevention are poor.

Aims: To evaluate the risk of pregnancy-related recurrent VTE and OC with or without LMWH.

Methods: Single-center retrospective cohort study of fertile women (age 18-40 years) referred to our Center from 2000 to 2018 for a thrombophilia screening with at least a previous VTE and at least one pregnancy following VTE ended within September 2018. Women with antiphospholipid antibodies were excluded. Data on pregnancy-related VTE, pregnancy outcomes, and the use of LMWH were collected.

Results: 208 women were included and the first pregnancy following VTE was considered. No thrombotic or bleeding events were observed in 140 pregnancies with LMWH, while 10 out of 68 (15%) pregnancies without LMWH were complicated (7 deep vein thrombosis, 2 pulmonary embolism and one cerebral vein thrombosis). Nine women (90%) with recurrent VTE had had a previous hormone-related event. The incidence of miscarriages was lower in pregnancies with LMWH than in those without, whereas late OC and terminations were similar among the two groups (Table 1). The incidence of late OC was higher in women with thrombophilia who received LMWH (18%) than in those who did not (7%) (Table 2). The incidence of terminations was doubled in women with thrombophilia (12%) than in those without (6%).

TABLE 1 Pregnancy outcomes with or without low molecular weight heparin antithrombotic prophylaxis

	Pregnancy LMWH + N= 140	Pregnancy LMWH- N= 68	RR (95%CI)
Full term	93 (72%)	30 (49%)	1.5 (1.1-1.9)
Miscarriage	15 (12%)	19 (31%)	0.4 (0.2-0.7)
Late obstetrical complication	22 (17%)*	12 (20%)†	0.9 (0.5-1.6)
Termination	10 (7%)	7 (10%)	0.7(0.3-1.7)

*9 small for gestational age, 7 preeclampsia, 1 eclampsia,4 placental abruption and 1 stillbirth. †8 small for gestational age, 1 preeclampsia, 1 eclampsia, 1 placental abruption and 1 stillbirth.

TABLE 2 Risk of pregnancy-related VTE and obstetrical complications in women with or without thrombophilia

	Thrombophilia + N=85			Thrombophilia - N=123		
	LMWH + N=67	LMWH - N=18*	RR (95%CI)	LMWH+ N=73	LMWH - N=50	RR (95%CI)
VTE	-	3 (21%)	-	-	7 (15%)	-
Pregnancy outcome						
Full term	43 (71%)	6 (43%)	1.6 (0.9-3.1)	50 (73%)	24 (42%)	1.4 (1.0-1.9)
Miscarriage	7 (12%)	7 (50%)	0.2 (0.1-0.5)	8 (12%)	12 (21%)	0.5 (0.2-1.0)
Late obstetrical complication	11 (18%)	1 (7%)	2.5(0.4-18.0)	11 (16%)	11 (23%)	0.7 (0.3-1.4)
Termination	6 (9%)	4 (22%)	0.4 (0.1-1.3)	4 (5%)	3 (6%)	0.9 (0.2-3.9)

* 7 women had multiple thrombophilia defects.

Conclusions: LMWH prophylaxis during pregnancy resulted effective and safe for the prevention of recurrent VTE and miscarriage, but not of late OC, particularly in women with thrombophilia. A previous hormone-related VTE was a prevalent condition for pregnancy-related recurrent VTE. Women with thrombophilia decide for terminations twice more than those without.

PB2522 | Investigation of Risk Factors of Deep Vein Thrombosis during Pregnancy Using a CFD Model

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Background: Deep vein thrombosis (DVT) is the formation of a blood clot within the deep vein vessels. DVT becomes fatal when it leads to pulmonary embolism (PE) causing complication such as heart attacks and strokes. During pregnancy, a hypercoagulable state is required to prevent women from haemorrhage during childbirth. Also, compression of the iliac vein and the hormonal changes makes pregnant women more susceptible to DVT. PE occurs in about 1 in 1000 pregnancies in the developed world and 4 in 1000 in the developing world, making it the leading cause of maternal death in the world. Computational models of DVT have been developed in the past, however, there have been few studies on the effects of physiological changes on DVT during pregnancy.

Aims: The aim of this study is to understand how the physiological changes during pregnancy affect the clot formation during DVT in the femoral vein.

Methods: Transient computational fluid dynamics (CFD) simulations were carried out in ANSYS Fluent (ANSYS, Lebanon, NH). A 2-D idealized vein vessel of diameter 11.84 mm and length 60 mm was used to simulate the clot formation in a healthy adult woman. This study adopted a thrombin-based continuum model to simulate DVT in the left femoral vein of a 27-year-old pregnant woman with thrombin being supplied at an injury zone 30% of the vein length and circumference. The investigation was carried out by comparing clotting patterns in a pregnant to a non-pregnant state by a parametric

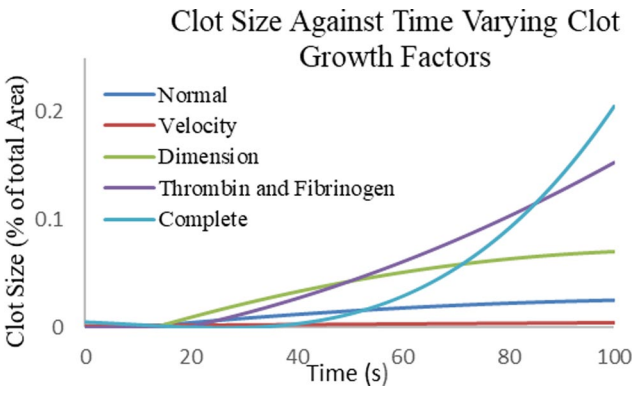

FIGURE 1 Surface clot size average for a healthy woman compared with clot size when velocity, dimension and biochemistry are varied

study of velocity, blood pressure, vein geometry, and thrombin concentration.

Results: Initial results illustrated in Figure 1 showed that the hormonal changes had the greatest effect while the reduction in velocity had the least effect on the clot size.

Conclusions: In future work, we plan to study the effect of anticoagulant medication using the model.

PB2523 | Low Molecular Weight Heparins in the Prevention of Placenta-Mediated Complications in Carriers of the *Prothrombin G20210A* Mutation

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Background: Our earlier prospective cohort study which included 140 women with the *prothrombin G20210A* mutation reported that prothrombin activity can be considered as a predictive marker for the development of preeclampsia (PE). When plasma prothrombin activity is >171% at the preconception stage, the risk of PE is predicted in 86% of cases (AUC-0.863; p< 0.0001), when prothrombin activity is >181.0% at the gestational age of 7-8 weeks, it is predicted in 84% of cases (AUC-0.840; p< 0.0001). The absence of studies aimed at examining the benefit of low molecular weight heparin (LMWH) administration due to the high prothrombin activity in carriers of the *prothrombin G20210A* mutation to prevent gestational complications was the reason for this study.

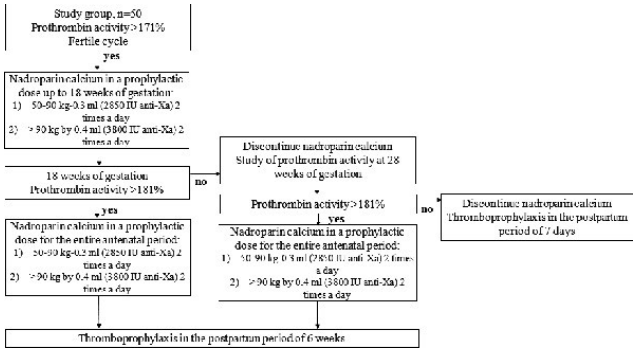

FIGURE 1 The algorithm of nadroparin calcium administration to the patients with prothrombin G20210A gene mutation in terms of prothrombin activity

Aims: To determine the effect of LMWH on the prevention of preeclampsia and fetal growth restriction (FGR) since the preconception period in carriers of the *prothrombin G20210A* mutation with an increased suprathreshold prothrombin activity.

Methods: A single-center randomized controlled trial of 80 pregnant women with the *prothrombin G20210A* mutation was conducted. The criterion for inclusion into the study was the plasma prothrombin activity >171% at the preconception stage. The study group consisted of 50 patients received weight-adjusted heparin prophylaxis since the fertile cycle (Figure 1). The control group included 30 pregnant women who did not receive LMWH prophylaxis.

Results: Analysis of pregnancy outcomes showed that the use of nadroparin calcium in prophylactic doses in carriers of the *prothrombin G20210A* genotype with a prothrombin activity level >171% at the preconception stage reduces the absolute risk of PE by 46.7% (p=0.0001), including a severe form by 30.7% (p=0.0001) and FGR by 30.7% (p=0.0001).

Conclusions: In carriers of the *prothrombin G20210A* genotype with a prothrombin activity level >171%, LMWH administration is pathogenetically justified and leads to a significant reduction in the number of gestational complications.

PB2524 | Approach to Venous Thromboembolism Risk in Women with Physical Disability in Pregnancy - Multidisciplinary Survey

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Background: Venous thromboembolism (VTE) during pregnancy is associated with significant morbidity and mortality. International guidelines recommend risk assessment during antepartum and postpartum period to inform VTE prophylaxis. Pregnancies in women with chronic physical disability (WWCPD) are increasing, VTE risk assessment can be challenging.

Aims: We aimed to evaluate physicians approach to VTE risk assessment of WWCPD during pregnancy.

Methods: A cross-sectional study consisting of an anonymous self-administered electronic questionnaire was sent to Hematologists, Maternal-fetal Medicine Specialists (MFM) and Internal Medicine Specialists across Canada.

Results: The survey was active from August 2019 to November 2019. Seventy-three participants responded to the survey, and 55 (75.3%) completed the survey. The survey sample consisted of 33 (60%) MFM specialists and 22 (40%) IM specialists (IM): 14 (25.5%) hematologists and 8 (14.5%) general internal medicine physicians. Participants were primarily from the Province of Ontario (58.2%), and the majority (94.5%) reported assessing less than five WWCPD during pregnancy yearly.

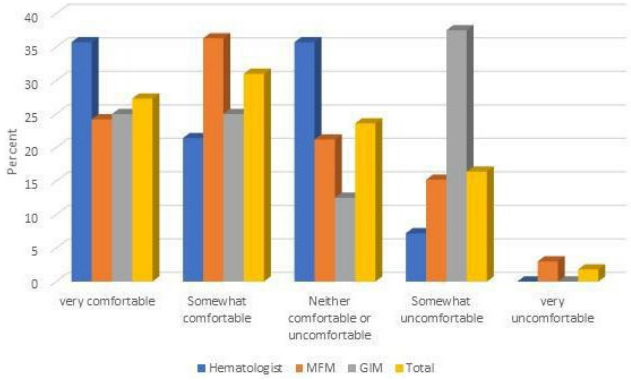

FIGURE 1 Physician's Comfort level with thromboprophylaxis for a woman that requires use of a wheelchair or has limited mobility in pregnancy

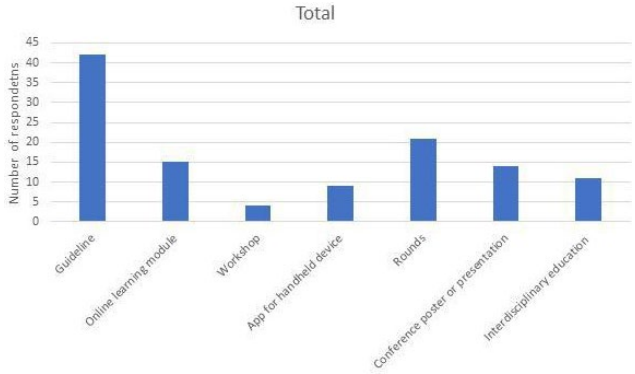

FIGURE 2 Physician's preferred method of education on the topic

There was considerable variability regarding antithrombotic treatment strategy for the hypothetical case scenarios. The majority of MFM Specialists favoured antepartum and postpartum VTE prophylaxis for women using wheelchairs during pregnancy but not for women using walking aids. Most IM specialists favoured no antepartum or postpartum thromboprophylaxis for WWCPD using wheelchairs or walking aids unless they developed other risk factors. Most respondents favoured antepartum (67.3%) and postpartum (65.5%) VTE prophylaxis for women experiencing pregnancy within a year of spinal cord injury.

Conclusions: Our study suggests a variable approach to thromboprophylaxis in WWCPD and that most MFM specialists were comfortable with starting VTE prophylaxis. As well, MFM physicians appear to be more likely to consider thromboprophylaxis than IM physicians. Ongoing collaboration between MFM and IM, as well as further research to understand the actual risk of VTE is needed to improve evidence-based antenatal and postpartum care in this complex population.

PB2525 | Thromboprophylaxis during Pregnancy - 10-year Review of Women Seen in the Haematology Clinic at King's College Hospital (KCH)

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Background: Venous thromboembolism (VTE) continues to be the leading cause of direct maternal death in the UK since 1985. At KCH, we utilise weight-based dosing of enoxaparin for thromboprophylaxis, based on RCOG criteria, during pregnancy (20mg od for women weighing
< 50kg, 40mg od (50-100kg), 80mg od (≥100kg), 120mg od (≥150kg)). The optimal dosing of thromboprophylaxis remains a topic of debate in the antenatal population.

Aims: To review the impact, of prescribing LMWH thromboprophylaxis in pregnant women on bleeding and VTE, seen between 01/01/2009 and 31/12/2018 in the haematology clinic at KCH.

Methods: All women referred to the haematology clinic and commenced on LMWH thromboprophylaxis antenatally were identified retrospectively from the electronic patient record (EPR) with data collected on demographics, the specific indication and dose of thromboprophylaxis prescribed. Bleeding, VTE and adverse events, were identified and recorded. Descriptive statistics was used to analyse the data.

Results: Over the 10-year period, 486 women had recognised risk factors for VTE and prescribed LMWH; mean age 34 (SD+/-5.7), mean pre-pregnancy weight 83.4kg (SD+/-23.8), mode gravida 2. The commonest indication for prescribing thromboprophylaxis (table 1) was thrombophilia (43%), with many women also having a history of VTE (36%).

Most women (76.1%) were prescribed enoxaparin 40 mg daily. Nine (1.9%) women had objectively confirmed thromboembolic events with all of these women having a prior history of VTE. Bleeding

TABLE 1 The most prevalent VTE risk factors taken into account by clinicians when LMWH thromboprophylaxis was prescribed

Risk factors	n (%)
Thrombophilia	211 (43)
Previous VTE	176 (36)
BMI > 30 kg/m2	92 (19)
Family history of VTE	65 (13)
Age > 35 years old	60 (12)
Parity > 3	55 (11)
Medical comorbidities	39 (8)
IVF/ART	38 (8)
Previous pre-eclampsia	16 (3)

was reported in 7.2%, with no major bleeding and most classified as nuisance bleeding (epistaxis) reported at scheduled reviews. A significant number of women (17.3%) reported bruising at the injection sites.

Conclusions: The low incidence of VTE in this cohort demonstrates the safety and efficacy of weight-based enoxaparin thromboprophylaxis during pregnancy.

PB2526 | The Role of Antithrombotic Therapy in the Prevention of Adverse Pregnancy Outcomes

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Background: Despite the progress in understanding the pathogenesis of pre-eclampsia (PE), recurrent miscarriage and the progress in the clinical hemostasiology biology and pharmacology of new anticoagulants, antiplatelet agents, a study of their anticoagulant properties, the prevention and treatment of these «diseases of pregnancy» has not changed a lot.

Aims: To evaluate the role of antithrombotic therapy in adverse pregnancy outcomes.

Methods: We investigated 500 women with history of fetal loss syndrome, 80 - venous thromboembolism (VTE), 100 - placental abruption, including 10 patients with multiple pregnancies after IVF, 100 - the intrauterine fetal death, 160 - severe pre-eclampsia and 500 healthy women of the control group. Prevention of thromboses with LMWH was initiated since the fertile cycle and during pregnancy in all groups except in patients with pre-eclampsia diagnosed during pregnancy. The treatment included LMWH, the Omega-3 antioxidant, folic acid, B vitamins and low dose aspirin in patients with APS and platelet hyperactivity.

Results: Alive and healthy children were born in absolute majority of women with history of fetal loss syndrome (96%). After preventive therapy in patients with placental abruption, repeated events were not recorded and 100% of patients gave birth to live babies. No cases of severe or moderate pre-eclampsia were observed in the group of women with history of recurrent pre-eclampsia, who received the preventive treatment since the fertility cycle. Mild pre-eclampsia in a late period of the 3rd trimester developed in 16% of the women. The treatment initiated after the onset of pre-eclampsia, was ineffective. 48% of women developed mild pre-eclampsia and 52% showed deterioration of the disease with the development of severe pre-eclampsia.

Conclusions: Our studies and over 10 years of clinical experience indicate the presence of etiopathogenetic relation between thrombophilia and obstetric complications and the high efficacy of prophylaxis with anticoagulants when it starts early, since the period of preconception.

PB2527 | Efficiency of Inhibition of Thrombin Generation and Stimulation of Fibrinolysis in vitro Fertilization Programs

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Background: Experimental studies have demonstrated that thrombin and plasmin interact with a wide range of proteins that can alter the physiological processes of implantation and trophoblast development.

Aims: To determine the value of excessive thrombin generation and hypofibrinolysis in the occurrence of IVF failures and the possibility of their therapeutic correction.

Methods: A prospective randomized controlled trial was conducted in 765 patients. 25 laboratory parameters were analyzed including evaluation of thrombin generation (endogenous thrombin potential - ETP and thrombin peak) and determination of the index of fibrinolysis-activating endothelial ability (FAEA=t-PA/PAI-1)*100%. To correct the findings, nadroparin calcium and Intermittent Pneumatic Compression (IPC) were used.

Results: It was found that excessive thrombin generation in blood plasma and/or low fibrinolytic activity in the IVF cycle were observed in 78.8% of cases. The critical thresholds of these shifts associated with the risk of unsuccessful attempts of IVF cycles were calculated: ETP>1900nmol/min (OR=27.8, p=0.0001), Peak trombini>360nmol/l (OR=22.8; p=0.0001) and the FAEA>11% (OR=11.5; p=0.003).

The algorithm of correcting the findings in the hemostatic system in women in the IVF cycle is shown in figure 1. In the groups of patients with critical thresholds of the found shifts during the treatment according to the specified algorithm at the end of the IVF program, a decrease in the ability to generate thrombin in terms of ETR and thrombin peak by 13.9% (p=0.0041) and 11.6% (p=0.0038), respectively, and an increase in fibrinolytic activity according to the FAEA index by 2.1 times (p=0.0001) were observed. Regardless of the

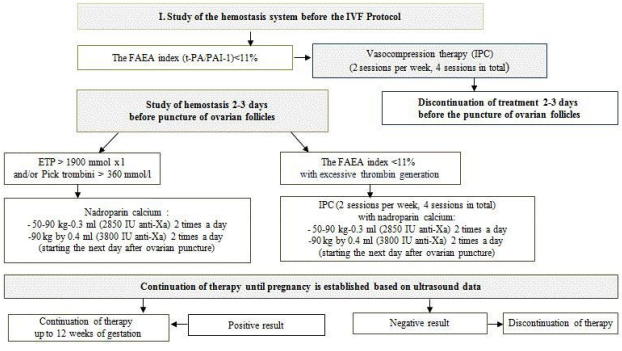

FIGURE 1 The proportion of successful pregnancies in IVF programs for therapeutic correction

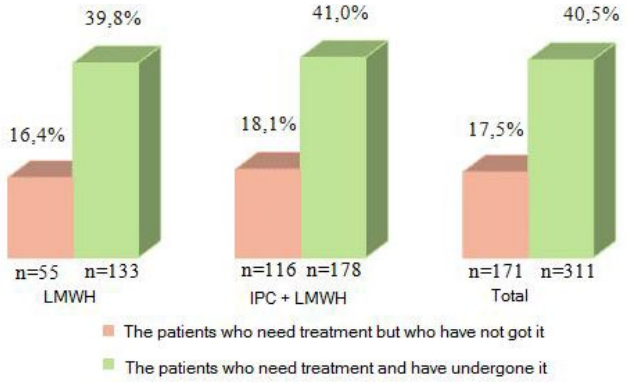

FIGURE 2 The algorithm of detecting and correcting the findings in the hemostatic system in women in the IVF cycle

method of therapeutic effect, the onset of pregnancy in the group of patients receiving treatment increased by 24.0% overall (Figure 2).

Conclusions: In the IVF cycle, 78.8% of cases show excessive plasma thrombin generation in blood plasma and/or low fibrinolytic activity, therapeutic correction of which increases the chance of pregnancy by 24.0% [p=0.0001; NTT:4.35, 95%CI(3.18-6.90)].

PB2528 | Peculiarities of the Hemostasis System in Pregnant Women with Severe Preeclampsia

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Background: Numerous studies on the state of the blood coagulation system in preeclampsia have revealed its disorders in the form of various coagulopathies and thrombocytopathies, in particular the development of thrombocytopenia in pregnancy complicated by preeclampsia.

Aims: Based on the study of hemostasiological parameters, to assess the state of the blood coagulation system in women with severe preeclampsia.

Methods: We conducted a study of the blood coagulation system in 72 pregnant women in the third trimester, of which 32 were with a physiological pregnancy and 40 pregnant women with severe preeclampsia. The age of the women surveyed ranged from 21 to 41 years. We study the state of platelet, procoagulant and fibrinolytic links of the hemostatic system and their assessment were carried out in the dynamics of observation.

Results: A significant decrease in the average platelet count in women with severe preeclampsia relative to healthy pregnant women in the third trimester of pregnancy (161.2 ± 9.6 × 109 / l and 193.5 ± 8.7 × 109 / l) (P < 0.05).

When studying platelet aggregation activity, we found that platelet aggregation activity was significantly higher in the main group -112.7 ± 3.5 compared with the control group — 99.8 ± 3.3 (P < 0.05). When studying APTT, we found its shortening in the main group to 27.3 ± 1.3 sec, compared to the control group - 35.0 ± 2.07 sec (P < 0.01). The concentration of fibrinogen, the main substrate of blood coagulation, was slightly higher in pregnant women with preeclampsia - 4.0 ± 0.2 g / l than in women with physiological pregnancy - 3.5 ± 0.17 g / l.

Conclusions: An analysis of the observations of patients indicates the staged development of platelet hyperaggregation with subsequent hypercoagulation. Confirmation of this is the high platelet aggregation, hyperfibrinogenemia and shortening of APTT.

PB2529 | Histone H4 Plasma Levels in Women with Pregnancy Complications

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Background: Recent published studies revealed the possible role played by the Nucleosomes — circulating complexes comprising DNA tightly wrapped around a core of Histone proteins (H2A, H2B, H3, H4) — in pregnancy complications.

Aims: The purpose of our study was to evaluate global coagulation in a group of 89 women with obstetrical complications encompassing Preeclampsia (PE), Pregnancy-induced Hypertension (PIH), Small for Gestational Age (SGA), Preterm Labor (PTL), Placental Abruption (AP) and Intra Uterine Fetal Death (IUFD).

Methods: 89 consecutive referrals of pregnant women with one of the following diagnoses of obstetrical complications — PE, PIH, SGA, PTL, AP, IUFD — were enrolled. During the same period of time, we also enrolled 89 healthy pregnant women matched with cases for age (± 1 year) and gestational age (±2 weeks). We collected a blood sample from each patient to assess the TG global coagulative profile by CAT method and to evaluate H4 Histones plasma levels with ELISA.

Results: Among the 89 cases (mean age 33 ± 5 yrs), n. 12 (13.5%) had a diagnosis of PE, n. 14 (15.7%) PIH, n. 9 (10.1%) SGA, n. 14(15.7%) PTL, n. 23(25.8%) AP and n. 17 (19.2%) IUFD. Overall, patients with obstetrical complications showed significantly higher average Endogenous Thrombin Potential (ETP) values (1690.2±365.1 nM*min) compared to the control population (1451.3 ± 210.7 nM*min, p< 0.0001). The average levels of H4 Histone in women with obstetrical complications were significantly higher (152.6 ±100.1 AU) vs. heathy controls (110.7±54.3 AU, p=0.0007).

Conclusions: In conclusion, our study highlights a hypercoagulability state in women with obstetrical complications compared to the control population, characterized by a significant increase of ETP values and of H4 Histone plasma levels. Larger prospective studies are required to confirm these findings.

PB2530 | Lack of Disease Modification of HbF on Pregnant Women with Sickle Cell Disease

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Background: Pregnancy in women with sickle cell disease has an increased risk of complications for both mother and infant. While sickle cell mothers have an increase in painful episodes, infection, pulmonary complications and thromboembolic events, there is also a higher risk of stillbirth and low birth weight for the fetus/neonate. Increased vaso-occlusion, viscosity and hypercoagulability in both the mother and the placenta are thought to explain the increased incidence of placental infarction and necrosis that lead to these higher rates of adverse perinatal and fetal outcomes. Higher hemoglobin F (HbF) levels correlate generally with a better prognosis in sickle cell disease patients but it is unclear whether the protective effect of a higher affinity hemoglobin holds true for the uteroplacental circulation.

Aims: We hypothesized that HbF levels might have an effect on pregnancy outcomes such as spontaneous abortions, pre-eclampsia, or low birth weight.

Methods: We reviewed the obstetrical history of a cohort of 61 female sickle cell patients. Demographics, age of mother at delivery, past medical history of mother (crises, medications, transfusions, avascular necrosis), number of spontaneous abortions, adverse perinatal outcomes, gestational age at delivery, birth weight, and complications.

Results: Patient HbF levels did not correlate with number of spontaneous abortions ($r = 0.083$, p-value = 0.52) and there were no pairwise differences in HbF levels in patients who had a history of spontaneous abortions versus those without ($p = 1.0$). We also found that there was no difference in HbF levels between mothers who had experienced pre-eclampsia and those who did not ($p = 0.89$) and no significant correlation with birth weight of first child ($r = 0.26$, $p = 0.33$).

Conclusions: We could not find a mitigating effect of HbF on pregnancy, perinatal adverse outcomes or childbirth in women with sickle cell disease.

INSIGHTS Study Clinical Trial NCT02156102.
NIH Project Number: ZIA-HG200394-06.

PB2531 | Pregnancy Complications and Outcomes in Women with Moderate and Severe von Willebrand Disease (VWD)

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Background: Women with VWD are at increased risk of peripartum bleeding complications, especially those with significant coagulation defects.

Aims: Obstetric management and outcomes in women with moderate and severe VWD who are registered at the Katharine Dormandy Haemophilia and Thrombosis Centre and delivered at Royal Free Hospital.

Methods: Retrospective cohort analysis of data obtained from multidisciplinary (MDT) clinic and maternity electronic records.

Results: 36 deliveries to 21 women were identified; 15 severe and 6 moderate VWD cases. All women had MDT birth plans.

Early pregnancy bleeding was seen in 5 pregnancies; all received Tranexamic Acid (TXA) and 1 required platelet and clotting-factor concentrate (CFC).

There were 4 pregnancies complicated with minor antepartum haemorrhage (< 50ml blood-loss); all treated with TXA and 1 given CFC. Increment, to a variable degree, was observed in third-trimester VWF:Ag and FVIII levels. In 43% of cases, out of which 75% had moderate VWD, VWF:CB remained < 50iU/dL in third trimester.

Mean gestational age at delivery was 38+3 weeks (range 37-42); 25% delivered by elective Caesarean section (LSCS), 56% vaginal delivery and 19% underwent emergency LSCS. All women received intrapartum TXA; additional cover with CFC given in 15 cases and DDAVP in 4 cases.

Regional analgesia/anaesthesia was administered in 64% of deliveries with haemostatic cover (17 epidurals, 6 spinal anaesthesia). No bleeding complications noted.

Mean blood-loss at LSCS was 590ml and 250ml after vaginal delivery. There were two cases of primary postpartum haemorrhage (PPH); one retained placenta and the other one due to surgical loss. There was one case of secondary PPH related to non-compliance with postpartum haemostatic prophylaxis.

All women received postpartum prophylaxis with TXA up to 6 weeks; 42% required CFC cover between 5 days-6 weeks.

Conclusions: This study demonstrates the important role of MDT management in minimising risk of peripartum bleeding complications and optimising uptake of regional analgesia/anaesthesia.

PB2532 | Alternative Complement Pathway Activation in the Severe Hypertensive Disorders of Pregnancy

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Background: Preeclampsia (PE) accompanied by proteinuria and/or other organ failures is categorized as one of hypertensive disorders of pregnancy (HDP). Recently, mutations of complement-related genes that predispose atypical hemolytic uremic syndrome (aHUS) have been reported in PE. This finding suggests that complement dysregulation should be involved in the pathogenesis of PE.

Aims: This study was conducted to investigate the relationship between HDP and abnormality of complement System.

Methods: We enrolled 4 normal pregnant individuals with a history of HDP and 10 HDP patients including 3 cases with gestational hypertension (GH) (severe:1, non-severe:2), 7 cases with PE (severe:5, non-severe:2). We measured plasma levels of complement-related factors (C3, C4, CH50, sC5b-9, Ba, C5a, CFH, anti-CFH antibody). In biochemical analyses, we classified plasma samples based on the symptoms, the severity, and the time of sampling (during pregnancy, early postpartum [within 3 days after delivery], and late postpartum [over 4 days after delivery]). We compared the plasma levels among the groups and non-pregnant healthy volunteers using mixed effect model. Genetic analysis of complement-related genes has been performed.

Results: Two severe HDP patients showed continuously elevated values of anti-CFH antibody, suggesting alternative pathway activation. None had rare variations in known aHUS predisposing genes. Pregnant individuals consecutively had higher levels of sC5b-9 before and after partum. Patients with severe HDP tended to have higher levels of Ba (an activation marker of alternative pathway). Patients with PE and severe HDP showed high Ba levels in the early postpartum period. Patients with PE had lower levels of C4 and CH50 before and after delivery. All pregnant individuals showed increased levels of C3, C4 and CH50 in the late postpartum period.

Conclusions: The activation of alternative pathway should be involved in the severity of HDP. Pregnancy-related thrombotic microangiopathy may be mainly induced in the late postpartum.

PB2533 | Mean Platelet Volume from Adolescent to Adult and during Pregnancy

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Background: Platelets play a critical role in both hemostasis and thrombosis. Mean platelet volume (MPV) is a potential biological marker of platelet function. Higher MPV was shown to be associated with traditional cardiovascular risk factors such as diabetes, obesity, hypertension and hypercholesterolemia and is associated with an increased risk for both arterial and venous thrombosis. However, there is currently no report linking MPV to pregnancy or pregnancy loss.

Aims: To define the change of MPV from adolescent to adult and throughout pregnancy.

Methods: We evaluated MPV and platelet counts from adolescent to adult throughout pregnancy in women who delivered at Tongji University Shanghai Tenth People's Hospital between Jan 1, 2017 and December 31, 2018. These MPV and platelet counts were compared with those of nonpregnant women who were included in Tongji University Shanghai East People Hospital from June 1, 2017 to September 30, 2019.

Results: Among the 314 deliveries that occurred during the study period, 258 women had sufficient data for our analyses. Among them the median MPV at the 12th week of gestation, 9.20 fL, was significantly lower than that in the 13,1261 nonpregnant women, 10.50 fL (P< 0.001), with a decrease by 12.4%. Then the MPV gradually increased until delivery by 21.3%. By contrast, mean platelet counts decreased by an estimated 23.8% during pregnancy. In adulthood, MPV kept constantly stable while platelet counts gradually decrease (female:11.8%, male:17.0%) regarding to age in adults. Contrast to the steady pattern of MPV in adult men and nonpregnant women, there was a sudden fall of MPV in late adolescent, no matter female or male.

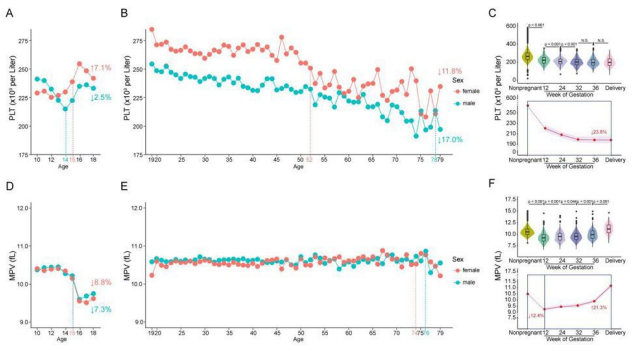

FIGURE 1 Trends of MPV and platelet counts from adolescent to adult and throughout pregnancy

Conclusions: MPV keeps stable in adults while decreased in late adolescent and early in the first trimester during pregnancy.

PB2534 | Deviations in Red Blood Cell Gas Transport Function in Pregnant State

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Background: Disturbance in red blood cell (RBC) gas transport function can lead to hypoxic state and it is significant to detect in pregnant state. In the early stages of pregnancy, hypoxia causes the appearance of embryo pathophysiology. It seems necessary to develop diagnostic system for prediction the risk of hypoxia by detailed characterization of morphological and functional properties of red blood cells. It consist of shape parameters, hemoglobin content, the rate of anion permeability (active state of Band 3 proteins) and critical membrane tension.
Aims: To establish the essential deviations in RBC gas transport functions in healthy and pregnant state.
Methods: After informed consent a blood sample was taken from a donor by venipuncture and collected in a vacuum tube containing anticoagulant and used in experiments at room temperature (22 °C). All donors were divided in three groups: healthy pregnant women after 22 gestation week and with arterial hypertension and healthy donors. Experiments were carried out on scanning flow cytometer (SFC, fabricated by CytoNova Ltd., Novosibirsk, Russia, http://cyto.kinetics.nsc.ru). This approach allows to investigate RBC gas transport function in terms of diameter, thicknesses, volume, surface area, sphericity index, spontaneous curvature, the rate of anion permeability and the critical relative expansion of a membrane, hemoglobin content.
Results: Total 35 pregnant women and 30 donors were included in this study. Accordidng to obtained data healthy pregnant women have decreased by 32% native rate of anion permeability compare with healthy donors group. However, the ration between native and activated anion permeability among these groups remains the same with accuracy around 6%.
Conclusions: For the first time the mean values and distributions of RBC gas transport fuction was obtained for pregnant state. Such investigation is the small step to diagnostic system which capable of determine the risk of hypoxic state by detailed RBC analysis.

PB2535 | Antithrombotic Therapy for Pregnant Woman with Bland-White-Garland Syndrome and Prosthetic Mitral Valves

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Background: Among all causes of maternal mortality from non-obstetric disorders, heart defects of different etiology are 15-20%, and the specific gravity of this pathology in the structure of maternal mortality and morbidity remains high due to the fact that these patients traditionally constitute a high-risk group for the development of thromboembolic disorders and death.
Aims: The management of pregnancy with a high risk of cardiovascular complications.
Methods: We report the case of 32-year-old woman with Bland-White-Garland (BWG) syndrome (the anomalous origin of the left coronary artery from the pulmonary artery, ALCAPA). In 2011 she had a reimplantation of the left coronary artery in the ascending aorta with the plastic of the pulmonary artery and mitral valves repair. She suffered from cardiac dysrhythmia by the ventricular extra systole, paroxysmal form of supraventricular tachycardia, heart failure (HF) II functional class (FC) NYHA. She was on warfarin therapy. This is her 2nd pregnancy. She refused to switch on LMWH/UH because in the previous pregnancy there was constant bleeding that ended in a miscarriage.
Results: The entire pregnancy she was given warfarin under the control of INR. There were repeated hospitalizations due to complaints of paroxysmal atrial flutter. At 38 weeks the patient had given birth to a healthy full-term baby girl without malformations, 2750 g, 47 cm and an Apgar score of 8/9 by cesarean section as planned.
Conclusions: Experience of pregnancy management in women with arterial conduits with ALCAPA is small. It should be kept in mind that outside of pregnancy, artificial valves are a direct indication to for a lifelong anticoagulant therapy. However, if out-of-pregnancy anticoagulation is provided by oral anticoagulants (mainly warfarin in most of the countries), then during the pregnancy there are different schemes of anticoagulant therapy. In this case warfarin was the method of choice.

PB2536 | Early Plasma Transfusion and Maternal Outcomes in Women with Persistent Postpartum Haemorrhage: Does the Amount of Resuscitation Fluids Matter?

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Background: In a recent study initiation of plasma transfusion during the first 60 minutes of persistent postpartum haemorrhage was not associated with more favourable maternal outcomes. Some argued that this absence of effect may have been due to too high volumes of resuscitation fluids. The RCOG guideline recommends to infuse less than3.5L of fluids before blood transfusion.

Aims: To quantify the association of plasma transfusion within 60 minutes after diagnosing persistent PPH and adverse maternal outcome among women who received less than 3.5L of resuscitation fluids, and similarly among women who received more than 3.5L.

Methods: We performed a secondary analysis in a retrospective cohort of women with persistent PPH. Women were enrolled between 2011 and 2013 in 61 hospitals. The outcome was a composite of maternal mortality, hysterectomy and arterial embolization. In a time-dependent propensity score-matched analysis, women with transfusion of plasma early, i.e. within 60 minutes following PPH diagnosis, were matched to women without early plasma transfusion; with sensitivity analyses of plasma within 120 and 180 minutes.

Results: The cohort consisted of 1216 women with persistent PPH; seven women died (0.6%), 62 underwent hysterectomy (5.1%) and 159 arterial embolization (13.1%). Among women who had received less than 3.5L fluids there was no difference in outcome between those who had received plasma early and those who had received plasma later, adjusted odds ratio 1.02 (95% confidence interval 0.44 to 2.40, 77 pairs); among women who had received more than 3.5L the odds ratio was 0.78 (0.25 to 2.38, 34 pairs).

Conclusions: These findings do not support the notion that the previously observed absence of association between early plasma transfusion and maternal outcome was due to too high volumes of resuscitation fluids. Whether early plasma transfusion prevents adverse maternal outcomes when the volume of resuscitation fluids is much lower than 3.5L remains to be established.

PB2537 | Rotational Thromboelastometry (ROTEM) in Obstetric Population

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Background: ROTEM is a point of care testing (POCT) that offers a therapeutic approach to postpartum hemorrhage (PPH), this test is not considered a standard of care for this complication. Normal references ranges are different in pregnant women reflecting a hypercoagulable state. Recently the Women Health SSC of the ISTH published the current evidence of these POCT not only in PPH but in other risk conditions in pregnancy (bleeding, hypercoagulability and/or thrombosis).

Aims: To evaluate the use of ROTEM in obstetrics in a single high complexity hospital.(Retropective. May 2018-July 2019).

Methods: ROTEM was indicated during delivery in eight pregnancies: Group A (PPH): 2 patients with placenta *acreta*; 2 uterus hypotonia (1 with hypovolemic shock);1 cesarean section that needed to be reoperated. Group B (other risk conditions): 1 severe idiopathic pulmonary hypertension (IPH) medicated with prostanoids and unfractionated heparin (UFH); 1 antiphospholipid syndrome (APS) with acute venous thrombosis (VT) near delivery, with thrombocytopenia and UFH; 1 twin pregnancy with thrombocytopenia.

ROTEM ^{R} sigma: standard panel comprises EXTEM, FIBTEM and INTEM; ADPTEM was used in the patient with prostanoids and HEPTEM in 1 patient with UFH.

Results: Group A: normal results, allowing to distinguish between surgical and coagulopathic bleeding. Group B: the ADPTEM in IPH detect an abnormal platelet aggregation and platelet transfusion was indicated; in the twin pregnancy with thrombocytopenia (70.000 $mm^{3)}$ tests were normal and useful to define spinal anesthesia .The patient with APS and VT with UFH the HEPTEM (use to detect residual heparin) was normal and cesarean section was performed without complications, an hypercoagulable state was detected too.

Conclusions: Most women with PPH have no coagulopathy, where the value of ROTEM base management is to avoid unnecessary transfusion that can increase the basal hypercoagulable state (Group A). Other potential areas for use in obstetrics (Group B) need further investigation.

PB2538 | Pregnancy Outcomes in Women with Budd Chiari Syndrome or Portal Hypertension

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Background: Women with Budd Chiari Syndrome (BCS) and/or portal hypertension are at high risk of pregnancy complications and adverse outcomes. Decreased liver function, underlying prothrombotic disorders combined with physiological changes during pregnancy underlie the observed high rates of maternal and fetal morbidity. There is no consensus on optimal treatment strategies throughout pregnancy. Clinical practice with regard to counselling these women planning for pregnancy is heterogenous and some physicians advise against pregnancy.

Aims: To evaluate management and outcomes of pregnancies in women with BCS and/or portal hypertension.

Methods: We performed a retrospective single center cohort study between 2008 and 2019. Women with confirmed BCS and/or portal hypertension who conceived at least once were enrolled and study outcomes were assessed up to 12 weeks after delivery or 6 weeks after pregnancy loss. The primary outcome was maternal pregnancy-related mortality and birth of a living fetus. Secondary outcomes included maternal, fetal and obstetrical complications.

Results: Eighteen women (3 BCS of whom 2 with portal hypertension and 15 with portal hypertension) conceived at least once (24 pregnancies overall). Ten women had a history of venous thrombosis, mostly portal vein thrombosis (N=7). Underlying prothrombotic disorders included myeloproliferative neoplasms (N=4), protein C deficiency (N=2), antiphospholipid syndrome (N=1) and cirrhosis (N=5) [Table 1]. Six of ten women with a history of thrombosis received low-molecular-weight heparin during pregnancy. Of 18 first pregnancies, 12 resulted in live birth, 5 in pregnancy loss and 1 was ectopic [Table 2]. Six women conceived twice. No maternal deaths or thrombotic events were observed.

Conclusions: Adverse pregnancy outcomes in women with BCS and portal hypertension are common. However, in our small cohort, the chance of term live birth of 56% seems too high and maternal mortality too low to consider BCS or portal hypertension an absolute contra-indication.

TABLE 2 Pregnancy outcomes

Pregnancy outcomes	All patients (N=18)	Patients with prior VTE (N=10)
Live birth, n (%); gestational age, median (range)	12 (67); 39 (27- 41)	5 (50); 40 (39 - 41)
Pregnancy loss, n (%); early miscarriage <10 weeks GA, late miscarriage 10-20 weeks GA, Fetal death >20 weeks GA	5 (28); 3 (17), 1 (6), 1 (6)	4 (40); 3 (30), 1 (10), 1 (10)
Ectopic pregnancy, n (%)	1 (6)	1 (10)
Birth weight total (grams), median (range)	2993 (850 - 4165)	3035 (2725 - 3250)
Mode of delivery, n (%); vaginal, caesarean section	8 (67), 4 (33)	4 (80), 1 (20)
Antithrombotics during pregnancy, n (%); LMWH, Aspirin	6 (33); 6 (33), 0	6 (60); 6 (60), 0

TABLE 1 Patient characteristics

Patient characteristics	All patients (N=18)	Patients with prior VTE (N=10)	Portal vein thrombosis (N=7)	Budd Chiari Syndrome (N=3)
Age at conception (years), mean ± SD	30.3 ± 6.3	30.4 ± 6.3	29.1 ± 5.9	30.3 ± 4.6
BMI (kg/m^2), mean ± SD	23.5 ± 3.3	23.7 ± 2.9	22.1 ± 2.5	25.3 ± 1.9
History of miscarriage, n (%)	4 (22)	3 (30)	2 (29)	0
Myeloproliferative neoplasms, Protein C deficiency, Antiphospholipid syndrome, Cirrhosis	4 (22), 2 (11), 1 (6), 5 (28)	3 (30), 2 (20), 1 (10), 0	2 (29), 2 (29), 0, 0	1 (33), 0, 1 (33), 0
Antithrombotics prior to pregnancy, n (%); LMWH, VKA, DOAC	6 (33); 1 (6), 5 (28), 0	6 (60); 1 (30), 5 (50), 0	3 (43); 1 (14), 2 (29), 0	2 (67); 0, 2 (67)

PB2539 | Risk Factors for Severe Preeclampsia in Obstetric Antiphospholipid Syndrome (OAPS) with Conventional Treatment

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Background: Pre-eclampsia (PE) is a cause of maternal/fetal mortality, but its treatment and prevention are neither clear nor effective.
Aims: The aim of our study was to identify risk factors for developing severe PE (sPE) in patients with OAPS treated with conventional treatment (CT).
Methods: 102 OAPS women were recruited (Tables 1-2) and treated only with CT: low dose aspirin 100 mg/day + low molecular weight heparin 40 mg/day. At the end of their following pregnancy, it was analyzed the association between sPE and well-known risk factors obesity, metabolic syndrome, chronic hypertension, hypertriglyceridemia, diabetes, age, tabaquism, previous sPE, triple/double/single positivity of antiphospholipid antibodies (aPL), high titers of aPL, systemic lupus erythematosus, late pregnancy loss, previous intrauterine growth restriction (IUGR). Informed consents were obtained for all participants.
The odds ratio (OR) was estimated to assess the strength of association between sPE and the studied variables (SPSS statistical software).
Results: After CT, 18 (17.6%) suffered pregnancy losses: 8 (7.8%) pregnancy losses >20 weeks (fetal losses) and 10 (9.8%) early and late miscarriages, while in 84 (82.3%) cases live births were achieved. Besides, 9 patients presented with sPE (8.9%) and 11 (10.8%) with IUGR.
Patients with double and simple positivity of aPL decreased their sPE rates compared with their obstetric history (3/87=3.5% vs 15/87=17.2%). Remarkably, CT was only partially effective in the prevention of sPE for patients with triple positivity of aPL (6/15=40% vs 3/15=20%).
Triple positivity of aPL OR=24.70 ([4.27-142.92]; $p< 0.001$) and history of sPE OR=7.11 ([1.13-44.64]; $p=0.036$) proved to be the only strong risk factors for developing sPE with CT, and they were present in 66.6% (12/18) of patients with history of sPE.
Conclusions: As triple positivity for aPL and previous sPE turned out to be risk factors for sPE in patients with OAPS under CT, these patients could benefit from receiving further treatment.

TABLE 1 Demographic and clinical characteristics of patients with OAPS

Number of patients	All (n=102)	%
Age	31 (28-36)	
History of previous:		
*severe preeclampsia	18	17.6
*intrauterine growth restriction	17	16.7
Systemic Lupus Erythematosus	11	10.8
History of thrombosis	6	5.9
Pregnancy loss x<10 weeks	71	69.6
Pregnancy loss 10 to 20 weeks	34	33.3
Pregnancy loss x>20 weeks	46	45.1

TABLE 2 Laboratory characteristics of patients with OAPS. aPL: antiphospholipid antibodies; aCL: anticardiolipin antibodies; aβ2GPI: anti-β2 glycoprot

Number of patients	All (n=102)	%
Lupus anticoagulant	63	61.8
aCL >40 (GPL/MPL)	46	45.1
aCL >80 (GPL/MPL)	12	11.8
aB2GPI >40 (UG/UM)	33	32.4
aB2GPI >80 (UG/UM)	12	11.8
aB2GPI and aCL high titers	20	19.6
Simple positivity of aPL	76	74.5
Double positivity of aPL	11	10.8
Triple positivity of aPL	15	14.7

PB2540 | F1+2 in Preeclampsia: Is it a Good Hemostatic Marker?

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Background: Preeclampsia (PE) is associated with endothelial dysfunction, which can trigger abnormal activation of hemostasis.
Aims: To evaluate prothrombin fragment 1+2 (F1+2) levels, an indicator of thrombin generation, as a potential marker for prognosis and monitoring of PE, compared to D-dimer (DDi).
Methods: Ethical aspects have been observed previously (CAAE 0530.0.203.00-09). A total of 59 pregnant women participated in the study, including 19 with severe PE, 22 with mild PE and 18 normotensive pregnant women (controls). F1+2 and DDi levels were determined by Elisa. Statistical analysis was performed by ANOVA and $P< 0.05$ was considered significant.
Results: No difference was found for F1+2, comparing the average values for women with severe PE (2.50±1.07nmol/L), mild PE (2.45±0.85nmol/L) and controls (2.94±1.09nmol/L). Conversely, measurement of DDi levels revealed a marked exacerbation of coagulation in severe PE (1497.8±435.3ng/mL) compared to mild PE (1060.3±259.2ng/mL) and controls (1146.6.8±311.2ng/mL) (Dusse et al., Clin Chim Acta. 2013,415:226-9).

Conclusions: According to DDi levels results, an increase conversion of prothrombin into thrombin resulting in higher levels of F1+2, was expected in severe PE. However, it was not obtained. Our results are in line with a previous one (Cadroy et al., Br J Obstet Gynaecol. 1993, 100(5):416-20) who did not observe an increase of F1+2 in PE. Therefore, accuracy of F1+2 determination to assess thrombin generation has been questioned. According to Cadroy et al., some F1+2 molecules are retained on the catalytic phospholipid surface where they were formed, not being released into the circulation. It results in underestimated plasma levels of F1+2. Concluding, F1+2 levels, unlike DDi, failed as a potential hemostatic marker for prognosis and monitoring of PE.

Support: CNPq (404353/2016-9) and FAPEMIG/BRAZIL (PPM-00764-16).

PB2541 | Maternal Carriage of the Annexin A5 M2 Haplotype Combined with Inherited or Acquired Thrombophilia Would Synergistically Increase the Risk of Suffering Intra Uterine Growth Restriction

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Background: Annexin A5 (ANXA5) is a protein abundantly expressed in normal placenta where it was proposed to exert an anticoagulant function. As the carrier status of the *M2/ANXA5* promoter haplotype results in a significantly reduced expression of placental ANXA5, it was proposed to be involved in the etiology of obstetric complications of vascular origin. However, different studies yielded conflicting evidence, similarly to the controversial role of inherited thrombophilia.

Aims: Our aim was to assess the association between maternal carriage of the *M2/ANXA5* haplotype and obstetric pathologies in which placental vascular complications could have a key role.

Methods: 283 women with recurrent pregnancy loss (RPL) and 114 parous controls without pregnancy complications were recruited. Patients were stratified according to the timing of miscarriages: early (<10 weeks), late (≥10 weeks) and fetal losses (≥20 weeks) and having suffered or not intra-uterine growth restriction (IUGR) and/or preeclampsia (PE). Differences in the maternal *M2/ANXA5* carriage rate between groups were assessed using the chi-square test. The *odds ratio* (OR) was estimated by binary logistic regression adjusted for potential confounding variables.

Results: Our results showed an association between maternal carriage of *M2/ANXA5* and IUGR, but neither association was found with the timing of pregnancy losses nor with PE (Fig. 1) However, logistic regression analysis showed that maternal carriage of *M2/ANXA5* would not be an independent risk factor for IUGR but when combined with either acquired or hereditary thrombophilia, it would increase the odds of suffering this obstetric pathology (OR=6.26; 95%CI [1.99-19.06]).

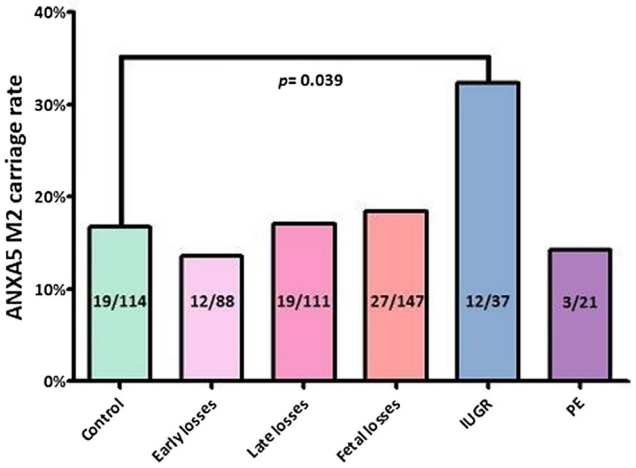

FIGURE 1 Maternal M2/ANXA5 carriage rate among clinical subgroups of patients and controls

Conclusions: As with inherited thrombophilia, the role of the ANXA5 M2 haplotype in obstetric complications of vascular origin has been a matter of debate. Our results showed that maternal carriage of *M2/ANXA5* would not be associated with the timing of RPL or PE, but when combined with either inherited or acquired thrombophilia might act synergistically increasing the risk of IUGR.

PB2542 | The Impact of Hydroxychloroquine on the Prevention of Severe Preeclampsia in Patients with Obstetric Anti-Phospholipid Syndrome

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Background: Patients with Anti-phospholipid Syndrome may be stratified in terms of risk for future thrombotic and/or obstetric events, depending on the number of positivity of antiphospholipid antibodies (aPL: lupus anticoagulant, anticardiolipin antibodies, aβ_2Glicoprotein I antibodies), titers of aPL, and the clinical manifestations associated with aPL. However, there are few studies exploring the effects of additional treatment in high-risk obstetric APS (OAPS) patients on preventing severe preeclampsia (sPE).

Aims: The aim of our study was to retrospectively evaluate the efficacy on improving pregnancy outcomes of having added hydroxychloroquine to conventional treatment in patients who were considered high-risk OAPS.

Methods: We selected 42 patients with OAPS with at least one of the following high-risk factors: triple positivity of aPL and/or history of previous sPE (Table 1). Patients were grouped according to the

TABLE 1 Demographic and clinical characteristics of OAPS patients before treatment

Variables	All (n=42)	Group A (n=30)	Group B (n=12)
History of previous severe Preeclampsia	54.7% (23/42)	60% (18/30)	41.7% (5/12)
History of intrauterine growth restriction	23.8% (10/42)	16.7% (5/30)	41.7% (5/12)
Systemic Lupus Erythematosus	16.7%(7/42)	16.7% (5/30)	16.7% (2/12)
Triple Positivity aPL	54.7% (23/42)	50.0% (15/30)	66.6% (8/12)
Double Positivity aPL	2.4% (1/42)	0.0% (0/30)	8.3% (1/12)
Simple Positivity aPL	42.8% (18/42)	50.0% (15/30)	25.0% (3/12)
Lupus Anticoagulant	80.9% (34/42)	80.0% (24/30)	83.3% (10/12)
aCL High titers (GPL/MPL) x>80	35.7% (15/42)	26.7% (8/30)	58.3% (7/12)
aβ2GPI High titers (UG/UM) x>80	38.1% (16/42)	36.7% (11/30)	41.7% (5/12)

TABLE 2 Severe Preeclampsia rates in Group A vs Group B after treatment

	Severe Pre-eclampsia		
	NO	YES	
Group A (CT)	18 (60.0%)	12 (40.0%)	p=0.03
Group B (CT+Hidroxyicloroquine)	11 (91.7%)	1 (8.3%)	

treatment received and their pregnancy outcomes were compared. Group A (n=30): patients treated with CT: low dose aspirin 100 mg/day + low molecular weight heparin 40 mg/day vs Group B (n=12): patients with CT+ hydroxychloroquine 400 mg/day. A chi-squared test was used to assess the association between sPE and treatment in high-risk OAPS patients (SPSS 23.0).

Results: After treatment, the pregnancy outcomes were: Group A: pregnancy loss 16.7% (5/30), sPE 40.0% (12/30), prematurity 26.6% (8/30); Group B: pregnancy loss 8.3% (1/12), sPE 8.3% (1/12) and prematurity 0.0% (0/12). Patients with CT+HCQ showed significantly lower sPE rates compared with patients with only CT (p=0.03) (Table N°2). Hence, treatment with hydroxychloroquine would be associated with a protective response on preventing sPE, in OAPS patients with high-risk factors.

Conclusions: In our cohort, CT was only partially effective in the prevention of sPE. Additional treatment with hydroxychloroquine might benefit OAPS patients with high-risk factors (as triple positivity of aPL and/or history of sPE) by preventing them from developing sPE.

PB2543 | Antithrombotic Therapy (Nadroparin Calcium) in the Prevention of Pregnancy Loss in aPL-Positive Women

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Background: As it is known the pregnancy loss is a marker and the most striking manifestation of antiphospholipid syndrome (APS). Pregnant women with antiphospholipid antibodies (aPL) circulation has a potential risk of placental insufficiency and fetal growth restriction development.

Aims: The evaluation of aPL profile in patients with recurrent pregnancy loss.

Methods: 146 women with history of recurrent miscarriage and 60 age matched healthy pregnant women were examined for anticardiolipin (aCL), anti-annexin V, anti-b2-GPI, anti-prothrombin (aPt) antibodies and LA circulation. Of them, 74 women were in the I trimester of pregnancy and 72 admitted in II and III trimesters.

Results: 34.2% women were diagnosed APS. LA circulation - 14%, anticardiolipin - 31.5%, anti-annexin V - 31%, anti-b2-GPI - 22.6, anti-prothrombin - 10.3%. Combination of LA, anti-b2-GPI, anticardiolipin was in 12.1%, LA, anti-annexin V and anti-b2-GPI - in 13.7%, anti-prothrombin and anti-b2-GP I - in 8.9%, LA, annexin V and b2-GPI - in 7.9% and was associated with more severe complications. All patients received anticoagulant therapy with nadroparin calcium. In women treated before the pregnancy early miscarriage in the next pregnancy occurred in 1.6%. There was no antenatal death

or stillbirth. In women started the therapy in II or III trimesters the frequency of obstetric complications was higher (p< 0.05) but still significantly lower compared with their history without therapy.

Conclusions: 34% fetal loss cases were associated with antiphospholipid syndrome. The combination of various antibodies at the same time in women with more severe obstetric history demonstrates the diagnostic value of the determination of different groups of aPL. LMWH (nadroparin calcium) was effective for prevention of recurrent thromboembolism and obstetric complication.

PB2544 | Impaired Platelet Contractility in Women with a History of Miscarriage

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Background: Platelets are blood cells that have multiple non-hemostatic and hemostatic functions, including platelet-driven contraction of blood clots and thrombi that can modulate the course and outcomes of thrombosis. Hemostatic disorders are among the most important pathogenic factors in recurrent pregnancy loss (RPL). We hypothesized that impaired platelet-mediated contraction of blood clots can increase the obstructiveness of microthrombi and aggravate microcirculatory disorders in the placenta, thus promoting RPL.

Aims: To study the kinetics of clot contraction in the blood of patients with a history of RPL in combination with platelet functions and hemostasis.

Methods: Clot size during thrombin-initiated contraction was tracked optically in clots made from the blood of 50 women with a history of RPL and 30 parous women without obstetric complications in the past. In parallel, platelet functionality was assessed using flow cytometry before and after TRAP-induced activation, in combination with hemostatic and hematologic blood tests. All women gave written informed consent as required by the Ethical Committee of KFU.

Results: In non-pregnant women with a history of RPL, blood clot contraction was significantly impaired in combination with hypercoagulability. The impaired clot contraction was associated with platelet dysfunction, manifesting as reduced expression of P-selectin and phosphatidylserine in response to stimulation via PAR-1 receptors. Partial platelet refractoriness likely results from continuous activation and metabolic exhaustion.

Conclusions: These results indicate that impaired contraction of blood clots combined with chronic hypercoagulability form a premorbid background in patients with a history of RPL. The impaired platelet functionality may be a pathogenic factor that promotes thrombotic complications during pregnancy. Prevention of systemic platelet activation may be considered as a potential therapeutic target to reduce the risk of RPL.

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PB2545 | Assessment of Endocan as a Marker for Preeclampsia and Its Clinical Forms

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Background: Preeclampsia is a disease specific from pregnancy. It is classified according to the age of symptoms onset as early (< 34 weeks) and late (≥34 weeks). Endocan is a biochemical marker of endothelial dysfunction that is potentially associated with immunoinflammatory response. It is known that endothelial dysfunction and inflammatory process are important features in preeclampsia (PE).

Aims: To evaluate endocan levels in preeclamptic women as a potential marker for prognosis and monitoring of PE.

Methods: This case-control study included 80 Brazilian pregnant women, 40 with severe PE and 40 normotensive pregnant women (Controls). From those 40 with severe PE, 20 had the early form and 20 the late form. Endocan levels were investigated by enzyme-linked immunosorbent assay. Statistical analysis was carried out using SPSS 19.0. Data normality was tested by Shapiro-Wilk test. The differences in endocan levels between the PE and normotensive groups were assessed by Mann-Whitney test. P values < 0.05 were considered statistically significant. Ethical aspects have been observed previously (CAAE 0530.0.203.00-09).

Results: No significant difference was observed between PE (0.388ng/mL [0.346-0.516]) versus normotensive pregnant (0.393ng/mL [0.321-0.623]) women (P=0.870). Additionally, no significant difference was found comparing early (0.385ng/mL [0.311-0.459]) versus late PE (0.407ng/mL [0.313-0.500]).

Conclusions: Our data showed no association between endocan levels and PE occurrence in Brazilian pregnant women, as well as the early and late form. These findings suggest that endocan seems not to be a potential marker for prognosis and monitoring of PE.

Support: CNPq (404353/2016-9) and FAPEMIG (00764-16).

PB2546 | Personalized Control of Anticoagulant and Antiplatelet Therapy for Pregnant Women with a Complicated Obstetric Anamnesis Is Pathogenetically Justified for the Purpose of Prevention of Placenta-associated Complications

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Background: Currently low-weight-molecular heparins (LWMH) are prescribed to pregnant women to prevent venous thromboembolic complications. There're no recommendations for personalized selection of LWMH and acetylsalicylic acid (ASA) for women with recurrent pregnancy losses (RPL) to improve the outcome of pregnancy.
Aims: To prove the effectiveness of selection of LWMH and ASA for women with RPL to improve the outcome of pregnancy.
Methods: 120 women with RPL were included in the research. LWMH and ASA were administered individually when pathological hypercoagulation was detected based on D-dimer, XIIa-dependent fibrinolysis and thrombodynamics tests (global test) at the pregravidar preparation stage (group 1), or from the moment of pregnancy (group 2), or at any stage of pregnancy (group 3). During pregnancy the control of these tests was carried out at the end of LWMH effect every 4 weeks, the doses were corrected in case of deviation from the reference values.
Results: Group 3 patients didn't receive NMG and showed more pronounced chronometric hypercoagulation at the inclusion stage according to the thrombodynamics test (increased speed parameters) and the highest percentage of fixation of spontaneous clot formation (33%).). The negative correlation (-0.71) between the time of XIIa-dependent fibrinolysis and the size of the clot was found in group 3. This shows the importance of detecting of decrease in the activity of fibrinolysis as a marker of pathological hypercoagulation. The dose of enoxaparin was assigned from 0.2 ml to 1.0 ml per day and didn't depend on weight. The dose of ASA is from 50 mg to 150 mg per day. Successful carrying a child: 1st group - 91%, 2nd group - 87%, 3rd group - 76%.
Conclusions: These data indicate the pathogenetic role of disorder of fibrinolysis system in the formation of pathological hypercoagulation of women with RPL. Individual laboratory control of anticoagulation during pregnancy allows to improve pregnancy outcome.

PB2547 | Disorders in the Fibrinolytic System as a Possible Factor in the Failure of Assisted Reproductive Technologies

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Background: The factors of success of assisted reproductive technologies (ART) are known: the quality of embryos, endometrial receptivity, and hemostasis (fibrinolysis) which plays an important role in the regulation of the implantation process. Hypofibrinolysis is a known cause of failures in ART. But some patients with ART failures show a state of hypocoagulation and hemorrhagic complications.
Aims: to study the state of the fibrinolysis system of women with a history of ART failures during ART procedure, to evaluate the effectiveness of drugs that affect hemostasis in ART cycle in order to increase the frequency of pregnancy.
Methods: A prospective single-center research included 130 patients with a history of ART failures who were planning pregnancy using ART method. The approval of the local ethics Committee was obtained, patients signed an informed consent. Evaluation of the fibrinolysis system (D-dimer, XIIa-dependent fibrinolysis) and thrombodynamics (TD) were monitored at the stages: 2-3 days after oocyte puncture or 3-4 days before cryoembryon transfer, and then 2-3 days, 4-5 days, and 8-9 days after embryo transfer. Low-weight-molecular heparins (LWMH) were prescribed in hypercoagulation / hypofibrinolysis, and tranexamic acid (TA) in hypocoagulation / hyperfibrinolysis.
Results: The analysis included 111 patients. 140 embryo transfers were performed, including 68 transfers of cryoembryos. Pregnancy was achieved in 43.6% cases. In 80% protocols LWMH was prescribed and pregnancy occurred in 41% cases. In 18.6% protocols TA was prescribed and pregnancy occurred in 53.8% patients. 2 women refused to take medication. Cut-off points are determined based on tests: for the Vst and D parameters in the TD and the fibrinolysis system parameters to make a decision about the choice of the drug (LWMH or TA) at the point of study before embryo transfer.
Conclusions: Controlled fibrinolysis is a possible strategy for increasing the frequency of pregnancy in ART programs.

PB2548 | Reduction of Thromboembolic Disorders in Pregnancy and Postpartum Period: Role of National Pregnancy Heart Team

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Background: DVT and PE rank fourth place in Ukraine among the causes of maternal mortality. The National pregnancy heart team (NPHT) has been formed in 2013 by common efforts of National Institute of Cardiovascular Surgery (NICVS) and Institute of Pediatrics, Obstetrics and Gynecology.

Aims: To reduce maternal mortality and disability rates from DVT/PE by improving diagnostic and treatment programs in pregnancy and puerperium including activities of NPHT.

Methods: There were analyzed eight cases of DVT/PE in pregnancy and puerperium, occurred at 2014-2018. Seven patients were transferred to the NICVS and one patient was treated by NPHT on site in the regional perinatal center. There were performed four thrombectomies with CPB, 1 cava-filter insertion after failed thrombolysis, three patients successfully passed through the LWMH (enoxaparin).

Results: There were no cases of maternal mortality of severe maternal morbidity. The uneventful course of pregnancy resulted in full-term birth occurred in two cases, one pregnancy resulted in miscarriage of twin gestation in 18 gestational weeks, all the other cases concerned the puerperal period. The debriefing of all the cases allowed to establish the new approach for thromboprophylaxis in Ukraine, based on the risk stratification in every pregnant within her first prenatal visit, hospitalization for non-pregnancy related disorders, hospitalization for pregnancy disorders, labour and delivery, ambulance stage after discharge from maternity institution. The diagnostic algorithm for VTE, as well as for timely treatment of DVT/PE, choice of optimal method (or combination of methods) for treatment, especially in group of high risk of mortality was presented for the cardiology, cardiosurgery departments and perinatal centers in Ukraine.

Conclusions: A multidisciplinary approach at all stages of VTE diagnosis in pregnant women / women in childbirth is a necessary component of cardiac and obstetric care for pregnant women with VTE and enables efficient use of resources (avoiding duplication of functions).

PB2549 | Prevalence and Management of Post Partum Hemorrhage in Latin America: An Overview of Systematic Reviews

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Background: Incidence of post partum hemorrhage (PPH), a leading cause of maternal morbidity and mortality, is increasing worldwide. Social and economic implications of this complication are significant. Data from the developing countries, where access to health care can be challenging, is scarce.

Aims: To investigate the prevalence, management and outcomes of PPH in Latin American countries.

Methods: A comprehensive overview was performed to assess: a) prevalence of PPH, defined as blood loss > 500 mL, and prevalence of severe PPH (blood loss >1000 mL). b) management: use of uterotonic agents, and tranexamic acid and transfusion requirements. c) outcomes: mortality and hysterectomy.

Data source: major electronic databases and hand-searching of abstracts presented at scientific meetings (Hematology, Obstetrics and Gynecology, and Intensive Care Medicine), and reference lists checking of the retrieved articles. Data abstraction was performed following a predetermined data abstraction form.

Results: We identified 118 potentially eligible publications. Only one systematic review (SR) fulfilled our inclusion criteria (Calvert C et al. PLoS ONE 2012; 7: e41114). The SR quality, using AMSTAR-2 tool, resulted in low quality (lack of detailed description of the primary studies and of the risk of bias). Eleven studies from Latin American and Caribbean region were included in the SR (23.129 women). The

TABLE 1 Prevalence of PPH in Latin America

	Prevalence of blood loss per 100 women giving birth	Prevalence of blood loss per 100 women giving birth based only on studies which used objective method of blood loss measurement
PPH	8.2 (6.1-10.4)	11.9 (6.0-19.5)
Severe PPH	3.3 (1.8-5.2)	4.1 (1.9-7.0)

estimated prevalence of PPH is shown in Table 1. Regarding management of delivery: active management was reported in 4 studies (36%); no information about transfusions or use of tranexamic acid was provided. There was no data about mortality and hysterectomy.
Conclusions: PPH is a prevalent complication in Latin America. However, high quality SR evaluating the prevalence, causes and management of post-partum hemorrhage in Latin America are warranted.

PB2550 | Long-term Incidence of Venous Thromboembolic Recurrence After a First Venous Thrombotic Episode During Pregnancy

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Background: Pregnancy is considered as a major transient risk factor for venous thromboembolic (VTE) disease, but the long-term recurrence rate is poorly documented.
Aims: Determine the long-term incidence rate after a first VTE during pregnancy.
Methods: We performed a study on 595 consecutive patients who presented a first VTE during pregnancy or 6 weeks puerperium and who were referred in our institution between January 2000 to December 2015. VTE was defined as pulmonary embolism (PE) or deep venous thrombosis (DVT). We collected from our database demographics data, characteristics of first VTE and data on thrombophilia screening. Incidence rate was assessed after phone call or after a new visit in our department. The type of event (PE or DVT), presence and type of a provoked risk factor and biological thrombophilia were studied as predictors for VTE recurrence.
Results: 595 women were followed during 16 ± 14 years. Mean age at first VTE event was 29 ± 6 years. Anticoagulation therapy was conducted during 9 ± 13 months. VTE recurrence rate was 32% (188/595 patients). Mean age at VTE recurrence event was 42 ± 15 years. Type of recurrence was: 18% PE (n=33) and 82% DVT (n=153). 24% of recurrence occurred during new hormonal exposition (pregnancy or contraception, n=43), 26% after surgery or immobilization (n=48) and 50% were unprovoked (n=90). Prevalence of congenital thrombophilia was similar in patients with or without recurrence (28% vs 26% respectively; p=0.5). Results were similar when we restricted the analysis to severe thrombophilia (ie natural anticoagulant deficiencies, homozygosity or double heterozygosity for FV or FII G20210 variants: 9% vs 7%;p=0.64). ABO blood group distribution was also similar in patients with or without recurrence (73% vs 70% respectively; p=0.6).
Conclusions: Long-term recurrence of VTE after a first event during pregnancy is high, this recurrence is frequently unprovoked and challenges prevention strategy.

PB2551 | Outcomes in Women with Mechanical Heart Valves Anticoagulated with Low Molecular Weight Heparin During Pregnancy

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Background: Management of pregnant women with mechanical heart valves (MHVs) is complicated, as they are at risk for thrombosis, bleeding and cardiac complications.
Aims: We sought to determine the various risks that occur in these women.
Methods: We performed a retrospective analysis of all women managed at a tertiary Canadian centre who had a MHVs and received LMWH throughout pregnancy.
Results: We identified 42 pregnancies (28 women), all of which had a single MHV; 96% had a bileafet or tilting-disc valve, and 71% were in the mitral position. Although 13 (46%) had prior cardiac events, all were in New York Heart Association functional class I/II at conception. LMWH was commenced at diagnosis of pregnancy. Of the available peak anti-Xa levels, based on published recommendations, 46% were in therapeutic or supra-therapeutic range, while 54% were sub-therapeutic. Three pregnancies sustained thromboembolic complications [peri-partum valvular thrombus; transient ischaemic attacks in the first trimester, prompting change to warfarin; and a cortical infarct at 24 weeks followed by a cardiac arrest, maternal death and perimortem caesarean delivery at 26 weeks' gestation]. In the case of maternal mortality, peak anti-Xa levels were in the therapeutic range. In addition to the thromboembolic complications, there were 7 cases of major haemorrhage, three cases of heart failure, three cases of endocarditis, and two arrhythmias. All cases except the perimortem caesarean section and another with bacteremia, received regional analgesia/anaesthesia for delivery. The 42 pregnancies resulted in nine miscarriages, three pregnancy terminations, one stillbirth and 28 live births, one of which resulted in a neonatal death. There were no congenital anomalies.
Conclusions: Despite multidisciplinary care, women with MHVs receiving LMWH during pregnancy remain at risk of adverse outcomes and are a high-risk cohort. While focus on thromboembolic complications and anti-Xa monitoring is important, women are also at risk for serious bleeding and cardiovascular complications.

PB2552 | Hemostasis Disorders in Tunisian Women with Severe Post Partum Heamorrage : A Case Control Pilot Study

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Background: Postpartum hemorrhage (PPH) is the leading cause of maternal death worldwide. The incidence of inherited bleeding disorders in women with PPH is not well established. Hence, knowing such conditions may improve the maternal prognosis.

Aims: The aim of this study is to establish the prevalence of chronic hemostasis disorders discovered in primagravida with severe immediate PPH in the Tunisian population

Methods: It is a transversal study including all primagravida parturiant in our delivery unit who presented with severe immediate PPH (estimated blood loss> 1000 ml) during a 2 years period (01/07/2017-30/06/2019). Women with known pre existant inherited blood disorders were excluded. Platelet aggregation and ATP release were performed using different agonists (ADP, Collagen, Arachidonic Acid and Epinephrin. Ristocetin was used to assess platelet agglutination. Levels of coagulation factors, including factor XIII were assessed by ROTEM analysis. All blood samples were taken at the time of the diagnosis of severe PPH and 3 months after delivery.

Results: During the study, 25 694 women delivered in our unit from whom 32% primagravida. From the 8 222 primagravida, 21% had PPH and 6% severe PPH (493 patients). The mean age of this group was 26 +/- 5.82. An inherited blood disorder was already diagnosed in 29 patients from whom only 3 were in the severe PPh group.

The prevalence of hemostasis disorders in primagravida with severe PPH in our population was 29% (96% platelet function abnormalities, 12% VWD and 12% coagulation factors deficiency). The blood sample results were concordant in each patient at the time of delivery and 3 months after in 95% of the cases. A factor VII deficiency was only noticed in 4 cases.

Conclusions: The first delivery in asymptomatic young women is a challenge for the hemostasis of the parturient and severe PPH may be secondary to a chronic unknown hemostasis disorder.

PB2553 | First Direct Evidence in Humans of a Severe Antithrombin Defect Causing Embryonic Lethality

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Background: Antithrombin deficiency is a major thrombophilia. In animal models, the absence of antithrombin is lethal. However, role of this deficiency in embryonic states in humans has only been suggested by indirect observations.

Aims: To investigate the potential association of antithrombin deficiency and embryonic lethality in humans by means of case-based analysis.

Methods: Characterization of a thrombophilic family with antithrombin deficiency and history of foetal and neonatal deaths. Plasma antithrombin was analyzed by functional anti-FXa and immunological methods (western-blot in different electrophoretic conditions). Sanger's sequencing of *SERPINC1* was performed in peripheral blood mononuclear cells, as well as in donated tissue from an early miscarriage.

Results: Clinical and molecular characterization of the thrombophilic family is shown in Figure 1. The index case is a 32-year old woman (II-3) with venous thromboembolism (VTE) and two miscarriages (G4:P2:A2:L2), who had type-I antithrombin deficiency with plasma disulphide-linked dimers. Antithrombin deficiency was caused by an intronic splicing variant (c.1154-14G>A) of paternal origin (who also had VTE) that led to an in-frame insertion of 4 residues. The second partner of the patient (II-4) was an asymptomatic carrier of a Budapest 3 variant (p.Leu131Phe). Whilst heterozygous carriers of this type-II/HBS deficiency had no history of VTE (N=7), a homozygous subject (III-7) died from perinatal aortic thrombosis. Genotypic characterization of one of the early foetal losses (III-4) revealed a

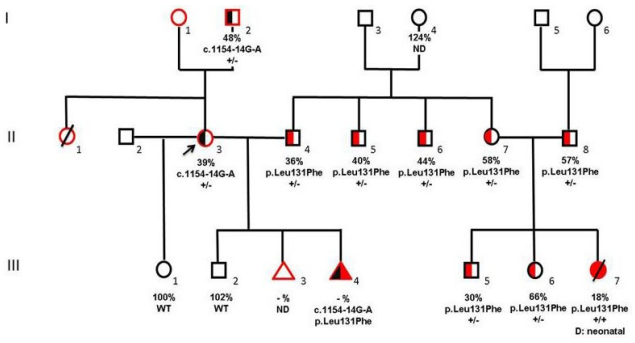

FIGURE 1 Clinical and molecular characterization of a thrombophilic family with antithrombin deficiency and foetal losses

double heterozygosity for c.1154-14G>A and Budapest 3 pathogenic variants.

Conclusions: We have characterized an exceptional thrombophilic family with an excellent genotype-phenotype association concerning antithrombin defects: from mild heterozygous type-II/HBS deficiency (asymptomatic), to severe type-I and very severe homozygous type-II/HBS deficiencies (with early and neonatal thrombosis, respectively), to the extraordinary severe double heterozygosity of these two variants, that cause embryonic lethality. Thus, this is the first direct evidence in humans that very severe antithrombin deficiency causes embryonic lethality.

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PB2554 | Pregnancy Associated Bleeding in Women with Inherited Bleeding Disorders

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Background: Women with inherited bleeding disorders are at increased risk for bleeding complications during pregnancy and the postpartum period, particularly postpartum haemorrhage (PPH). But bleeding during the preganancy is not well documented.

Aims: This retrospective study evaluates pregnancy management through the Inherited Bleeding Disorders in a tertiary Tunisian maternity, the clinical factors associated with pregnancy-related abnormal bleeding and assesses tranexamic acid use in the postpartum and postabortum prevention of bleeding in these patients.

Methods: It is a retrospective study including 100 pregnancies, from 28 women with inherited bleeding disorders. For each pregnancy, we evaluated patient characteristics (age, haemostatic factor levels) and delivery conditions (term, mode of delivery, postpartum complications and treatment) in relation to abnormal postpartum or postabortum bleeding and/ or thrombosis.

Results: This cohort revealed that 52% of pregnancies terminated at the first trimester with spontaneous abortion with an increased risk of immediate postabortum hemorrage (IPAH)with increased age at the time of abortion (mean age: 33.1 years with IPAH, 28.5 years without IPAH, P = 0.03). The incidence of severe PPH was 24% with an increased risk with birth by vaginal delivery (P = 0.04). Women treated preventively with tranexamic acid postpartum and postabortum had less severe bleeding (P = 0.04) with no thrombotic complications.

Conclusions: Pregnancy is a high risk situation of bleeding in women with inherited bleeding disorders. The first trimester is associated with a high risk of hemorragic abortion that may compromise the future fertility of the patients.

PB2555 | Women's Experience of Bleeding and Adverse Events Associated with Postpartum Pharmacological Thromboprophylaxis: A Prospective Cohort Study

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Background: Pharmacological thromboprophylaxis is commonly recommended for the prevention of postpartum venous thromboembolism.[1] The proportion of women who experience minor bleeding and other adverse events has not been well-characterized.

Aims: Describe women's experience of minor bleeding and other adverse events in the postpartum period and compare self-reported events among those who did and did not receive postpartum pharmacological thromboprophylaxis.

Methods: A prospective cohort study was undertaken at the Rotunda Hospital, Dublin. Using convenience sampling, a paper-based questionnaire was distributed to postpartum women. Women were asked about minor bleeding and other adverse effects which occurred since childbirth. All women were followed up by telephone to ascertain minor bleeding and adverse events during the first 6 weeks after discharge. Data were analysed using descriptive statistics and multivariable logistic regression.

Results: In total, 479 women completed the questionnaire during their postpartum admission (Table 1). Over 40% reported a minor bleeding or adverse event since childbirth (n=202, 42.2%). The most frequently reported events were bruising, gingival bleeding and skin rash (Table 2). Of the 320 women who completed the follow-up questionnaire, one in three reported a minor bleeding event or adverse event in the six weeks since discharge from hospital (n=107, 33.4%).

TABLE 1 Cohort characteristics

Table 1: Study characteristics

Characteristic	All women (N=479)	LMWH exposed (N=246)	LMWH unexposed (N=233)
Age ≥35 years	223 (46.6%)	135 (54.9%)	88 (37.8%)
Parity ≥3	58 (12.1%)	39 (15.9%)	19 (8.2%)
Smoker	26 (5.4%)	16 (6.5%)	10 (4.3%)
Overweight or obese	231 (48.2%)	152 (61.8%)	79 (33.9%)
Multiple pregnancy	4 (0.8%)	4 (1.6%)	0 (0%)
Thrombophilia	14 (2.9%)	13 (5.3%)	1 (0.4%)
Previous VTE or family history of Venous thromboembolism	17 (3.6%)	16 (6.5%)	1 (0.4%)
Severe medical co-morbidity	13 (2.7%)	13 (5.3%)	0 (0%)
Pre-eclampsia	5 (1.0%)	5 (2.0%)	0 (0%)
Intrauterine growth restriction	9 (1.9%)	7 (2.9%)	2 (0.9%)
Postpartum Infection	2 (0.4%)	2 (0.8%)	0 (0%)
Prolonged labour >12 hours	5 (1.0%)	5 (2.0%)	0 (0%)
Mode of delivery			
- Planned caesarean section	108 (22.6%)	87 (35.4%)	21 (9.0%)
- Emergency caesarean section	71 (14.8%)	69 (28.1%)	2 (0.9%)
- Operative instrumental delivery	78 (16.3%)	33 (13.4%)	45 (19.3%)
- Spontaneous vaginal delivery	222 (46.4%)	57 (23.2%)	165 (70.8%)
Delivery outcome			
- Live born	478 (99.8%)	245 (99.6%)	233 (100%)
- Stillborn	1 (0.2%)	1 (0.4%)	0 (0%)
Manual removal of the placenta	5 (1.0%)	4 (1.6%)	1 (0.4%)
Postpartum haemorrhage >1000ml or receipt of blood transfusion	24 (5.0%)	22 (8.9%)	2 (0.9%)

TABLE 2 Self-reported minor bleeding and adverse events

Table 2: Self-reported minor bleeding and adverse events

Self-reported minor bleeding or adverse events during postpartum admission							
	All women (N=479)	LMWH during postpartum admission (N=246)	No LMWH during postpartum admission (N=233)	OR (95%CI)	p-value	aOR (95%CI)‡	p-value
Self-reported minor bleeding	**163 (34.0%)**	**104 (42.3%)**	**59 (25.3%)**	2.2 (1.5-3.2)	<0.01	1.8 (1.0-3.2)	0.04
Bruising	70 (14.6%)	50 (20.3%)	20 (8.6%)				
Blood in stools	19 (4.0%)	10 (4.1%)	9 (3.9%)				
Hematemesis	0 (0.0%)	0 (0.0%)	0 (0.0%)				
Gingival bleeding	78 (16.3%)	46 (18.7%)	32 (13.7%)				
Epistaxis	28 (5.6%)	20 (8.1%)	8 (3.4%)				
Self-reported adverse event	**76 (15.9%)**	**56 (22.8%)**	**20 (8.6%)**	3.1 (1.8-5.4)	<0.01	1.8 (0.8-3.8)	0.14
Caesarean section or perineal wound complication	21 (4.4%)	17 (6.9%)	4 (1.7%)				
Skin rash	62 (12.9%)	45 (18.3%)	17 (7.3%)				
Allergic reaction*	5 (1.0%)	4 (1.6%)	1 (0.4%)				
Self-reported minor bleeding or adverse events in the first six weeks after discharge from hospital							
	All women (N=320)	LMWH during postpartum admission (N=161)†	No LMWH during postpartum admission (N=159)	OR (95%CI)	p-value	aOR (95%CI)‡	p-value
Self-reported minor bleeding	68 (21.3%)	41 (25.5%)	27 (17.0%)	1.7 (1.0-2.9)	0.06	1.2 (0.5-2.8)	0.66
Bruising	18 (5.6%)	12 (7.5%)	6 (3.8%)				
Blood in stools	32 (10.0%)	18 (11.3%)	14 (8.8%)				
Hematemesis	2 (0.6%)	2 (1.3%)	0 (0.0%)				
Gingival bleeding	26 (8.2%)	16 (10.0%)	10 (6.3%)				
Self-reported adverse event	61 (19.1%)	34 (21.1%)	27 (17.0%)	1.3 (0.7-2.3)	0.35	0.9 (0.4-2.1)	0.76
Caesarean section or perineal wound complication	32 (10%)	18 (11.3%)	14 (8.8%)				
Skin rash	33 (10%)	20 (12.4%)	13 (8.2%)				
Allergic reaction*	3 (0.9%)	2 (1.3%)	1 (0.6%)				

*Defined as allergic or anaphylactic reactions including symptoms such as throat or lip swelling, difficulty breathing, severe skin reactions, including skin blistering.
†Includes 23 women who received a prescription for LMWH after discharge
‡Adjusted for maternal age, parity, overweight/obesity, smoking status, severe medical co-morbidity, thrombophilia, previous VTE or high-risk family history, multiple pregnancy, mode of delivery, prolonged labour, birth outcome, postpartum infection, pre-eclampsia, intrauterine growth restriction, manual removal of

Women who received postpartum thromboprophylaxis had increased odds of minor bleeding events and adverse events during their postpartum admission, compared with those who did not receive pharmacological thromboprophylaxis (OR 2.2, 95%CI:1.5-3.2, P< 0.01 and OR 3.1, 95%CI:1.8-5.4, P < 0.01, respectively). However, when adjusted for underlying population-level differences, only self-reported minor bleeding remained significant (aOR 1.8, 95%CI 1.0-3.2, P 0.04). Similarly, no difference in the odds of self-reported minor bleeding or adverse events was observed in the first six weeks after discharge among those who received postpartum thromboprophylaxis (Table 2).

Conclusions: Women commonly report minor bleeding and other adverse effects in the postpartum period.

PB2556 | Fertility Issues in Women with Inherited Bleeding Disorders

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Background: Women with diagnosed inherited bleeding disorders have the wright to a normal reproductive life. Even if many efforts are put into the quality of life of such patients and especially regarding their menstrual blood loss, very few studies have focused on their fertility issues except for the evaluation of pregnancy related bleeding.

Aims: This transversal study aims to evaluate the knowledge, attitudes and perceptions of women with inherited bleeding disorders about fertility and contraception.

Methods: It is a transversal study including 100 non pregnant married women diagnosed with inherited bleeding disorder at our hospital and referred for a gynecological consultation. We excluded women under 18 and over 40 and women alredy under contraception. We used a 4 item chart to assess the child bearing desire of the patient, her knowledge about the pregnancy associated risks for her, her awareness of the risk of transmission of her bleeding condition to her descendence and the risks of contraception.

Results: The mean age in our cohort was 25 +/- 3.1 years. Most patients were nulligravida (68%) and only 12% had at least one child. 88% of the patients reported a desire of parenthood and of child bearing. The barriers to that were their doctor's advice against pregnancy and their fear of transmitting the illness to their descendence. Only 22% had spoken about their fertility issue with their hematologist and 18% had a previous fertility consultation. Only 5% have started a fertility treatment. But 71% had at least a previous contraception. None had a hormonal one.

Conclusions:

Fertility is a still a tabou in young women with inherited bleeding disorders in Tunisia:

PB2557 | The Attitudes of Haemophilia Carriers towards Prenatal Diagnosis and Mode of Delivery

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Background: Prenatal diagnosis (PND) is an integral aspect of care provided to carriers of haemophilia as knowledge of gender and haemophilia status of the fetus determines obstetric management.

Aims: Analysis of attitudes towards PND.

Methods: Retrospective cohort analysis.

Data collected through review of medical records of carriers of haemophilia carriers wo attended for obstetric management.

A standardiser questionnaire was sent by post to all eligible carriers.

Results: We present a 10-year (2005-2016) review of PND in carriers of haemophilia offered at the Royal Free Hospital, London.

41 carriers of haemophilia had obstetric care in 73 pregnancies. Uptake of non-invasive diagnosis (NIPT) was 90% and invasive PND was 15%. This was reduced compared to previous two decades (20% and 35% in 1985-1995 and 1995-2005, respectively). Year of pregnancy (p= 0.0010), severity of haemophilia (p= 0.004) and family history of haemophilia complications (p= 0.0012) were factors that significantly influenced uptake of invasive testing. The rate of termination of pregnancy was 1.4% (1/73);lower than 5/90 (5.5%) in the previous decade.

59 deliveries (including 13 female and 45 live male births) wee managed; mode of delivery was a planned CS in 66% and haemophilia was the primary indication in 59% cases. The CS rate was 38% in male babies compared to 14% female counterparts.

27% of carriers who responded to the questionnaire would not opt for invasive testing in pregnancy due to fear of fetal loss. All 41 respondents stated that they would opt for NIPT to confirm the haemophilia status of the fetus should this become available, and that they would find this approach less stressful.

Conclusions: The options for haemophilia are expanding with a drive towards achieving non-invasive approaches. A non-invasive test to confirm the haemophilia status of the fetus is of exceptional importance and required urgently.

PB2558 | ABO Blood Groups and Inherited Thrombophilia in Women with Abortion

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Background: The association between congenital thrombophilia and pregnancy loss has been debated in several studies with conflicting results.
Aims: To evaluate the prevalence of both non-O blood group type and inherited thrombophilia (e.g antithrombin, protein S and C deficiencies, factor V Leiden and prothrombin variant) in a group of healthy women with a personal history of miscarriage.
Methods: A group of 156 women with a personal history of abortion were enrolled among non-related caregivers of patients hospitalized in our Medical Department between June and December 2019. Seven hundred twelve healthy women without a personal history of miscarriage were recruited among blood donors as controls. Age below 18 yrs, personal history of venous and/or arterial thrombosis or acquired thrombophilia were exclusion criteria. The Odds Ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the risk of pregnancy loss.
Results: The combination of non-O group and thrombophilia was present in 25 (16%) cases and 51 (7.2%) controls (OR 3.27; CI 1.85 to 5.76); women with both O blood group and thrombophilia were 10 (6.4%) among cases in comparison to 34 (4.8%) controls (OR 1.96; CI 0.91 to 4.22). Finally, non-O group without thrombophilia was present in 73 (46.8%) cases and 307 (43.1%) controls (OR 1.59; CI 1.07 to 2.36).
Conclusions: Our results underline a significant higher prevalence in women with abortion of either non-O blood type or thrombophilia. The combination of both (e.g. non-O blood type and thrombophilia) further increases this risk. Larger studies are warranted to confirm our results and to clarify the clinical implications of our findings.

PB2560 | Hold Your Breath for 498 (Alternative) Diagnoses in Pregnant Women Suspected of Pulmonary Embolism

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Background: Diagnostic management of suspected pulmonary embolism (PE) is challenging during pregnancy because physiological pregnancy-related complaints can mimic symptoms of PE. The YEARS-algorithm has proven safe for exclusion of PE in pregnancy (Artemis study: failure rate 0.2%, 95%CI 0.0-1.2). Pulmonary embolism was present in only 4.0% of women at baseline. Little is known about the diagnoses in pregnant patients in whom PE was excluded.
Aims: To study which diagnoses were present among pregnant women with a suspicion of PE but in whom PE was ruled out.
Methods: We performed a descriptive analysis using data from the Artemis (NTR5726) study. Information on the final diagnosis was prospectively collected from medical charts combined with patients' narratives at the follow-up visit 3 months after initial suspicion of PE or after delivery. Dyspnea was classified as the predefined category 'physiological pregnancy-related complaints' if 1) no alternative diagnosis was found on imaging at time of presentation, 2) during follow-up, no other disease was diagnosed that could explain symptoms at time of PE suspicion, and 3) patient reported that complaints persisted until the end of pregnancy and disappeared after delivery.
Results: We analyzed data from all 498 patients included in the Artemis study. Final diagnoses are listed in Table 1. With increasing trimester of pregnancy, the prevalence of pulmonary embolism

TABLE 1 Final diagnoses in pregnant women with clinically suspected PE

Diagnosis, n (%)	All patients (n=498)	1st trim (n= 74)	2nd trim (n= 193)	3rd trim (n= 231)
Pulmonary embolism at baseline	20 (4.0)	5 (6.8)	8 (4.1)	7 (3.0)
Other pulmonary causes (pneumonia or respiratory tract infection ǂ, asthma and pleural effusion) (ǂ pneumonia or respiratory tract infection was one category, this was a clinical diagnosis, confirmation on imaging was not required)	90 (18.1)* (* largely driven by incidence of pneumonia or upper respiratory tract infection)	18 (24.3)	33 (17.1)	39 (16.9)
Cardiac causes (amongst others sinus tachycardia, heart failure, aortic valve stenosis)	13 (2.6)	0 (0.0)	8 (4.1)	5 (2.2)
Pregnancy-associated causes (Physiological pregnancy-related complaints, pre-eclampsia and HELLP syndrome) (HELLP; hemolysis elevated liver enzymes and low platelets)	99 (19.9)	7 (9.5)	39 (20.2)	53 (22.9)
Vasovagal syncope & anemia	12 (2.4)	3 (4.1)	5 (2.6)	4 (1.7)
Gastroesophageal reflux, epigastric pain and other abdominal causes § (§ other abdominal causes: pyelonephritis, adrenal necrosis, nephrolithiasis, cholecystolithiasis, obstipation)	29 (5.8)	4 (5.4)	11 (5.7)	14 (6.1)
Hyperventilation, anxiety or stress-related	40 (8.0)	5 (6.8)	19 (9.8)	16 (6.9)
Musculoskeletal and myalgia	69 (13.9)	12 (16.2)	27 (14.0)	30 (13.0)
Other causes ¶ or cause remained unknown (¶ Other causes: amongst others deep vein thrombosis during follow-up (only one case, 90 days after suspected PE), sickle cell crisis, viral infection other than respiratory tract infection, parietal chest pain, pneumoperitoneum, medication-induced. 14 patients had a diagnosis in category 'other causes'.)	126 (25.3)	20 (27.0)	43 (22.2)	63 (27.3)

decreased (6.8%, 4.7% and 3.0%), while the proportion of pregnancy-related causes increased (9.5%, 20.2% and 22.9%). The final diagnosis was physiological pregnancy-related complaints in 90(18.1%) patients, musculoskeletal causes or myalgia in 69(13.9%) patients and pneumonia or upper respiratory tract infection in 62(12.4%) patients.

Conclusions: Across pregnancy trimesters, the prevalence of pulmonary embolism decreased while the proportion of pregnancy-related causes increased. In pregnant women with suspected PE, one in 6 had physiological pregnancy-related complaints. Better understanding of presentation and differential diagnoses may improve the diagnostic management of pregnant patients with suspected PE.

PB2561 | Assessment of Post-partum Haemorrhage Risk among Women with Thrombocytopenia: A Cohort Study

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Background: Post-partum haemorrhage (PPH) is a leading cause of maternal death globally. Several risk factors for PPH have been

TABLE 1 Study population - demographic, clinical and gynaecological characteristics

	Platelet count < 100.000/mcl	Platelet count > 150.000/mcl
Age (mean, range)	35 (20 - 46)	35 (20 - 46)
Platelet count (*103/mcl) (mean, range)	86 (46 - 100)	237 (151 - 414)
Caucasian	73 (81%)	72 (79.9%)
Nulliparous	56 (62%)	43 (47%)
Blood Group O/non O	41 (45%) / 49 (55%)	40 (44%) / 50 (56%)
Cesarean sections / vaginal deliveries	59 (65%) / 31 (35%)	59 (65%) / 31 (35%)
PPH (number, percentage)	33 (36.7%)	10 (11.1%)
PPH among cesarean sections	22 (37.3%)	7 (11.9%)
PPH among vaginal deliveries	11 (35.3%)	3 (9.7%)

previously described, including uterine atony, retained placenta/membranes, maternal age>35y, foetal macrosomia and coagulopathies. A recent study found that thrombocytopenia conferred an increased risk of postpartum haemorrhage for caesarean or vaginal birth after a previous caesarean.

Aims: The aim of the study was to assess the PPH risk among women with platelet count below 100.000/mcl, regardless the type of delivery.

Methods: We performed a cohort study including consecutive women delivering in our Gynaecological Clinic between November 2018 to November 2019, with less than 100.000/mcl platelets at birth. Consecutive women with more than 150.000/mcl platelets were selected as controls and matched for age, type of birth and ethnicity.

Postpartum haemorrhage (PPH) was defined as ≥500mL blood loss for vaginal deliveries and ≥1000mL in caesarean sections, according to the WHO criteria. As estimates of relative risks, odds ratios (ORs) with their 95% confidence intervals (95% CI) were calculated according to the method of Woolf.

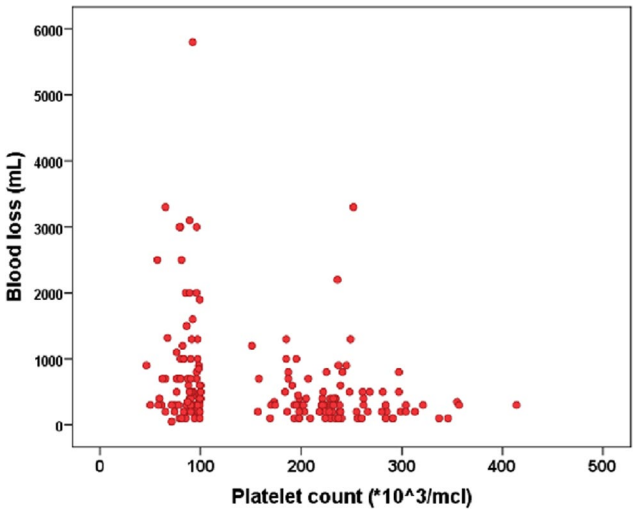

FIGURE 1 Blood loss and platelet count at birth

Results: Ninety women with less than 100.000/mcl platelets at delivery (platelet count range 46.000/mcl - 100.000/mcl) and ninety women with more than 150.000/mcl platelets were enrolled in the study. Mean age was 35 years (range 20-46). Demographic and clinical characteristics are listed in the table. The risk of PPH was 5-fold higher in women with thrombocytopenia compared to healthy controls (OR 4.6; 95% CI 2.1-10.1). When stratifying the analysis for the type of birth, the risk appeared to be slightly higher for vaginal delivery than for caesarean section (ORs 5.1 and 4.4 respectively).

Conclusions: A platelet count below 100.000/mcl appears to be a significant risk factor for PPH, and could help clinicians to identify women at higher-risk before delivery.

PB2562 | Impact of 399C/T Polymorphism of Tissue Factor Pathway Inhibitor on Risk of Recurrent Pregnancy Loss in India

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Background: Recurrent pregnancy loss (RPL) is a complex, multifactorial disease, with a frequency of 0.5-3% in all couples trying to conceive. Low TFPI level increased risk of RPL in the West. However, the association of TFPI levels with its polymorphisms and their role in Indian RPL patients have not been studied yet.

Aims: To analyse the distribution of TFPI polymorphisms, their effects on TFPI levels and risk of RPL in India.

Methods: RPL women with at least 3 consecutive pregnancy losses before 20 week's gestational age and equal number of healthy women with, at least one naturally conceived pregnancy studied. Plasma TFPI levels were determined by ELISA and its normal range determined (Mean±2SD of TFPI levels in controls). TFPI polymorphisms 287T/C, 536C/T and 399C>T were detected by PCR-RFLP and allele specific PCR respectively.

TABLE 1 TFPI levels according to genotypes of TFPI polymorphisms and risk of RPL

Polymorphisms	TFPI levels ng/ml (Mean±SD)	OR (95% CI)	ORA (95% CI)
399C/T CC CT TT	43.32±14.227 32.8 ±13.559 31.42±9.880 0.000*,0.001$,0.087#	1.0 3.384(0.63-17.99) 4.061(1.85- 8.89)	1.0 3.48(0.61-19.87) 4.72(2.02-11.01)
287T/C TT TC CC	43.77±13.15 38.89 ±14.71 37.51±13.83 0.100*, 0.109$, 0.478#	1.0 2.092(1.005-4.354) 3.254 (0.8312.74)	1.0 2.44(1.092-5.44) 3.85(0.92-16.11)

Results: 70 RPL patients, median age 34 years range (21-44 years) were recruited. Mean TFPI level was significantly lower in patients (37.21±13.86 ng/ml) than controls (48.04±13.30 ng/ml, p=0.001). Moreover, 8 patients with RPL had low TFPI (< 21.44 ng/ml), whereas no control had low TFPI. The prevalence of CT and TT genotypes of 399C>T polymorphism was significantly higher 22 (31.4%) in patients than 9 (12.8%) controls (p=0.01). 536C/T polymorphism was not detected in our studied population. The prevalence of 287T/C polymorphism was higher 19 (27.1%) in patients than 15 (21.4%) controls but difference was not statistically significant (p=0.55). Relation between the TFPI levels and their genotypes are shown in table.

Conclusions: Low TFPI level predisposes to RPL. 399C/T polymorphisms associated with low levels of TFPI and RPL. 287T/C polymorphism neither associated with TFPI level nor with risk of RPL. Thus, TFPI 399C/T polymorphisms detection may help in identifying high risk of RPL in India. A study with large sample size is required to confirm these findings.

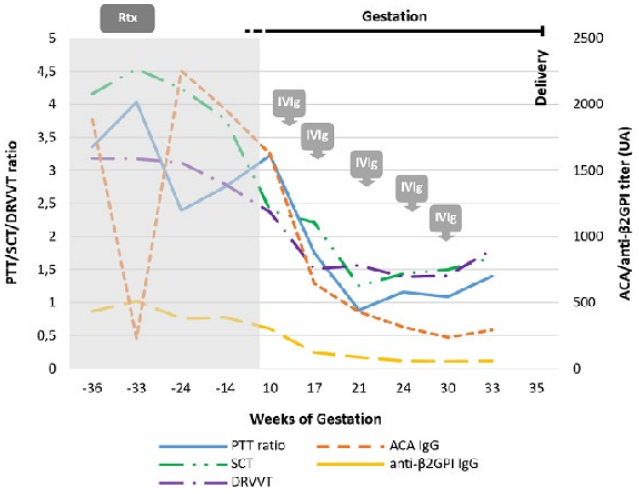

FIGURE 1

PB2563 | Rituximab before Pregnancy in Refractory Obstetrical Antiphospholipid Syndrome

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Background: Antiphospholipid syndrome (APS) is a severe acquired thrombophilia abnormality associated with an increased risk of thrombosis, recurrent miscarriage and late complications. The combined use of low-dose aspirin and low-molecular-weight heparin usually improves pregnancy outcome, but in 30% of women with obstetric APS is unsuccessful.

Aims: -

Methods: -

Results: An Egyptian 32-year-old obese (BMI 37.5) woman with triple APA at a high titer and homozygous factor V Leiden had recurrent deep vein thrombosis of the lower limbs since the age of 13 years and two early miscarriages at the age of 25 and 29 years. She arrived in Italy at the age of 30 years and referred to our Center during her third pregnancy. Since the first weeks she started enoxaparin 8,000 IU bid (body weight 102 kg), aspirin 100 mg od, intravenous immunoglobulins 400 mcg/kg for 5 days monthly and prednisone 1 mg/kg od. At week 26 she developed preeclampsia and fetal growth restriction and at week 27 the fetus died (May 2018). On Oct 2018 (off-label) rituximab 375 mg/m^2 (700 mg) once a week for four weeks was administered and the next pregnancy was planned after six months from the last administration. She had her forth pregnancy on Apr 2019 and started prophylaxis with enoxaparin 10,000 UI bid. We observed a progressive decrease of antiphospholipid antibody titer during pregnancy (figure), that ended with vaginal delivery at week 35 (Dec 2019) of a male newborn of 2,820 g, healthy despite mild immunodepression.

Conclusions: Rituximab could be a therapeutic approach in pregnant women with triple APS and previous obstetrical complications. This is the first case in which rituximab has been effectively used before a pregnancy in a patient with refractory obstetrical APS.

PB2564 | Utility of Fibrinogen Levels and Rotational Thrombo Elastometry (ROTEM) in Predicting Post Partum Hemorrhage

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Background: Post Partum Hemorrhage (PPH) is a common cause of maternal mortality. Whatever the initial etiology of PPH, dilutional

coagulopathy sets in all patients. As d-dimer values increased in the third trimester these patients are wrongly diagnosed as DIC. The major coagulation abnormally associated with PPH is acquired fibrinogen deficiency as per recent studies. Coagulation tests are time consuming and ROTEM is emerging as a point of care device in PPH.

Aims: To study correlation between fibrinogen levels and FIBTEM parameters on ROTEM in women delivering after34 weeks of gestation. The secondary objective is to study the utility of FIBTEM and fibrinogen levels in predicting PPH

Methods: 100 pregnant women in third trimester who were admitted for delivery were enrolled. Pre and post delivery (1 hour) /on onset of PPH samples were collected and studied. Tests performed -CBC, PT, APTT, fibrinogen, d-dimer and ROTEM.

Results: 16 out of 100 women suffered from PPH. The reference ranges of fibrinogen, PT, APTT, d-dimer and ROTEM parameters in pregnant women pre delivery and post delivery established. Pre and Post delivery fibrinogen < 2 mg/L was 100% predictive of PPH. Clot amplitude of FIBTEM at 5 minutes -A5 had a positive correlation with fibrinogen levels with r^2 =0.713. CFT and CT on FIBTEM had a negative correlation with fibrinogen. On the basis of ROC curves fibrinogen < 2.79g/L was found to be the best cut off to predict PPH with 92% accuracy. FIBTEM A5 values < 16.5 mm had 81% predictive accuracy for PPH.

Conclusions: Plasma fibrinogen levels and FIBTEM A5 can be used to predict PPH and to guide blood transfusion in on appropriate manner once PPH occurs. Further studies are required to validate the application of ROTEM in management of PPH.

PB2565 | Successful Pregnancy Outcomes in Patients with Protein S Deficiency Treated with Daily Fondaparinux Regimen

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Background: Physiological hypercoagulable state is a normal condition in pregnancy, but if it happened in parallel with other conditions such as thrombophilia, it will be aggravated and lead to pregnancy complications. Protein S deficiency is an inherited thrombophilia associated with recurrent pregnancy loss. The use of anticoagulants, such as fondaparinux, as treatment of recurrent pregnancy loss, is still debatable.

Aims: To investigate the effect of fondaparinux in pregnant women with recurrent pregnancy loss and protein S deficiency.

Methods: Cases of four patients with recurrent pregnancy loss and protein S deficiency were reported. All patients have experienced at least two recurrent miscarriages. There was no anatomical abnormality, medical condition such as autoimmune and metabolic disorders, and other thrombophilia. The level of protein S, d-Dimer, fibrinogen, and INR were monitored during pregnancy. Fondaparinux in a dose of 2.5 mg daily (at the same time each day) was given to all patients from the day when their pregnancies were confirmed to 24 hours before delivery. Live birth and complication as pregnancy outcomes were assessed.

Results: The mean age of our patients was 31.75 y. o. The level of protein S in all patients decreased in the third trimester. The mean of protein S in the first trimester was 38.75% and the third trimester was 29%. Meanwhile, the level of d-Dimer and fibrinogen in our patients were increased. Despite these results, we continued using fondaparinux at the same dose. All patients delivered singletons live births by cesarean section at 37-38 weeks of gestation. There was no abnormal bleeding or other complications in the mother and child.

Conclusions: Treatment with fondaparinux 2.5 mg daily might have a benefit to women with recurrent pregnancy loss and protein S deficiency.

PB2566 | Five Years of Evaluation of Postpartum Haemorrhage in a Tertiary Portuguese Hospital

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Background: Worldwide postpartum haemorrhage (PPH) remains the leading cause of maternal mortality (WHO, 2018). There are no universally accepted definitions of PPH, which vary according to the use of estimated blood loss, quantities of blood transfused or evaluation of decreases in haemoglobin.

Aims: To evaluate the prevalence of PPH and severe PPH, blood transfusion management and mortality in a Portuguese tertiary maternity.

Methods: All parturient who had requests for blood components from January 2015 to December 2019 were selected. We defined PPH when red blood cells units (RBCU) were transfused and severe PPH when the accumulated blood loss was >1000ml and/or transfusion of ≥3 RBCU.

Results: In a total of 16568 deliveries (3314/year), blood components were ordered for 608 women, mean age 32.4yr (range 16-46), with RBCU transfusion in 46% of them (PPH prevalence of 1.7%). 35 parturient received ≥ 3 RBCUs, with a severe PPH prevalence of 0.21%. Severe PPH was due mainly to uterine atony (51.4% of the cases). In severe PPH, 19 (54%) women were transfused with only RBCU, 14 with RBCU and fibrinogen; fresh frozen plasma and platelet units were transfused in 10 and 6 women, respectively. Tranexamic acid was administered to 12 women. Two women (0.012% parturient) were transfused with >10 RBCU (massive HPP) in addition to other blood components and were submitted to hysterectomy. There were no cases of death, while nine women were admitted to the ICU and 6 undergone hysterectomies.

Conclusions: We found a lower PPH prevalence in comparison to the worldwide prevalence described before. As expected, severe

PPH was associated with uterine atony. The low prevalence and the absence of PPH associated deaths mirror the importance of an early diagnosis and management, in a multidisciplinary approach, according to the established institutional protocol.

PB2567 | Family History of Preeclampsia and Hypertension: A Predictor of Preeclampsia in a Resource Limited Country

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Background: Preeclampsia is the hypertensive pregnancy disorder accompanied by proteinuria occurring after 20th weeks of gestation. Despite of well-known role of genetic inheritance in preeclampsia, limited data are reported on the familial preeclampsia in Pakistani population.
Aims: The aim of this study was to determine if family history of preeclampsia and hypertension in Pakistani population may be used as efficient and cost-effective predictor variables for preeclampsia in resource limited country.
Methods: This case control study was conducted at the Liaquat University of Medical and Health sciences (LUMHS), Jamshoro, Pakistan. A total of 250 pregnant women (125 cases and 125 controls), matched for age, parity and ethnicity were recruited after approval from the Ethical Review Committee, LUMHS. A written informed consent was taken from all the participants. All the demographic and detailed clinical data including family history and previous history of preeclampsia was recorded and pedigree drawn.
Results: In the current study, risk for preeclampsia increased to 1.74 times in women with positive family history of hypertension, whereas risk further increased to more than 7 times with positive family history of preeclampsia (Table I). The majority of the patients reported affected mother, sister and aunt in their families. The maximum numbers of member affected were 4; reported in one family involving sister, aunt, first cousin and niece. The 26.3% patients with familial preeclampsia had previous history of preeclampsia.
Conclusions: The increased likelihood of preeclampsia among women with familial hypertension and preeclampsia and previous history of preeclampsia is consistent with genetic susceptibility affecting families. Ruling out family history may serve as a predictive variable for better prevention and management.

PB2568 | Reference Ranges for Hemostasis Parameters during ¨Pregnancy and Puerperium

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Background: Pregnancy is known to be associated with significant changes in the coagulation and fibrinolytic systems. As the consequence, the reference ranges defined in healthy adult population are not optimal for ensuring proper diagnosis of both hemorrhagic and thrombotic complications in pregnant women.
Aims: To define the reference ranges for coagulation parameters during pregnancy and puerperium period in women from the Krasnoyarsk area (Siberia, Russian Federation).
Methods: Blood samples were collected once from a total of 324 women (mean age=29.5 years, range: 18-43), during a routine visit related to their pregnancy (n=261) or during puerperium (n=63), after their informed consent was obtained. None had any complication during pregnancy, delivery, or puerperium. Samples were obtained at different time of pregnancy i.e. gestational weeks 1-12 (n=35), 13-21 (n=43), 22-28 (n=66), 29-34 (n=59), and 35-42 (n=58), as well as during postpartum day 1 (n=31) and day 3 (n=32). A control group of non-pregnant women was also evaluated (n=64). Routine and esoteric coagulation parameters were performed on a fully automated analyzer ACL TOP (IL) i.e. prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time, fibrinogen, factor (F)II,FV,FVII,FIX,FX,FXI,FXII, von Willebrand factor (vWF:Ag), D-dimers, antithrombin, protein C, free protein S and plasminogen, using reagents from the analyzer manufacturer.
Results: Pregnancy was associated with significantly shortened PT and aPTT when compared to non-pregnant women. Significant changes were demonstrated for most specific parameters. Particularly, the plasma levels of fibrinogen, D-dimers, FVIII FIX, FXII and vWF:Ag significantly correlated with gestational age, whereas free PS was negatively correlated. Altogether, these results suggested a prothrombotic state du during pregnancy. Even if changes in some parameters were amplified in the first days, a trend toward normalization was observed for most parameters during puerperium.

TABLE 1 Comparison of the family history of hypertension and preeclampsia in preeclamptics and controls

Family history (FH)	Preeclamptics	Controls	Odds ratio	95% Confidence Interval	P-value
+FH of hypertension	48 (38.4%)	33(26.4%)	1.74	1.01-2.97	0.0436
+FH of preeclampsia	19 (15.2%)	3(2.4%)	7.3	2.09-25.3	0.0018

Conclusions: The use of gestational age-specific reference ranges is critical for the accurate interpretation of hemostatic tests during pregnancy and puerperium.

PB2569 | Management of Thrombocytopenia in Obstetric Patients: A Retrospective Teaching Hospital Experience

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Background: Several causes of thrombocytopenia during pregnancy have been described, being gestational thrombocytopenia (GT) the most common. Monitoring platelet counts during pregnancy is essential to improve obstetric management face to the delivery.

Aims: Analyze the experience in the management of women with thrombocytopenia during pregnancy in our Hematology Unit.

Methods: We included women with thrombocytopenia during pregnancy diagnosed in our Unit from June 2018- January 2020. Sociodemographic, clinical and analytical variables of the pregnant women have been described.

Results: 23 women with thrombocytopenia during pregnancy were referred to manage in our Hematology Unit; a diagnosis of GT was established in 16 patients. Women were referred to our Unit at 37 week of gestation, median age was 37.5 years (32-43). Media platelet count at diagnosis was 98.5 x 10^9/L (67-115), platelet count post-delivery 144.5 x 10^9/L (80-267) and media platelet number before pregnant was 162.5 x 10^9/L (115-235).

31.25% women underwent a cesarean delivery and up to 12.5% an instrumental delivery. Epidural anesthesia was administered in 8 pregnant women. 2 patients presented obstetric complications (obstetric haemorrhage), requiring platelet transfusion. Only 3 patients had a pathological puerperium (because of anemia).

Conclusions: Multidisciplinary care including differential diagnosis of thrombocytopenia, evaluation of haemorragic risk of the delivery and proper treatment is required in the management of pregnant women with thrombocytopenia during pregnancy.

PB2570 | Hemophagocytic Lymphohistiocytosis in Pregnancy Evolving towards Fulminant Hepatitis: A Case Report and Complete Literature Review

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare disorder of excessive immune activation leading to hemophagocytic activity. It is excessively rare in the peripartum period and although few cases have been reported, no complete literature review is available. We describe the case of a 34-year-old healthy woman at 31 weeks' gestation presenting with fever and cytopenia, who developed fulminant hepatitis. Liver biopsy confirmed the diagnosis and she was treated with Decadron and Etoposide, which was changed to Anakinra to allow breastfeeding.

Aims: To determine presenting signs, symptoms, underlying etiologies and possible therapies for HLH in pregnant women. To determine maternal and fetal outcomes and establish diagnostic and therapeutic distinctions during the peripartum period.

Methods: We conducted a review of the literature of published cases of HLH in pregnancy using PubMed and cross-referenced each result. 48 cases were included, published between 1994 and 2019. Maternal characteristics, associated diagnoses, presenting symptoms, treatment, delivery and fetal outcomes were evaluated.

Results: Women diagnosed with HLH had a median age of 30 years old and a median gestational age of 22 weeks. 3 women were postpartum and 3 had a twin pregnancy. While chief complaints and investigations were variable, most common features included fever (95.8%) and cytopenia (100%). 25% had a non-contributory bone marrow biopsy. 87.5% of women received steroids and 27.1% Etoposide, the latter following delivery or termination of pregnancy. 41.7% had an infectious etiology and 20.8% an autoimmune disorder. 25.5% of patients died from complications of the disease. Fetal demise occurred in 25.6%, commonly because of prematurity or non-viability.

Conclusions: HLH is a challenging, life-threatening diagnosis in pregnancy. Treatment considerations include maternal health, fetal prematurity and treatment toxicity. Although safety data is lacking, Anakinra has been successfully used in pregnancy and provides a promising alternative to the current mainstay of therapy.

PB2572 | Management of Pregnancy in Severe Factor V Deficiency in Women with Varying Phenotypes

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Background: Severe Factor V deficiency is a rare disorder and literature on management of pregnancy is limited. We report on four pregnancies in two such women registered at our Centre.

Aims: The two patients with severe factor V deficiency had very different phenotypes and presentation. They both required very different approaches to the management of their pregnancies.The aim of this abstract is to shed light on the varying presentation and management-strategies that were required in four pregnancies in the two women.

Methods: Both women had no clear family history of factor V deficiency.

The first woman presented in her mid-20s and had three consecutive-pregnancies, none of which were complicated by antepartum-haemorrhage. In order to cover delivery she was given virally inactivated octaplas at onset of labour, and a pool of platelets for intra-platelet content of factor V. Epidural/spinal analgesia was not permitted. She also required tranexamic acid postpartum for 2 weeks.

The second woman who was in her late 20s had no previous history of bleeding and no surgical challenges. However, she presented on multiple occasions with significant antepartum haemorrhage which required hospital admission. On a couple of occasions the bleeding was severe enough to require cover with octaplas and tranexamic acid. On three other occasions the antepartum-haemorrhage episodes were managed with tranexamic acid alone.

Results: The three pregnancies in the first woman resulted in three vaginal deliveries of normal infants. Although she did not have bleeding she had severe back pain and symphysis pubis pain which were unrelated. The sole pregnancy in the second woman resulted in a normal vaginal delivery.

Conclusions: The clinical phenotypes of pregagncies can differ in severity despite similar factor V levels. Individualised treatment plans and close liaison is required between obstetricians, haematologists and gynaecologists to manage these complex pregnancies.

PB2573 | Optimizing Tranexamic Acid Dosing for Hemorrhage Prevention in Pregnant Women

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Background: International trials support the efficacy of tranexamic acid (TXA) for early treatment of severe hemorrhage. However, the optimal dose of TXA for hemorrhage prevention at delivery is unknown and often extrapolated from non-pregnant populations.

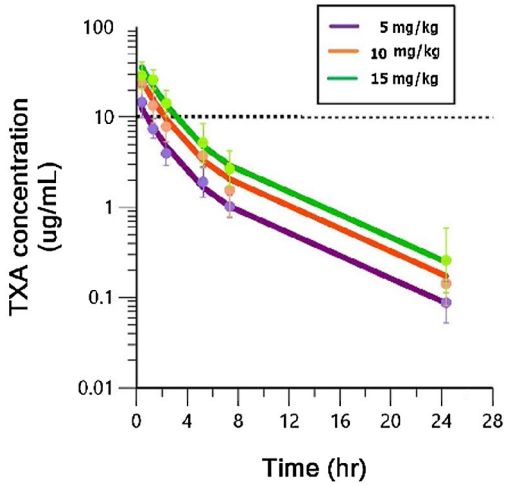

Figure 1 Observed mean (symbols) and population mean (lines) TXA concentration-time profiles after 15 mg/kg, 10 mg/kg and 5 mg/kg IV doses derived from 30 patients. The doses are normalized to a 70 kg patient. TXA concentration at 10 ug/mL is shown as the reference line. The error bars (1SD) are also shown.

FIGURE 1 PK data

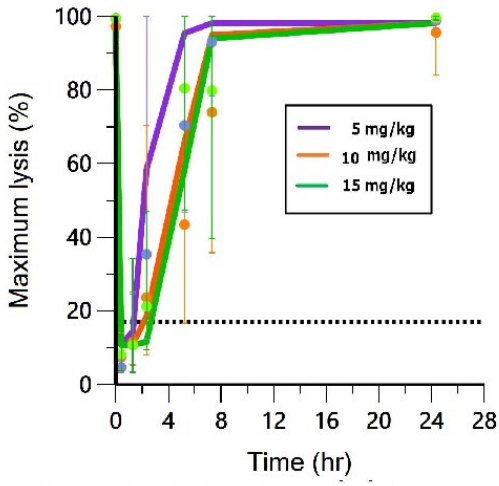

Figure 3 Observed mean (symbols) and population mean (lines) of maximum lysis-time profiles after 15 mg/kg, 10 mg/kg and 5 mg/kg IV doses derived from 30 patients. Maximum lysis at 17% is shown as the reference line. The error bars (1SD) are also shown.

FIGURE 2 PD data

Aims: Since pregnant women and newborns are particularly vulnerable populations, our objective was to determine the minimum TXA dose that effectively inhibits clot lysis.

Methods: We enrolled 30 pregnant women undergoing scheduled cesarean delivery in an open-label, nonrandomized, dose escalation study. Subjects were divided into 3 cohorts receiving 5, 10 or 15 mg/kg (max 1g) of intravenous TXA at time of umbilical cord clamping. Inclusion criteria were >34 wks gestation and normal renal function. Primary end points were pharmacokinetic and pharmacodynamic profiles. Rotational thromboelastometry (ROTEM) of tissue plasminogen activator-spiked samples was used to evaluate pharmacodynamic profiles 10 min, 30-60 min, 1.5-4 hr, 4-6 hr, 7-8 hr and 24 hr

after TXA administration. Safety was assessed by plasma thrombin generation, D-dimer, and TXA levels in breast milk.

Results: There were no serious adverse events including hemorrhage or venous thromboembolism. Plasma concentrations of TXA increased in a dose-proportional manner (Figure 1). Plasma TXA exceeded 10 μg/mL more than 1 hr after administration for all TXA doses tested. Maximum lysis was less than 17% for a majority of women, including all women receiving 5 mg/kg (Figure 2). Median estimated blood loss for cohorts receiving 5, 10, or 15 mg/kg TXA was 750, 750 and 700 mL, respectively. Plasma thrombin generation and D-dimer were not significantly different in any cohort. Breast milk concentrations of TXA were 1% or less than maternal plasma concentrations.

Conclusions: TPA-modified ROTEM enables assessment of TXA pharmacodynamic responses in real time. A single dose of approximately 0.5 g of intravenous TXA is sufficient to safely inhibit fibrinolysis for more than 1 hr after administration.

PB2575 | Role of Thrombin Generation Potential (TGP) in Cesarean Sections and Vaginal Deliveries Puerperium to Guide Thromboprophylaxis. Preliminary Results

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Background: Venous Thromboembolic Disease (VTD) is the leading cause of maternal mortality in the developed countries. The absolute risk of VTD in pregnancy is low so the risk needs to be stratified to justify pharmacological thromboprophylaxis[1].Caesarean section (CS) causes endothelial damage [2,3], it has five to nine times higher thrombosis risk than vaginal delivery (VD) [4,5,6,7].TGP could identify CS patients who would have benefit from thromboprophylaxis.

Aims: To determine and compare TGP in VD vs CS.

To evaluate TGP as an additional risk marker to guide thromboprophylaxis in CS.

Methods: We performed a cross-sectional observational analytical study at Pereira Rossell Hospital (Montevideo, Uruguay), between October 2018 and August 2019. Individual consent was taken. It was approved by institutional ethics committee.TGP was measured in the automated BCS® XP coagulation analyzer.

TABLE 1

Gestation	CS	VD	Total
1	34	51	85
2	18	43	61
3	12	27	39
4	3	12	15
5	1	6	7
6	2	4	6
7	-----	3	3
8	-----	1	1
9	-----	2	2
11	-----	1	1
Total	70	150	220

TABLE 2

	CS (media +/- SE)	VD (media +/- SE)	P value
Age (years)	25,7 +/- 0,7	25,6 +/- 0,5	0,903
BMI (kg/m²)	29,2 +/- 0,7	28,9 +/- 0,5	0,763
ETP AUC2 (ME)	577,72 +/- 20,75	419,35 +/- 8,02	< 0,001(*)
ETP AUC2 CAL	122,78 +/- 3,24	113,25 +/- 2,02	0,010(*)
ETPB CMAX	117,21 +/- 3,54	113,02 +/- 1,42	0,197
ETPB CAL	117,14 +/- 2,89	114,86 +/- 1,35	0,419

(*): significant values. SE: standard error.

Results: We studied 220 pregnant women, 70 CS and 150 VD. Characteristics of pregnancy and type of delivery are described in table 1.Women age, BMI and results of TGP are described in table 2. No differences were observed between maternal age and BMI in the two groups. The AUC2 ETP (ME) was statistically lower in group VD, p value < 0.001. The ETPB AUC2 CAL was statistically lower in group VD, p value p = 0.010.

Conclusions: TGP was statistically higher in patients with CS. Caesarean section was identified as a thrombin generating event associated with the greater endothelial damage it produces. Therefore, it could be an interesting biomarker for identify patients with a higher risk of VTD.