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Letter to the Editor

Decline in antibody responses to SARS-CoV-2 post-vaccination poses a risk to health care workers

Dear Editor,

We read with interest the article published in this journal by Pezzati et al. on the use of rapid lateral flow assays to detect antibodies induced by vaccination against SARS-CoV-2 infection. The authors concluded that a number of rapid lateral flow assays were useful in a qualitative assessment of vaccine responses [1]. SARS-CoV-2 caused the global COVID-19 outbreak originating in the Wuhan Province of China in late 2019 [2]. The first case of COVID-19 in South Africa was reported on 5 March 2020 [3], and the World Health Organization (WHO) declared a global pandemic on 11 March 2020 [4]. As of 25 February 2022, South Africa had >3.6 million laboratory-confirmed COVID-19 cases [5]. South Africa experienced several waves of the pandemic, owing to infection with different SARS-CoV-2 variants of concern (VOC), namely the Wuhan B.1 lineage variant (infection peak between June - August 2020), the Beta variant (infection peak between November 2020 - February 2021), and the Delta variant (infection peak between May - September 2021). The Omicron variant was first identified in South Africa and Botswana in mid-November 2021 [6], just before participants were recruited into this study. Omicron rapidly became the dominant infective variant in South Africa.

Health care workers (HCWs) are at high risk of exposure to COVID-19. In February 2021, South Africa, through the Sisonke phase 3B trial, began vaccinating HCWs against COVID-19 with the Johnson & Johnson vaccine [7]. Other vaccines, including the Pfizer Comirnaty and Oxford/Astra-Zeneca were also subsequently approved by the South African Health Products Regulatory Authority (SAHPRA). These vaccines are effective in preventing severe disease and hospitalization, however mild to moderate infections still occur. In a recent report, ~8.5% of HCWs vaccinated in the Sisonke trial contracted SARS-CoV-2 post-vaccination, with over 43% of infections caused by the Omicron VOC [8]. Breakthrough infections in HCWs have been reported in other studies, with participants being either asymptomatic or with mild to moderate symptoms [9]. A number of reports show waning antibody levels in both previously infected and vaccinated individuals [10,11], although the rate of decline varies amongst individuals.

The humoral response to SARS-CoV-2 infection is not clearly defined, though it is reported that most infected individuals produce Immunoglobin M (IgM) antibodies from 4 days post-symptom onset which peak between day 14 and 21 before declining. Immunoglobin G (IgG) levels start to rise between 7 and 14 days, although it is unclear how long these IgG antibodies are sustained [12]. In individuals vaccinated with the BNT162b2 Pfizer vaccine, antibody levels peaked between 4 and 5 weeks after the initial dose and decreased thereafter. After the second vaccine dose, antibodies were detected in >90% of participants but, again, decreased over time, especially in older recipients [11]. Similar trends in waning antibody levels have also been observed with other COVID-19 vaccines, with rapid decline in antibody levels reported in patients with obesity, autoimmune diseases, and other chronic inflammatory conditions [13]. While antibodies are not the only indication of protection against COVID-19, the presence of antibodies does decrease the risk of infection [14].

A number of laboratory-based assays are able to detect antibodies against SARS-CoV-2. Lateral flow immunoassays (LFIA) are rapid tests where a patient specimen (either whole blood, serum, or plasma) is placed onto a cassette containing the analyte of interest. If the patient has developed antibodies to SARS-CoV-2, the IgM and/or IgG test lines will change color indicating a positive result. LFIAs can be used in remote sites or point-of-care (POC) settings, as they require no other laboratory equipment, and can be performed by less skilled personnel.

We aimed to assess the seroprevalence of IgM and IgG antibodies to the spike protein of SARS-CoV-2 in HCWs using the Orient Gene COVID-19 IgG/IgM Rapid Test Cassette which detects antibodies to the N-terminal of the spike protein (S1) of SARS-CoV-2 [15]. We recruited HCWs at or affiliated with the University of Pretoria in South Africa between November 2021 and March 2022. We asked participants to provide written informed consent, to answer some questions relating to COVID-19 vaccination status and prior infection, and drew blood samples from them. This study was approved by the Human Research Ethics Committee at the University of Pretoria (approval number 680/2021).

One drop of whole blood was placed onto the cassette with 2 drops of buffer. Those that reacted with either the IgG or IgM band or both were considered seropositive. Data were analyzed using GraphPad Prism 9.3.1 for Windows. The participant characteristics, previous SARS-CoV-2 infection, vaccine data, and seropositivity are summarized in Table 1. Of the 203 total participants, the median age was 35 years (IQR 27–50), 160 (78.8%) were female, and 38.4% reported previous COVID-19 infection. Of the total participants, 195 (96%) were vaccinated, while 8 (4%) were unvaccinated. In total, 82.3% of the participants tested positive for antibodies to SARS-CoV-2.

The vaccinated participants (195) had a median age of 35 years (IQR 27–50), 79% were female, and 83.1% of this group tested positive for SARS-CoV-2 antibodies. Antibodies were tested on average 41 weeks (IQR 34–44) post vaccination. Thirty-six percent (36%) of this group reported testing positive for COVID-19, with 67% of infections occurring at a median of 24 weeks (IQR 17–33) post-vaccination. All participants who had COVID-19 tested antibody positive. Of the vaccinated participants who did not report previous COVID-19 (122), 74.6% were antibody positive. The majority of the vaccinated participants received the Johnson & Johnson vaccine (77.9%), while fewer received Pfizer (21%) or Astra-Zeneca

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Table 1
Study population characteristics.

			Overall	Antibody Negative	Antibody Positive
All participants, n (%)	Total		203	36 (17.7%)	167 (82.3%)
	Gender, n (%)	Female	160 (78.8%)	23 (63.9%)	137 (82%)
		Male	43 (26.9%)	13 (36.1%)	30 (18%)
	Age, median [IQR]		35 [27–50]	29 [24–42]	37 [28–50]
	Past COVID-19	COVID-19 positive	78 (38.4%)	4 (11.1%)	74 (44.3%)
	infection, n (%)	COVID-19 negative	125 (61.6%)	32 (88.9%)	93 (55.7%)
Vaccinated, n (%)	Total		195	33 (16.9%)	162 (83.1%)
	Gender, n (%)	Female	154 (79%)	20 (60.6%)	134 (82.7%)
		Male	41 (21%)	13 (39.4%)	28 (17.3%)
	Age, median [IQR]		35 [27–50]	29 [25–39]	38 [29–50]
	Vaccine type, n(%)	J&J	152 (77.9%)	33 (21.7%)	119 (78.3%)
		Pfizer	41 (21%)	0	41 (100%)
		Astra-Zeneca	2 (1%)	0	2 (100%)
	Past COVID-19	COVID-19 positive	71 (36.4%)	0	71 (100%)
	infection, n (%)	COVID-19 negative	122 (62.6%)	31 (25.4%)	91 (74.6%)
Unvaccinated, n (%)	Total		8	3 (37.5%)	5 (62.5%)
	Gender, n (%)	Female	6 (75%)	3 (50%)	3 (50%)
		Male	2 (25%)	0	2 (100%)
	Age, median [IQR]		32 [24–44]	42 [32–47]	30 [24–33]
	Past COVID-19	COVID-19 positive	5 (62.5%)	0	5 (100%)
	infection, n (%)	COVID-19 negative	3 (37.5%)	3 (100%	0

(1%). There were 8 unvaccinated participants of whom 6 were female. The median age was 32 years (IQR 24–44). Five participants reported prior COVID-19 infection and all had positive antibody tests. Those who had not had COVID-19 also did not test positive for antibodies.

Of interest, one vaccinated participant who tested positive for antibodies, reported testing positive for COVID-19 during the second, third, and fourth waves of the pandemic. This individual reported having an unspecified autoimmune disease. This suggests that the antibody-mediated defenses induced by vaccination with vaccines designed against the wild-type strain may offer suboptimal protection in certain autoimmune conditions.

These results show that 18% of healthcare workers tested in this study did not have detectable antibodies to SARS-CoV-2. All participants without prior vaccination or infection were sero-negative as were a quarter of those previously vaccinated but uninfected. Such HCWs should be prioritized for vaccination and booster doses due to their ongoing, and potentially high-risk, exposure to the pathogen.

We acknowledge that there are several limitations to this study. Firstly, the study population is a convenient sample of HCWs within a specific geographic location and may not be representative of the general population. Secondly, LFIAs are qualitative, and levels of antibodies cannot be determined. We aim to confirm these results with quantitative assays. Thirdly, most of these antibody tests were conducted several months after vaccination. It is therefore difficult to determine in seronegative participants if antibodies were induced initially at all, and whether they declined over time. In addition, we do not know whether the antibodies detected are protective against future infection, or whether antibodies induced by the vaccine are protective against different variants. Lastly, we only looked at antibodies against SARS-CoV-2, and did not assess their functionality or take immune responses induced by other immune cells (e.g. T cells) into consideration. We aim to explore these responses further in subsequent studies.

In conclusion, antibodies to SARS-CoV-2 can be induced by both prior infection and vaccination. All participants with previous COVID-19 were seropositive, irrespective of vaccination status. However, only 83.1% of the vaccinated participants with a history of COVID-19 and just 74.6% with no such history, were seropositive. This indicates that, while vaccination successfully induces antibodies to SARS-CoV-2 which persist several months after vaccination in 3 out of 4 participants, 25% are potentially unprotected. Regular antibody surveillance is important to assess the longevity of vaccine responses. Regular booster vaccination may be needed to increase antibody-mediated protection in certain individuals.

Declaration of interests

None.

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Single-cell RNA sequencing analysis of liver reveals the enhanced entry and release abilities of human adenovirus F41, partially explaining acute hepatitis in children

Dear Editor,

In this Journal, we described the incidence of pertussis, scarlet fever and hand-foot-mouth disease in China during Corona Virus Disease 2019 (COVID-19) pandemic.[1] Besides the change of incidence spectrum of infectious diseases, emerging and reemerging infectious diseases, such as acute hepatitis of unknown etiology in children and monkeypox, have occurred recently. The outbreak of acute hepatitis in children has been pointed to human adenovirus F41 (HAdV-F41) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[2] However, whether liver expresses host factors facilitating HAdV-F41 entry and release remains inconclusive. Moreover, whether SARS-CoV-2 is involved in the occurrence of this acute hepatitis of unknown etiology in children is still uncertain.

Interactions between the knob domain of the fiber capsid protein and host cell receptors are crucial for HAdV entry. Unlike other types of HAdVs, HAdV-F41 possesses a long fiber and a short fiber. The long fiber could bind to the Coxsackievirus and adenovirus receptor (CXADR), whereas the short fiber could interact with heparan sulfate (HS).[3] Additionally, the penton base of HAdV-F41 lacks the arginine-glycine-aspartic acid (RGD) motif, while RGD exists in other types of HAdVs for their association with RGD-binding integrins (integrin AVB1, AVB3, AVB5, AVB6, AVB8, A5B1, A8B1 and A2BB3) to facilitate viral entry. Instead, laminin-binding integrins (integrin A1B1, A2B1, A3B1, A6B1, A6B4 and A7B1) play an important role in HAdV-F41 virus entry by associating with its penton base.[4] N-Deacetylase and N-Sulfotransferase-1 (NDST1) is a key enzyme in HS biosynthesis, which replaces the N-acetyl groups at selected N-acetylglucosamine residues with sulfate groups. Heparanase (HPSE), the only known enzyme capable of degrading HS chain, could shed HS on the cell surface, and consequently increase virus release.[5] However, heparanase 2 (HPSE2) is a close homolog of heparanase that protects HS from shedding, which functions as an endogenous inhibitor of HPSE.[5] Interestingly, plasma HPSE activity and HS levels are elevated in COVID-19 patients, which indicates severer diseases.[6]

To investigate the mechanisms involved in the severe acute hepatitis in children, we took advantage of the Single-cell RNA sequencing (scRNA-Seq) data in cells derived from adult and fetal livers because children liver data is not available. Human scRNA-seq datasets from liver were obtained from Gene Expression Omnibus (GEO) under accession codes GSE134355.[7] After quality control and data normalization, 28,087 cells in adult liver and 18,042 cells in fetal liver were analyzed. Uniform Manifold Approximation and Projection (UMAP) was used for dimensional reduction and we clustered the 10 cell types through R package, Seurat v3.2.3[8] (Fig. 1A and B).

Next, we examined the transcription levels of potential host factors for HAdV-F41 including CXADR, NDST1, HPSE, HPSE2 and integrins in the liver of adults and fetuses. As shown in Fig. 1C, CXADR and NDST1 were co-expressed in most types of cells except stem cell and hematopoietic cell in adult liver. In fetal liver, CXADR transcripts were detected in every cell type but mesenchymal; in terms of NDST1, however, only minor expression was detected in 7 cell types of fetal liver, indicating less synthesis of HS in fetal liver than that in adult liver (Fig. 1C). Interestingly, HPSE was highly expressed in adult liver but seldom expressed in fetal liver, while HPSE2 expression in both livers illustrates a reverse pattern. Furthermore, there was less expression of potential RGD binding subunits of integrins (integrin AVB1, AVB3, AVB5, AVB6, AVB8, A5B1, A8B1 and A2BB3) and laminin-binding integrins (integrin A1B1, A2B1, A3B1, A6B1, A6B4 and A7B1) in fetal liver compared with that in adult liver (Fig. 1C). Taken together, both adult and fetal liver express host factors of HAdV-F41 entry and release including CXADR, NDST1, HPSE and integrins, which could support HAdV-F41 entry and release from liver. These data could partially represent the scenario in liver of children.

In the acute hepatitis recently reported, children firstly underwent an enteric symptom, which is consistent with the pathology of HAdV-F41 infection. Since HS specific glucuronidase HPSE is upregulated after SARS-CoV-2 infection,[6] the elevated HPSE would result in more shedding of HS to promote HAdV-F41 release. Besides, HAdV-F41 penton base utilizes laminin-binding integrins instead of RGD-binding integrins to facilitate virus entry. Interestingly, SARS-CoV-2 spike (S) protein possesses an RGD integrin-binding motif in its receptor binding domain.[9] SARS-CoV-2 S could induce endothelial inflammation through integrin $\alpha 5\beta 1$ signaling.[10] Therefore, we

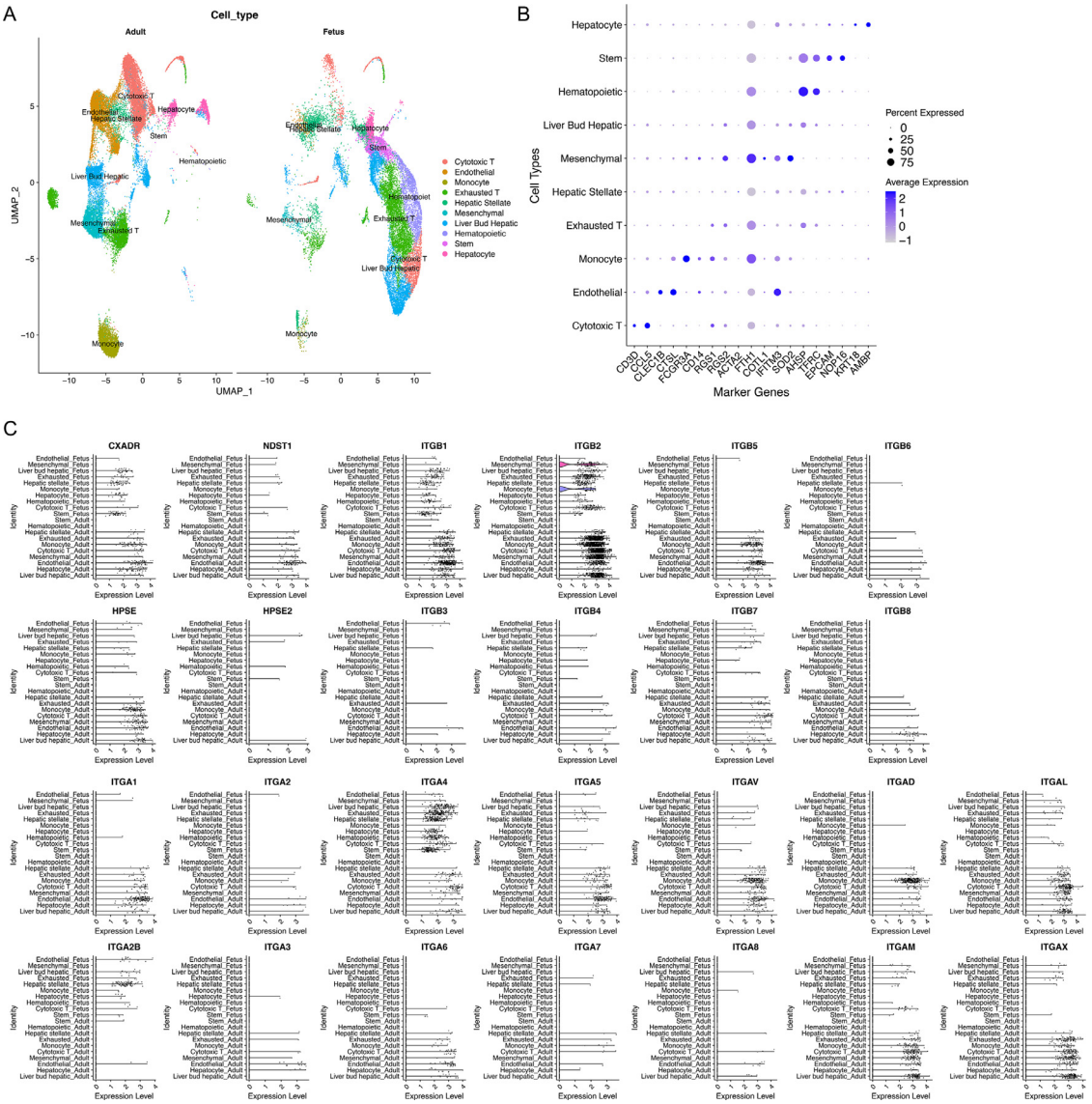

Fig. 1. scRNA-Seq data analysis of HAdV-F41 entry and release factors in human livers. (A) UMAP plot illustrating cell clusters identified from adult livers and fetal livers. Each dot represents an individual cell, colored by cell type. (B) Bubble plots showing expression levels of cell-type marker genes. The shadings denote average expression levels. The sizes of the dot are directly proportional to the percent of cells expressing the gene in a given cell type. (C) Violin plots showing the expression of CXADR, NDST1, HPSE, HPSE2 and subunits of integrins in adult and fetal livers.

speculate that there is synergism between HAdV-F41 and SARS-CoV-2 to activate more integrins for inflammation, which eventually causes severe acute hepatitis. Additionally, although both adult and fetus could be infected by HAdV-F41, the adult symptom would be mild or undetectable due to the maturation of their immune system. Taken together, HAdV-F41 and SARS-CoV-2 tend to work together to cause acute hepatitis in children.

In summary, we propose that potential entry and release factors expressed in both fetal and adult livers could lead to the incidence of HAdV-F41 infection. Upon SARS-CoV-2 infection, the release ability of HAdV-F41 will be enhanced due to the elevated HPSE activity. Moreover, SARS-CoV-2 S might work with HAdV-F41 penton base to activate a variety of integrins, leading to severe liver diseases. Notably, the information presented in this study was derived from scRNA-seq data in fetal and adult livers, which could approximately represent the children livers. In the future, it will be interesting to focus on children livers to elucidate the potential mechanisms of the incidence of HAdV-F41 infection in children, providing more clues to understanding acute hepatitis in children recently reported.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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The efficacy and safety of IBI314 on delta and omicron variant of SARS-CoV-2: First-in-human evidence

Dear Editor,

We have read with great interest the study by Martin-Blondel et al.[1] Reporting the outcome of neutralizing antibodies in Omicron and Delta-infected patients. Currently, there is few specific treatments recommended for COVID-19 particularly its variant strains, most of which are under emergency use authorization (EUA).[2–4] We generated potent SARS-CoV-2 neutralizing antibodies, IBI314A and IBI314B, against distinct and non-overlapping epitopes on the S protein from convalescent patients.[5] Herein, we reported the efficacy and safety of IBI314, consisting of 2 antibodies IBI314A and IBI314 at a 1:1 ratio, in two cohorts of Chinese COVID-19 patients who were infected with the Delta and Omicron variants.

Since November 2021, COVID-19 outbreaks caused by variant of SARS-CoV-2 emerged in multiple locales in China and is still ongoing. Two cohorts of patients infected with the variant of SARS-CoV-2 from Qinghai and Henan Province were hospitalized and given IBI314, intravenously at a dose of 1500 mg under compassionate use. Oropharyngeal and nasopharyngeal swabs were obtained prior to, on the day of (Day 0), and after IBI314 administration. Blood samples were collected for laboratory tests, including oxygenation index (PaO2/FiO2), peripheral oxygen saturation (SpO2), C-reactive protein (CRP), IgG and IgM. CT scans were evaluated by two independent radiologists. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was used and provided a cycle threshold (Ct) value, which is the number of cycles required for the fluorescent signal to cross the threshold for a positive test. A Ct value is a surrogate for viral load. A Ct value$>$40 denotes a negative test and a CT value$\leq$40 denotes a positive test.

A total of 61 cases included 10 cases from Qinghai Province and 51 cases from Henan Province received the compassionate use of IBI314. Table 1a and b summarized the baseline characteristics of patients in these two cohorts. Notably, there were 40% and 27.5% of severe cases in Qinghai and Henan cohort, respectively. Patients' symptoms and comorbidities reported at the time of hospitalization were described in Supplementary Tables 1 and 2.

Ct values of N gene and O gene of oropharyngeal and nasopharyngeal samples were plotted (Fig. 1A, B and 1G, H). After administration, the change of Ct values of both N and O genes exhibited a similar pattern, with a trend of increasing in Ct values, indicating a gradual viral clearance in all patients of both cohorts. A pooled analysis of partial pressure arterial oxygen (PaO2)/fraction of inspired oxygen (FiO2) referred to as oxygenation index (P/F) was performed in Qinghai cohort. A trend of increase in P/F was observed (Fig. 1C). Moreover, this indicator was replaced by SpO2, because of the incomplete data of patients' oxygenation index (P/F) in Henan cohort, and showed a stable trend (Fig. 1I). Furthermore, we used CRP to reflect the level of inflammation. After the treatment of IBI314, 71.43% (5/7) and 30.77% (8/26) of patients converted to a normal CRP level, from an abnormal value prior to administration in the Qinghai and Henan cohort, respectively (Fig. 1D and J). We also investigated the level of specific IgG and IgM to SARS-CoV-2. In comparison to the baseline median level of 3.77S/CO and 190.89 S/CO in Qinghai and Henan cohort respectively, the level of IgG was drastically increased in almost all patients after treatment,

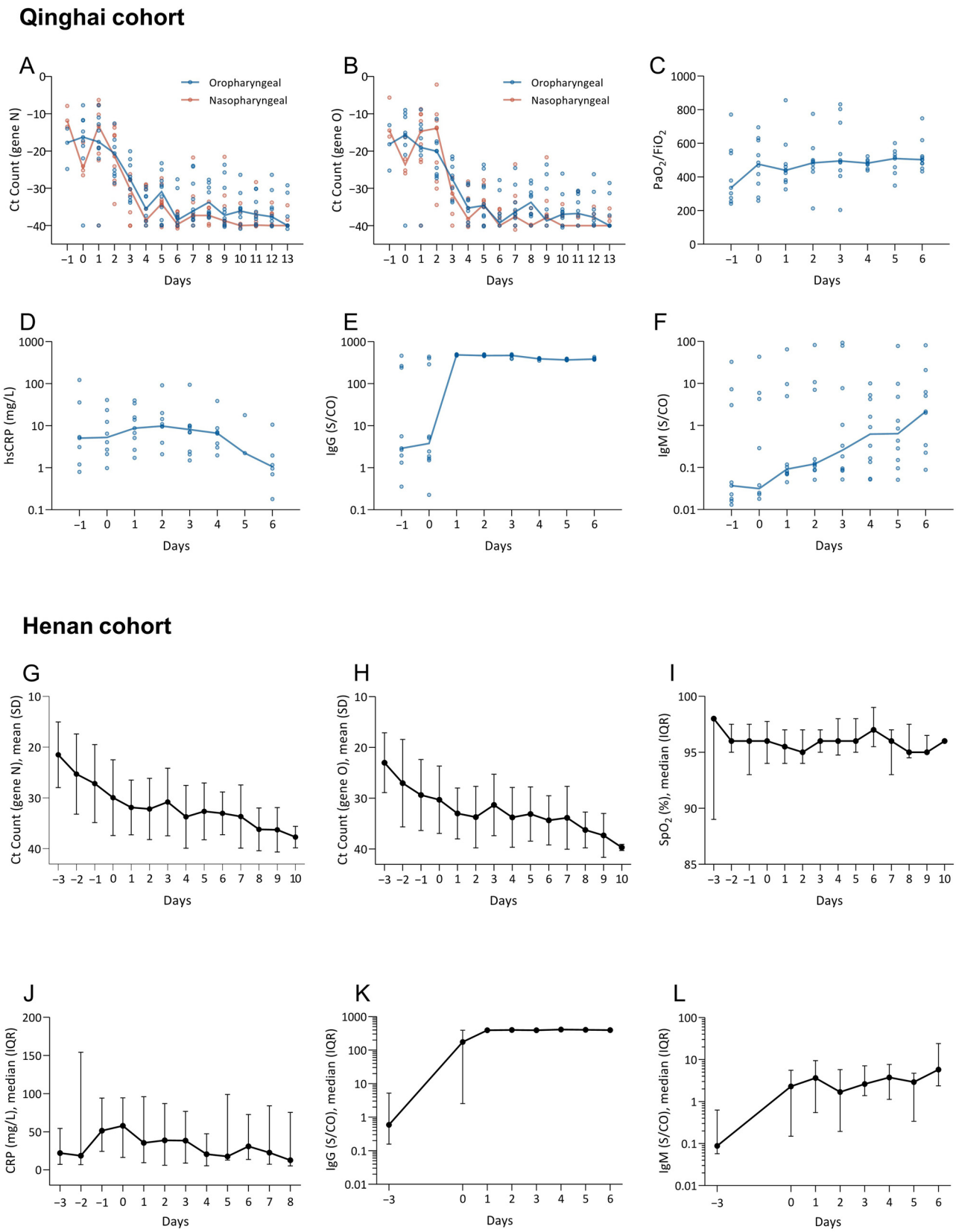

Fig. 1. Qinghai cohort: Changes of N gene (A), O gene (B), PaO2/ FiO2 (C), High sensitivity -CRP (D), IgG (E) and IgM (F) were evaluated prior to, on the day of (Day 0), and after IBI314 administration. Y-axis denotes the level of specific parameter measure and X-axis denotes the time in relation to IBI314 administration. Medians of various indicators in each study day were connected by lines.

Henan cohort: Changes of N gene (G) and O gene (H) in oropharyngeal swabs, SpO2 (I), CRP (J), IgG (K) and IgM (L) were evaluated prior to, on the day of (Day 0), and after IBI314 administration. Y-axis denotes the level of specific parameter measure and X-axis denotes the time in relation to IBI314 administration. Medians (or means) of various indicators in each study day were connected by lines.

Table 1a
Patient demographics and baseline characteristics in Qinghai cohort.

	All Cases(N = 10)	Mild(N = 2)	Moderate(N = 4)	Severe(N = 4)
Gender, n (%)				
Male	1 (10%)	0 (0%)	0 (0%)	1 (25%)
Female	9 (90%)	2 (100%)	4 (100%)	3 (75%)
Age (years)				
Mean ± SD	41.9 ± 16.64	31.5 ± 0.5	31 ± 2.55	58 ± 15.92
Median	33	31.5	31.5	58.5
Min: Max	27:80	31:32	27:34	35:80
Age group, n (%)				
≤ 65 years	9 (90%)	2 (100%)	4 (100%)	3 (75%)
> 65 years	1 (10%)	0 (0%)	0 (0%)	1 (25%)
Vaccination to disease onset (days)				
Mean ± SD	194.78 ± 88.54	245 ± 57	240 ± 77.62	101 ± 10.71
Median	170.5	245	278.5	99
Min: Max	89:302	188:302	107:296	89:115
Symptom onset to treatment (days)				
Mean ± SD	5.8 ± 3.31	3.5 ± 1.5	3.75 ± 1.79	9 ± 2.45
Median	5	3.5	3.5	10
Min: Max	2:11	2:5	2:6	5:11

*The most severe stage experienced by patient was used as the diagnostic type (mild, moderate, severe).

Table 1b
Patient demographics and baseline characteristics in Henan cohort.

	All Cases (N = 51)	Mild (N = 12)	Moderate (N = 25)	Severe (N = 14)
Gender, n (%)				
Male	21(41.18%)	5(41.67%)	10(40%)	6(42.86%)
Female	30(58.82%)	7(58.33%)	15(60%)	8(57.14%)
Age (years)				
Mean ± SD	67.63±17.21	70.25±17.35	60.08±16.25	78.86±12.05
Median	69	73.5	65	81
Min: Max	25:98	25:89	32:88	48:98
Age group, n (%)				
<65 years	16(31.37%)	3(25%)	12(48%)	1(7.14%)
65≤age<75	16(31.37%)	4(33.33%)	9(36%)	3(21.43%)
75≤age<85	12(23.53%)	3(25%)	3(12%)	6(42.86%)
≥85 years	7(13.73%)	2(16.67%)	1(4%)	4(28.57%)
Variant strains, n (%)				
Delta	47(92.16%)	11(91.67%)	23(92%)	13(92.86%)
Omicron	4(7.84%)	1(8.33%)	2(8%)	1(7.14%)
Symptom onset to treatment (days)				
Number of cases	N = 41	N = 9	N = 22	N = 10
Mean ± SD	8.41±3.75	8.22±3.67	8.36±4.19	8.7±3.09
Median	8	8	7	9
Min: Max	2:15	3:14	2:15	3:13

*The most severe stage experienced by patient was used as the diagnostic type (mild, moderate, severe).

reached the peak on Day 1, with a median level of 482.58 S/CO and 350.85 S/CO (Fig. 1E and K). Plateaued IgG levels were similar across all patients, and sustained after that. In Qinghai cohort, patients' IgM level increased gradually over the time, and the plateau was not observed (Fig. 1F). By contrast, the IgM level of patients in Henan cohort increased after administration (Day1) and were relatively stable in the following days (Fig. 1L).

The clinical symptoms of most patients were alleviated shortly after IBI314 treatment in both cohorts. In Qinghai cohort, all severe patients (N = 4) were transferred out of ICU after IBI314 treatment with a median time of 5 days, accompanying a significant improvement in CT scan (Supplementary Fig. 1). At the end of observation window, 3 patients (3/10) in Qinghai cohort and 28 patients (28/51) in Henan cohort achieved negative conversion for SARS-CoV-2, with a mean interval after administration of 11.7 and 5.8 days, respectively. IBI314 is generally well-tolerated with no serious adverse events or infusion-related reactions observed. Laboratory parameters including but not limited to white blood cell counts, lymphocyte counts, hemoglobulin, transaminases were assessed in every patient and no clinically significant abnormality related to IBI314 was observed. Only a few patients experienced elevation in ALT and AST, and leukocytes decreased (dropped below 4.0×10^9/L) after IBI 314 treatment. No death occurred.

The emergence of novel variants of SAR-CoV-2 presented a serious challenge for the development of therapeutic agents against COVID-19. Preclinical data indicated that IBI314 exhibited a high degree of mutation resistance and maintained potency against prevailing variants of concerns in pseudoviral neutralization and authentic virus neutralization *in vitro*.[5] This study expounded the efficacy and safety of IBI314 under compassionate use in two cohorts with mild to severe COVID-19, infected with the Delta and Omicron variant of SARS-CoV-2. Most patients showed a significant increase in the Ct value (corresponding to a decrease in viral load) shortly after IBI314 treatment, suggesting a rapid viral clearance. Other parameters including oxygenation index, SpO2, and CRP were also improved. What's more, almost all patients showed significant improvement in clinical symptoms shortly after IBI314 treatment.

Collectively, we presented the first-in-human data on the efficacy of IBI314 in patients infected with Delta and Omicron variant of SARS-CoV-2, indicating that IBI314 have a satisfying potency to reduce viral load and alleviate clinical symptoms in any stage of

cases with favorable safety. We also observed a trend that the earlier IBI314 was used, the better efficacy was achieved.

Declaration of Competing Interest

The authors have declared no conflict of interest.

Ethics approval

The compassionate use of IBI314 was approved by the ethics committee of Qinghai Provincial Fourth People's Hospital and Zhengzhou First People's Hospital, with written informed content from all participants.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.06.004.

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Evaluating the potential impact of COVID-19 passports in Lithuania

Dear Editor,

In this journal, Mendez-Brito and colleagues reviewed the effectiveness of non-pharmaceutical interventions (NPIs) against COVID-19. [1] We would like to contribute to this discussion with analyzing the recent experience of applying NPIs in Lithuanian.

Lithuania has been relatively successful in managing the first peak of a COVID-19 outbreak (March – May 2020). [2] However, the subsequent wave had much worse consequences. In December 2020, Lithuania had recorded unacceptably high COVID-19 morbidity and mortality indicators. The main reason for this was that previous Government seriously lagged behind in implementing adequate non-pharmaceutical measures. The newly elected Government imposed a strict lockdown (December 16, 2020), which lasted until June 30, 2021. [3] In order to prevent a long lasting lockdowns and to limit the spread of SARS-CoV-2 infections and to motivate for an uptake of vaccination, Lithuania introduced the COVID-19 vaccination passport (in Lithuanian *"Galimybių pasas"*). The COVID-19 passport was issued to people, who 1) are fully vaccinated; or 2) has a laboratory confirmed previous COVID-19 infection. Holders of these passports had the right to visit shopping malls, concerts, restaurants and other facilities. The use of COVID-19 passport was terminated on February 4th, 2022. The aim of this study was to evaluate a potential impact of COVID-19 passports to epidemiological situation in Lithuania.

We have evaluated the potential impact of the COVID-19 passport to epidemiological situation in Lithuania by the construction of three main possible scenarios without having a COVID-19 passport. Models of the three scenarios were constructed according to the simplified method of epidemic dynamics prognosis, which is described in a separate paper.[1] [4] The proposed approach is based on a modified *SIR* model with assumptions that the number of daily new infected cases n is the most reliable parameter allowing the estimate of the disease spreading due to lockdown measures where the number of infections is much smaller than the number of people to be potentially infected.

In order to damp daily and weekly fluctuations, n and estimated the parameter α were smoothed by moving average, where each average is calculated over a sliding window of length $N_s = 15$ days:

$$\langle n(t)\rangle = \frac{1}{N_s}\sum_{i=-n_s}^{n_s} n(t+i), N_s = 1 + 2n_s \tag{1}$$

where time t is measured in days.

The daily growth rate of new infection cases α was estimated on the basis of smoothed daily new infection cases $\langle n(t)\rangle$

$$\alpha(t) = \frac{\langle n(t+1)\rangle}{\langle n(t)\rangle} \tag{2}$$

$\alpha > 1$ when n is growing and n is falling when $\alpha < 1$. If acceleration of the infection is slowing, then α is reducing and n reaches peak when $\alpha(t)$ cross 1.

The parameter $\alpha(t)$ can be interpreted as the average daily reproduction number R_t [5].

The second assumption of the approach is that if lockdown condition is stable, then $\alpha(t)$ is constant and having value matching effectiveness of lockdown measures.

In order to predict COVID-19 disease spread in infected country or region with imposed lockdown measures, a model of the growth rate of new cases $\alpha_m(t)$ needs to be developed. Model of $\alpha_m(t)$ is constructed on expected conditions for virus spreading based on data of disease previous dynamics.

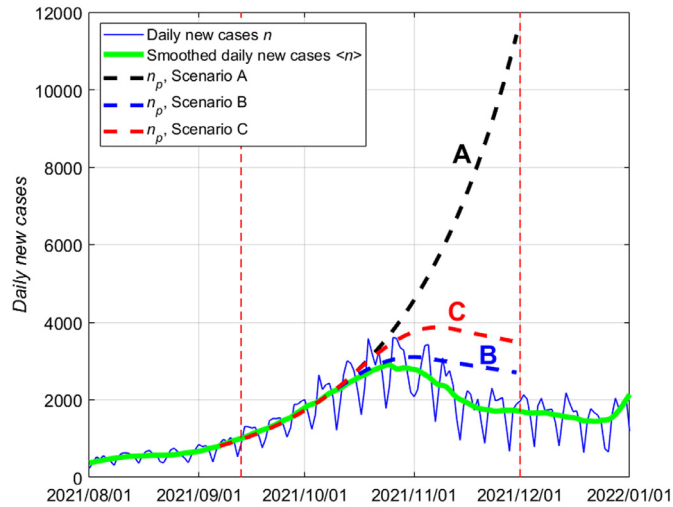

Fig. 1. Dynamics of the daily new cases n, daily new cases smoothed by moving average of a sliding window of 15 days, and modelled scenarios of the daily new cases n_p.

So, if we have model of $\alpha_m(t)$ we can predict number of daily new infections $n_p(t)$:

$$n_p(t) = \alpha_m(t) \cdot n_p(t-1) \tag{2a}$$

It is already known, from that non-pharmaceutical interventions limits population mobility and, consequently, contacts. [1] Reaction of the COVID-19 spread to lockdown measures comes with some delay of 2–4 weeks. [4]

The Lithuania COVID-19 passport was introduced on May 24, 2021, but was applied in the full validity only on September 13, 2021, when it became clear that the rise in SARS-Cov-2 infections were beginning to threaten the stability of the public health system. In early September, the average number of infections exceeded 670 infections per day (Fig. 1), 10 deaths per day, and the number of hospitalized COVID-19 patients at – 600.[2] As people returned from the summer holidays, contacts at workplaces and entertainment and retail events increased. [6] The introduction of COVID-19 passport has limited people-to-people contacts at work, public transport, shopping and entertainment, and retail events. Due to human behavior inertia, the effect of COVID-19 passport on contact reduction lasted for about 1–2 weeks.

The decrease in contacts with additional delays also started to affect the spread of the virus. The growth rate of new infection cases in early September was around $\alpha = 1.032$ and began to decline two weeks (since early October) since the introduction of COVID-19 passport. In the October last decade, α reached 1, marking the peak of COVID-19 infection (Fig. 2), after which the number of infections began to decline and α ranged from 0.974 to 0.998. Omicron was first identified in Lithuania on December 15, 2021, although it is likely that it appeared earlier. The infectivity of the Omicron strain was so high that COVID-19 passport quarantine measures were no longer sufficient to stop the new virus strain and α began to grow relentlessly and exceeded 1 in the second half of December, reaching 1.06 in early January 2022.

If the full validity of the COVID-19 passport would not been introduced on September 13, 2021, and if people's behavior (self-protection, mobility and other safety measures) did not continue to change, it is likely that the growth rate of new infection cases α would have adhered to the same value as before the introduction of COVID-19 passport ($\alpha_m = 1.032$) and the number of infections would have continued to grow exponentially until it reached

[1] Data for these calculations can be downloaded from https://storage.covid19datahub.io/rawdata-1.zip

[2] Data can be downloaded from https://storage.covid19datahub.io/rawdata-1.zip

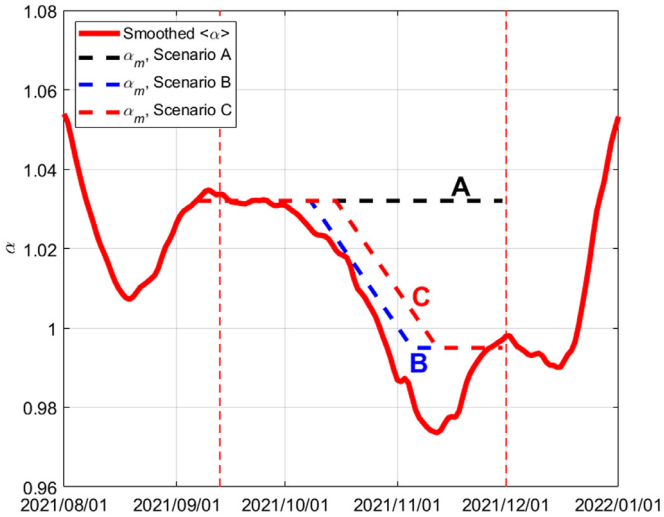

Fig. 2. Dynamics of the daily growth rate of new cases α smoothed by moving average of a sliding window of 15 days, and modelled scenarios of α_m.

11,000–12,000 infections per day by the end of December (Scenario A). Such a scenario is considered unlikely, as people were not only passively responding to the quarantine measures being introduced, but were also actively considering the potential risk of infection. Therefore, when the number of infections and their consequences (hospitalizations, intensive care units admissions, and deaths) exceed the psychological threshold, people themselves actively begin to beware and avoid contact. Against this background, we developed two other scenarios (B and C) related to human behavior such that when number of daily new infections reaches the psychological threshold of 1000 infections per day in Scenario B, people start responding to infections in late September (1 week later) and in early October (2 weeks later) for Scenario C.

There were a total of 163,307 officially registered COVID-19 infections in the period from the introduction of the COVID-19 passport on 13/09/2021 up to 01/12/2021. According to our calculations, under Scenario A 337,632 infections would be expected (i.e. +174,325 more were registered), while under Scenario B - 189,953 infections (+26,646), and under Scenario C - 220,368 infections (+57,061).

There was a total of 2065 COVID-19 deaths officially registered (deaths/infections ratio ≈ 1.26%) in the period from the introduction of the COVID-19 passport on 13/09/2021 until 01/12/2021. Our estimates suggest, that under Scenario A number of deaths from COVID-19 could have reached 4269 (+2204 more than it was), and for Scenario B – 2402 (+337) and Scenario C – 2787 (+722).

The COVID-19 passport policy has caused many debates in Lithuania. However, according to a Lithuanian National Broadcaster LRT poll (December 2, 2021), 53% of Lithuanians evaluated COVID-19 passports positively. [7] A study by Walkowiak et al. (2021) estimates, that Lithuania's strict policy resulted in a 12.34 p.p. increase in the vaccination rate in Lithuania. [8] According to our preliminary assessment, the COVID-19 passport could have helped to prevent approximately 26,000 – 57,000 COVID-19 infections and 330 - 720 deaths in the period between 13/09/2021 and 01/12/2021 in the country. More likely this effect could be even more significant, as we evaluated only the impact of COVID-19 passport to people's behavior. An increased vaccination level was not included in this analysis. Studies from other countries have reported similar positive impact of COVID-19 passports to epidemiological trends. [9,10] This suggests that vaccination passports could be an effective measure in tackling future pandemics. However, legal, social and ethical aspects should be considered.

Authors' contributions

Conceptualization: MS, AD. Data analysis and modeling: AD, GS, EM, RN. Writing-original draft: MS, AD. Writing-review and editing: MS, AD, GS, EM, RN.

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Declaration of Competing Interest

MS and AD are members of the Health Experts' Council under the President of the Republic of Lithuania.

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The resurgence of wild poliovirus in Pakistan and Afghanistan: A new setback for polio eradication

Dear Editor,

In a recent manuscript entitled" The negative impact of the COVID-19 Pandemic on immunization and the positive impact on Polio eradication in Pakistan and Afghanistan",[1] we described the looming threat of poliovirus outbreak in Pakistan and Afghanistan due to the disruption in immunization imposed by COVID-19 Pandemic. The concerns raised by the authors are proved correct as shown by the ongoing poliovirus outbreak in Pakistan and Afghanistan. In this paper, we would like to discuss the impact of the COVID-19 Pandemic on derailing the fight against polio in Pakistan and Afghanistan.

The global polio eradication initiative was established in 1988 when the wild poliovirus was endemic in 125 countries. Due to the tremendous efforts of the global polio eradication initiative, the number of polio cases has decreased by more than 99%, from 350,000 cases in 1988 to 118 cases in 2017. Immunizations have saved billions of lives and prevented countless illnesses and disabilities across the globe.[2]

Before the emergence of COVID-19, polio was considered one of the most challenging infectious diseases of global public health concern.

Pakistan and Afghanistan are the last two countries in the world where the wild poliovirus is still endemic as 100% (67.5% in Pakistan,32.5% in Afghanistan) of polio cases were reported in these two countries in the last five years.[3]

Unfortunately, eight wild poliovirus cases were reported from the North Waziristan area of Khyber Pakhtunkhwa (KPK) province of Pakistan. First wild polio case was reported on April 22 , 2022, and eighth polio case was reported on 6th June,2022. The southern part of KPK province bordering Afghanistan had been marked as the area at high risk after the identification of two wild polioviruses in the environmental samples in the last quarter of 2021 from D.I Khan and Bannu Districts.[4] Similarly, one case of wild poliovirus was reported in a 24-Month-old female child in Afghanistan in February 2022 in the Dila District of Paktika province[5] which is 126 km from the Waziristan area of Pakistan **Fig 1.** We fear that more children from the same area may be affected as the virus circulate in the upcoming peak season.

Table 1
Reported wild poliovirus cases in Pakistan and Afghanistan 2017–2022.

Year	Pakistan n (%)	Afghanistan n (%)	Total n (%)
2017	8 (3.1)	14 (11.2)	**22 (5.7)**
2018	12 (4.6)	21 (16.8)	**33 (8.5)**
2019	147 (56.5)	29 (23.2)	**176 (45.7)**
2020	84 (32.3)	56 (44.8)	**140 (36.3)**
2021	1 (0.38)	4 (3.2)	**5 (1.3)**
2022	8 (3.1)	1 (0.8)	**9 (2.3)**
Total	**260 (67.53)**	**125 (32.47)**	**385 (100)**

In 2021, the number of wild poliovirus-1 (WPV1) cases reported was only five (4 in Afghanistan and one in Pakistan), the lowest level in history. Before the COVID-19 pandemic, the polio cases rose from only 12 and 21 polio cases reported in 2018 to 147 and 29 cases in 2019 in Pakistan and Afghanistan, respectively. During the year 2020, the number of polio cases decreased in Pakistan and increased in Afghanistan. Details of reported polio cases in Pakistan and Afghanistan from 2017 to 2022 are presented in **Table 1**.

The latest wild polio cases in Pakistan and Afghanistan have raised to 11 global number of polio infections in 2022, includ-

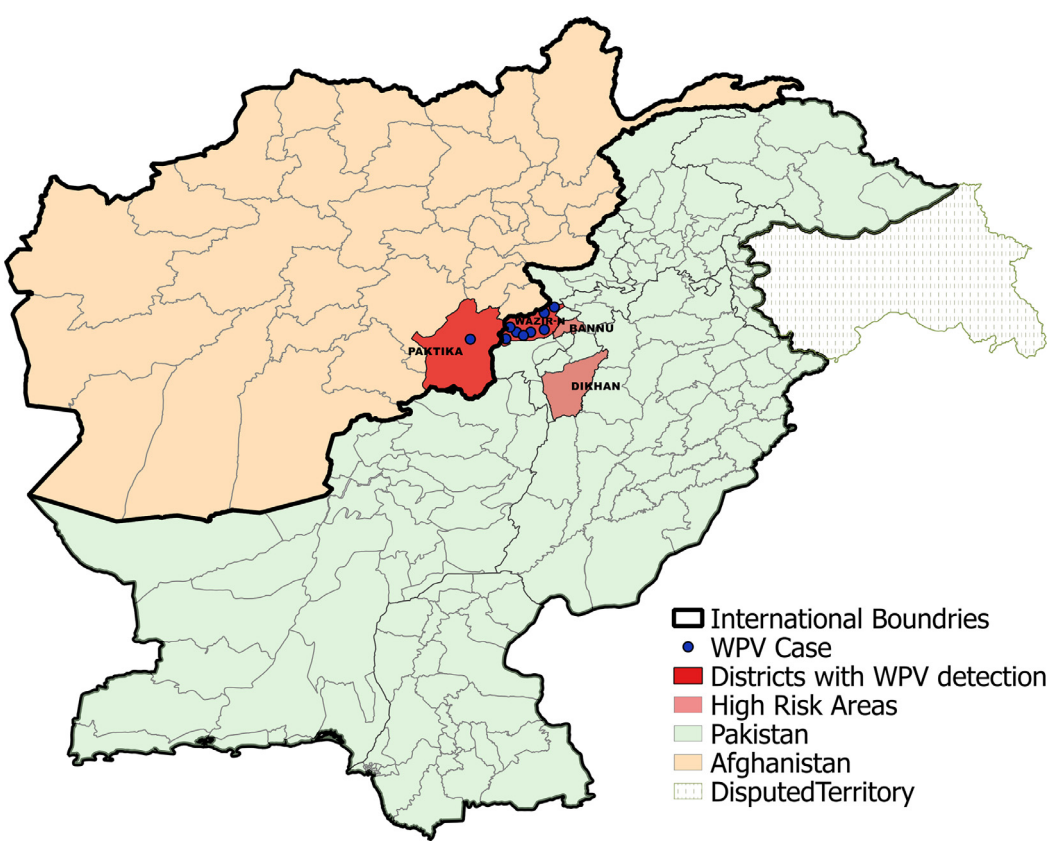

Fig. 1. Wild poliovirus outbreak in the border area of Pakistan and Afghanistan in 2022.

ing one in Malawi and one in Mozambique. Malawi has detected a case of wild polio in a 3-year-old child in Lilongwe District and the virus was genetically linked to the poliovirus circulated in Sindh province of Pakistan [6]. The resurgence of polio is a tragedy for the infected children and their families. It is very unfortunate for Pakistan, Afghanistan, and polio eradication efforts all over the world. With only five cases in 2021 in both countries, it was considered that the program is on track to have the lowest toll of polio cases in a decade. This temporary decline in polio cases was assumed to be due to the attribution of efficient awareness of vaccination among the general population. However, the scientific community was very surprised regarding the decline in polio cases in 2021 as the COVID-19 pandemic disrupted life-saving immunization services worldwide, particularly in developing countries including Pakistan and Afghanistan. Due to the disruption in immunization other vaccine-preventable diseases such as Measles showed an unpresidential upsurge and as result, more than 28,125 and 35,000 measles cases were reported in Pakistan and Afghanistan in 2021. COVID-19 pandemic had left more than 40 million children unimmunized in Pakistan [7] and 23 million children in Afghanistan [8].

The border area between Pakistan and Afghanistan from where recent nine wild polio cases were identified has already been considered a high-risk area for polio for the last many years [9] due to a variety of obstacles including security issues, hard to reach area, killing of polio workers, displaced population, low immunization coverage, vaccine hesitancy, vaccine refusal, lack of education and cross border movement of unimmunized population.

Suspension of the door-to-door polio vaccination owing to the COVID-19 pandemic in Pakistan and Afghanistan may not affect immediately but it might potentially result in future polio outbreaks and the international spread of the virus. The persistence and resurgence of polio in Pakistan and Afghanistan have been a warning call for intensifying efforts for polio eradication. Low immunization coverage, disruption in immunization in both countries along with Taliban control in Afghanistan threaten to reverse the tremendous achievements in polio eradication.

The government of Pakistan and Afghanistan along with the international health organizations should actively implement urgent immunization activities along with active surveillance in high-risk areas which will help eradicate polio from the world. Failure to eradicate polio now could result in a resurgence of infection, with as many as 200,000 to 300,000 new cases worldwide every year.

Declaration Competing Interest

The authors declare no competing interests.

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Long-acting HIV fusion inhibitor albuvirtide combined with ritonavir-boosted lopinavir for HIV-1-infected patients after failing the first-line antiretroviral therapy: 48-week randomized, controlled, phase 3 non-inferiority TALENT study

Dear Editor,

We read with great interest the article by Hou et al.[1] which described that the HIV-1-related mortality is still high despite the scaling up of antiretroviral therapy (ART). The constant proportion of late-stage presentation among treatment-naïve people living with HIV (PLWH), especially severely immunosuppressed PLWH, is considered to be one of the reasons. We think there should also be some other challenges to reduce the mortality, to improve the treatment efficacy and to improve the life quality of patients. For example, lifelong ART was associated with a set of challenges, including side effects and inconvenience of lifelong daily intake of single or multiple tablets.[2] Prolonged therapy may result in social stigma, poor treatment compliance, and drug resistance.[3] Therefore, more safe, effective, and convenient drugs are being searched for HIV treatment to provide better choices for patients.

Albuvirtide (ABT) is a long-acting injectable HIV fusion inhibitor targeting gp41, and was approved by the Chinese National Medical Products Administration (NMPA) in 2018.[4] It has a much longer half-life and can be intravenously infused once-a-week. Its anti-HIV-1 activity, as well as the safety and pharmacokinetics profile have been proved *in vitro* study and Phase 1/2 clinical trials. Considering the unavailability of long-acting drugs combined with once-a-week injectable ABT and drug accessibility, we selected ritonavir-boosted lopinavir (LPV/r) combined with ABT for its phase 3 study (named the TALENT study). We have previously reported the interim analyses of the TALENT study.[4] Here, we aimed to present the final results of the TALENT study with the 48 weeks of data. The study design and some of the results were described in the Supplementary materials. In this 48-week, randomized, controlled, open-label non-inferiority study conducted in

Table 1
Baseline characteristics of the HIV-1-infected patients.

Characteristics	ABT group (n = 185)	NRTIs group (n = 198)
Age (years), median (range)	41 (19–59)	40 (18–59)
Male, n (%)	139 (75.1%)	143 (72.2%)
Ethnicity, n (%)		
Han	181 (97.8%)	193 (97.5%)
Others	4 (2.2%)	5 (2.5%)
Plasma HIV-1 RNA (log_{10} copies/mL), n (%)	3.94±0.98	3.94±0.99
<100,000	157 (84.9%)	167 (84.3%)
≥100,000	28 (15.1%)	31 (15.7%)
CD4 T-cell counts (cells/μL), n (%)	230±190	210±160
<100	47 (25.4%)	47 (23.7%)
≥100	138 (74.6%)	151 (76.3%)
Time on first-line regimen (months), median (Q1-Q3)	28.8 (11.5–72.8)	27.0 (10.2–73.3)
Virus subtype, n (%)	N = 182	N = 192
B	90 (49.5%)	91 (47.4%)
CRF01_AE	59 (32.4%)	70 (36.5%)
CRF07_BC	15 (8.2%)	6 (3.1%)
Baseline resistance mutations, n (%)	n = 182	n = 192
≥2 classes (NRTI/NNRTI/PI)	131 (72.0%)	144 (75.0%)
Any 1 class (NRTI/NNRTI/PI)	148 (81.3%)	161 (83.9%)
any PI	3 (1.6%)	1 (0.5%)
NRTIs selected for use at study entry, n (%)		
Tenofovir and lamivudine	–	120 (60.6%)
Zidovudine and lamivudine	–	74 (37.4%)
Abacavir and lamivudine	–	3 (1.5%)
Tenofovir, zidovudine and lamivudine	–	1 (0.5%)

Data are n (%), n/N (%), and mean ± standard deviation.
NRTI: Nucleoside or nucleotide reverse transcriptase inhibitor. NNRTI: Non-nucleoside/nucleotide reverse transcriptase inhibitor. PI: Protease inhibitor.

12 hospitals in China, a total of 418 subjects who failed the first-line ART for the treatment of HIV-1, were enrolled and randomly assigned (1:1) to the two groups, receiving ABT plus LPV/r (ABT group) and LPV/r plus two optimized NRTIs (NRTIs group), respectively. Among them 401 subjects were allocated into the safety analysis set (SS), while 185 and 198 subjects entered the modified Intention-To-Treat (mITT) analysis set from the ABT and NRTIs groups, respectively (Fig. S1).

Patient characteristics at the study entry were not significantly different between the ABT and NRTIs groups (Table 1). Notably, more than 15% of subjects had a baseline viral load of >100,000 copies/mL. And approximately 25% of the subjects had a baseline CD4 T-cell count of <100 cells/μL.

As the primary endpoint, at week 48 in the mITT population, the percentages of subjects with HIV-RNA levels of <50 copies/mL in the ABT and NRTIs groups were 75.7% and 77.3%, respectively (Table S1); the difference was −1.6% with a 95% CI of −10.1 to 6.9% (Table S1 and Fig. 1A). According to the pre-specified non-inferiority margin of 12%, the ABT group was non-inferior to the NRTIs group (Table S1). The response rate in the per-protocol (PP) population and response rate defined as HIV-1 RNA levels lower than 400 copies/mL in the mITT population were consistent with the primary endpoint (Table S1 and Fig. 1B). No significant differences in virological outcomes were observed in the logistic regression analysis based on the group, baseline HIV RNA, baseline CD4 T cells, and sex. The decreases in HIV-1 RNA were 2.16±1.00 log_{10} copies/mL in the ABT group and 2.11±1.16 in the NRTIs group (Table S1 and Fig. 1C). The increases in CD4 T-cell counts were comparable between two groups (Table S1 and Fig. 1D).

The percentages of baseline genotypic resistance to at least one drug (NRTI/NNRTI/PI) were 81.3% and 83.9% in the ABT and NRTIs groups, respectively. Most viruses were resistant to NRTIs and NNRTIs. Resistance rates to protease inhibitors were 1.6% and 0.5% in the ABT and NRTIs groups, respectively (Table 1). Importantly, more than 70% of the subjects were resistant to more than two types of antiviral drugs, which revealed that the regimen of ABT plus LPV/r can guarantee two completely active drugs as the second line treatment for patients after failing the first-line treatment, even in the absence of drug resistance testing in the clinical practice. No drug resistance mutations related to gp41 were detected in cases with failed treatment for 48 weeks. This outcome demonstrated the high resistance barrier of ABT. Thus, there was no further resistance to the two-drug regimen, which is essential to avoid compromising future drug options in treatment-exposed HIV patients.

The changes in serum creatinine were assessed in both groups. There was a decreasing trend after 12∼48 weeks of treatment in the ABT group while an increasing trend was observed in the NRTIs group with TDF as the second-line drug. There were statistically significant differences between the two groups (P<0.05) at 12, 36, and 48 weeks. At week 48, serum creatinine in the ABT group was reduced by 3.34±13.15 μmol/L from the baseline, whereas serum creatinine in the NRTIs group with TDF was increased slightly by 0.53±14.10 μmol/L from the baseline. The ABT group showed a better effect on serum creatinine than the NRTIs group treated with TDF as a second-line drug, with a statistically significant difference (P<0.05) (Fig. 1E). The estimated glomerular filtration rate (eGFR) showed similar changes. The eGFR in the ABT group had an increasing trend after 12∼48 weeks of treatment, while that in the NRTIs group with TDF had a decreasing trend (Fig. 1F). Therefore, the ABT combined with the LPV/r regimen can serve as an alternative therapy for individuals who cannot tolerate TDF due to nephrotoxicity.

Till today, daily oral drug intake remains the most important determinant for sustained viral suppression and prevention of the emergence of drug-resistant viral strains, but the long-term compliance with drug intake might be hampered by many factors.[5] A promising approach to overcome the patient compliance challenge and prevent drug resistance is to develop long-acting formulations. Clinical safety and effectiveness in critically-ill hospitalized AIDS patients showed potential benefits of the combination of ABT with other effective antiretroviral drugs, e.g., Xu et al., re-

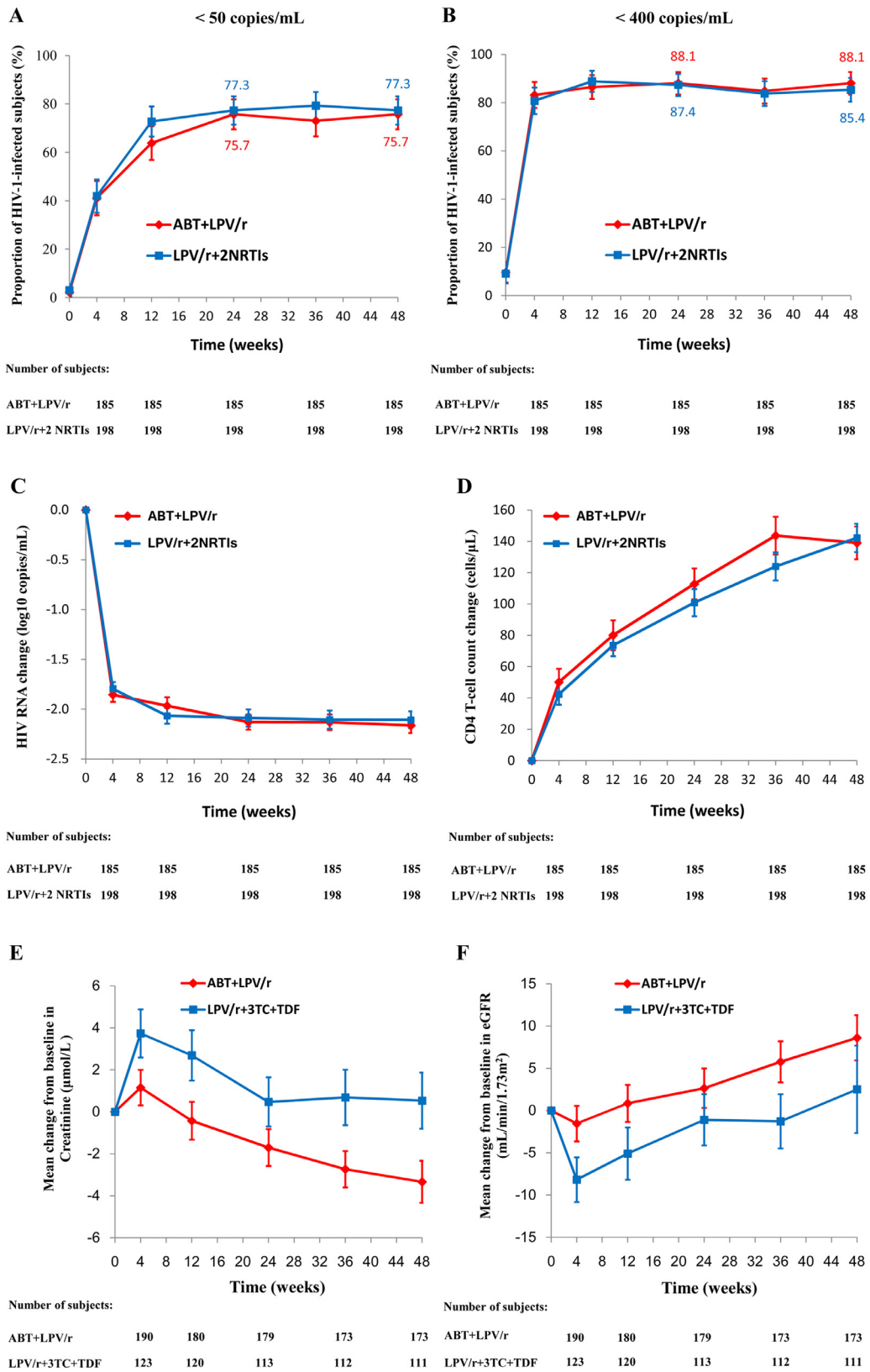

Fig 1. Characteristics of patients with plasma HIV-1 RNA levels below 50 and 400 copies/mL in the mITT analysis, changes of HIV-RNA and CD4 T-cell count, and Renal function. (A) Proportions of patients achieving plasma HIV-1 RNA levels below 50 copies/mL. (B) Proportion of patients achieving plasma HIV-1 RNA levels below 400 copies/mL. Patients were allocated (1:1) to the albuvirtide (ABT) plus ritonavir-boosted lopinavir (LPV/r) group (red) or to the LPV/r plus two optimized NRTIs group (blue). (C) Mean change from the baseline in HIV-RNA $\log_{10}$ (copies/mL). (D) Mean change from the baseline in CD4 T-cell count. (E) Mean changes from the baseline of serum creatinine (Scr). (F) Mean changes from the baseline of estimated glomerular filtration rate (eGFR). Error bars indicate standard errors.

cently found that daily treatment with ABT/3TC/TDF for 4 weeks followed by DTG/3TC/TDF was safe and effective in newly diagnosed HIV-infected patients with either severe liver dysfunction, HBV or Mycobacteria co-infection, or high HIV RNA copy numbers.[6] In addition, a recent cohort study on HIV post-exposure prophylaxis (PEP) in China showed that subjects in the ABT group (ABT+DTG or ABT+TDF+3TC) had significantly superior tolerability and adherence than those administered with the oral regimen DTG+TDF+3TC, and no subject was tested seropositive for HIV at the end of the study.[7]

As a new drug, ABT demonstrated characteristics such as high efficacy, good safety, and high drug resistance barrier, which can provide a new option to patients with unmet needs. More combination regimens containing ABT should be explored with different clinical situations that can highlight the advantage of ABT. Currently, a clinical trial of combination of ABT and a broadly neutralizing antibody (bNAb) 3BNC117 as long-acting maintenance therapy (intravenous administration every 2 or 4 weeks) in virologically suppressed subjects is ongoing in the United States to determine the dose, safety, and antiviral activity.[8-10] Further researches on combining ABT with integrase inhibitors or other long-acting drugs as a complete long-acting treatment are needed.

In conclusion, the TALENT study showed that ABT (320 mg/dose/week) combined with LPV/r, administered for 48 weeks in HIV-1-infected patients after failing the first-line ART, was non-inferior to that of the WHO-recommended standard second-line three-drug regimen and showed a good safety profile and did not impair renal function. Therefore, combining the results of previous studies, as the only long-acting fusion inhibitor administered intravenously once a week, ABT might be an efficient alternative not only for HIV treatment but also for its prevention.

Declaration of Competing Interest

The authors of this manuscript have read the journal's policy and have the following competing interests: CY, MH, XL, RJL, JHH, CCS, and DX have received salary support from Frontier Biotechnologies Inc. All authors had full access to all study data and analyses, and approved the final report. All other authors have declared that no competing interests exist.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.05.034.

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Suitability assessment of CD24 targeted-therapy in the cancer patients with COVID-19: Preliminary results from pan-cancer

Dear Editor,

The continuing coronavirus disease 2019 (COVID-19) pandemic has resulted in diverse implications at each stage of the cancer progression for individuals with a present or prior history of cancer. We read with great interest the recent paper published in the Journal of Infection by Afshar et al., who reported that the percentage of severe cases and deaths among COVID-19-infected cancer patients was higher than among COVID-19-infected patients without cancer, due to an abnormal immune function[1] . Mounting data shows that the severe incidence among cancer patients infected with severe acute respiratory syndrome coronavirus 2 was elevated (SARS-CoV-2).[2]

Glycosyl-phosphatidyl-inositol (GPI)-anchored glycoprotein CD24 was discovered as a phagocytic inhibitor (the "do not eat me" signal) that has a suppressive effect in tumor immunity through binding partners such as SIGLEC10, L1CAM, and P-selectin.[3] Genetic ablation or pharmacological inhibition of CD24 led to macrophage-dependent suppression of tumor development in vivo and enhanced survival.[4] These results resemble the evidence that soluble CD24 may reduce COVID-19-associated systemic immunopathology.[5] More recently, a randomized, double-blind, placebo-controlled phase 3 research confirmed that CD24-Fc is typically well tolerated and promotes clinical recovery in hospitalized COVID-19 patients requiring oxygen support.[6] However, the potential applicability of CD24-Fc in cancer patients infected with COVID-19 remains unclear.

We first analyzed CD24 expression in pancancer by using the TCGA/GTEx dataset. CD24 downregulation was detected in 6 malignancies relative to normal tissues, including adrenocortical carcinoma (ACC), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), acute myeloid leukemia (LAML), skin cutaneous melanoma (SKCM), and thymoma (THYM). In contrast, 19 malignancies demonstrated enhanced CD24 expression in established tumors (Fig. 1A).

CD24 expression was linked with the prognosis of six malignancies, according to univariate Cox analysis (Fig. 1B). Using the median value as the cutoff in lower grade glioma (LGG) and kidney renal clear cell carcinoma (KIRC), respectively, overall survival (OS) and progression-free interval were considerably decreased in CD24-low tumours (Fig. 1C, D).

Next, the CIBERSORT algorithm was used to assess the context-specific functions of CD24 programming tumor-infiltrating immune cells (TIICs) in the tumor microenvironment (TME). CD24 is negatively connected with CD8+ T cells and M1 macrophages, whereas it is favorably correlated with CD4+ T cells in LGG. In KIRC, CD24 had a positive connection with M2 macrophages and a negative relationship with memory B cells, M1 macrophages and Tregs (Fig. 1E), indicating that CD24 has a differential immunological role as a driving factor in various malignancies.[3]

The host inflammatory response, involving immune-cell hyperactivation and high amounts of circulating cytokines, has been

Abbreviations: COVID-19, The severe coronavirus disease 2019; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; CD24, Cluster of differentiation 24; ICB, Immune checkpoint blockade; TME, Tumor microenvironment; TIDE, Tumor immune dysfunction and exclusion; LGG, Brain Lower Grade Glioma; KIRC, Kidney renal clear cell carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; LIHC, Liver hepatocellular carcinoma; MESO, Mesothelioma; OS, Overall survival, PFS, Progression Free Survival; TCGA, The cancer genome atlas; GTEx, Genotype-Tissue Expression; KEGG, kyoto encyclopedia of genes and genomes.

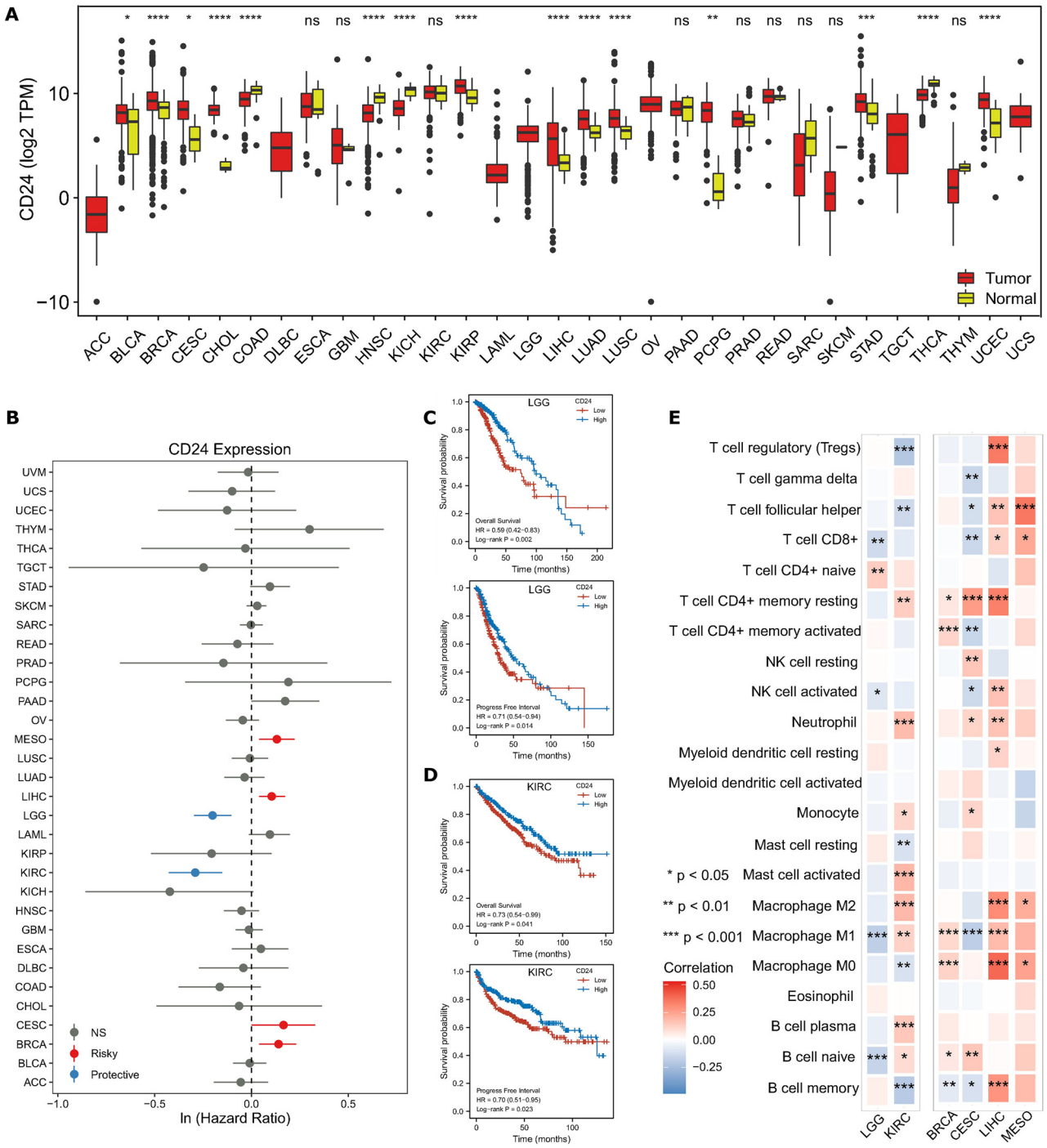

Fig. 1. Characterization of pan-cancer CD24 expression. (A) CD24 expression in human normal and tumor tissues statistically evaluated by the Mann-Whitney test. (B) Using univariate Cox regression, CD24 expression was linked with the survival of six kinds of cancer. (C) Kaplan-Meier plot with log-rank test of LGG samples stratified by the median CD24 value. The OS or PFS curves were shown in the top and bottom panels, respectively. (D) Effect of CD24 on survival in KIRC. (E) Correlation between CD24 expression and tumor-infiltrating immune cell infiltration levels.

demonstrated to explain severe COVID-19, resulting in a cytokine storm.[7] Consistent with recent findings that COVID-19 were severely inflamed but had limited T cell responses,[8] re-analysis of a single-cell RNA sequencing dataset (GSE158055)[9] revealed substantially elevated CD24 expression in epithelial and B cells (Fig. 2A). The inflammatory response was then assessed using the single-sample gene set enrichment analysis (ssGSEA) technique, which was based on the signature gene sets from the Molecular Signatures Database (MSigDB). CD24 expression exhibited either an anticorrelation or no significant connection with the ssGSEA score of LGG or KIRC. In contrast, the expression of CD24 was strongly correlated with inflammation in both BRCA and LIHC cohorts (Fig. 2B). These data indicated that CD24-Fc might have mitigated the systemic inflammation of LGG or KIRC.

To investigate the underlying mechanism, the protein-protein interaction (PPI) network of CD24 and its ligands was built using the STRING database (Fig. 2C). The functional enrichment analysis revealed that the network was significantly enriched with KEGG pathways associated with cells and molecules involved in local acute inflammatory response or tumorigenesis, such as cell adhe-

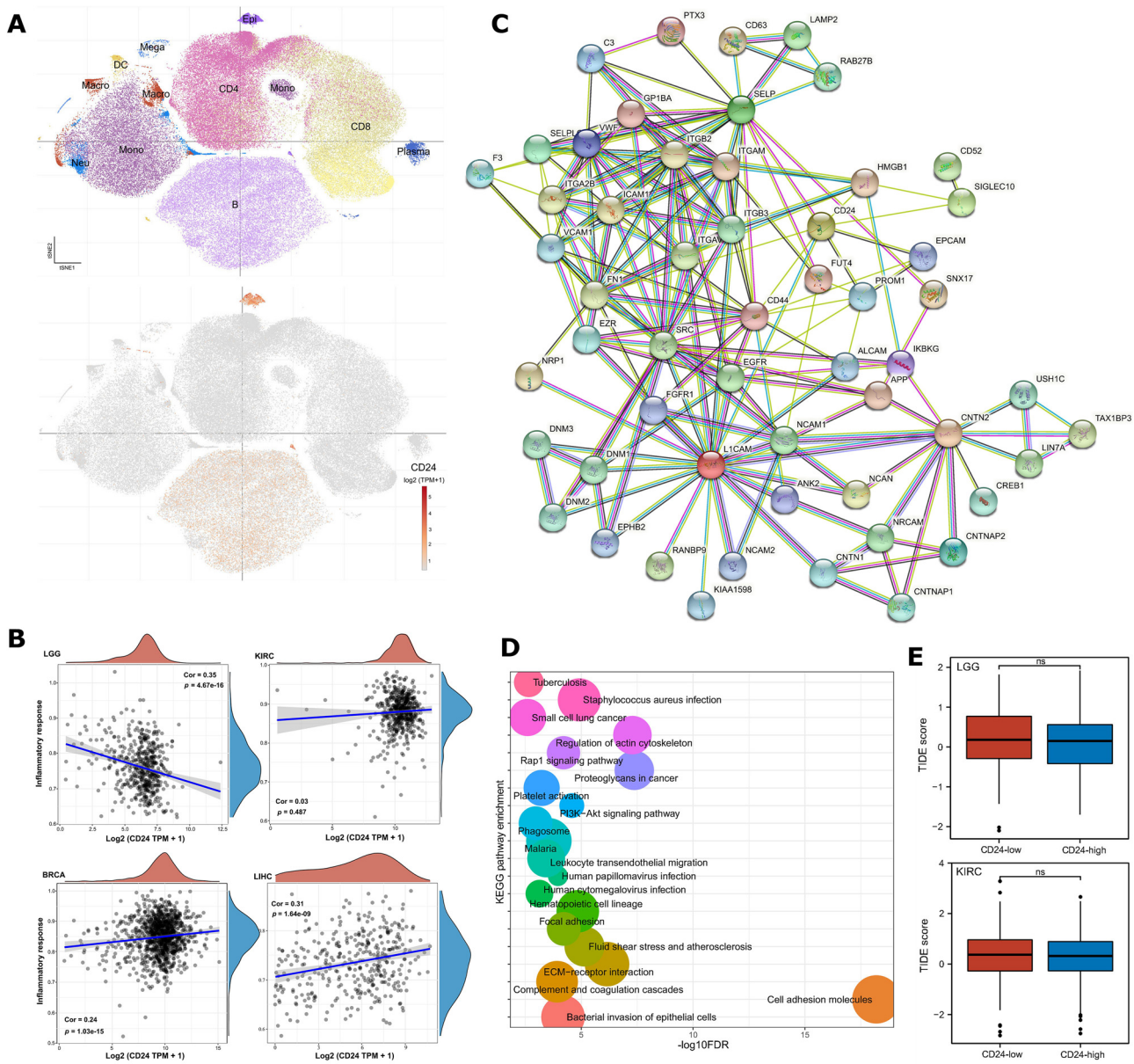

Fig. 2. Potential application of CD24-Fc in LGG and KIRC. (A) Annotation refinement of cell subsets within lineages using gene expression data from the NCBI GEO database with accession number GSE158055. The bottom panel displayed the CD24 expression of several cell lineages. (B) The correlation between CD24 and the inflammation score in various types of cancer. (C) CD24-related PPI network assembled using StringDB. (D) KEGG pathway enrichment of the network. (E) Differences in the TIDE score between CD24-high and CD24-low groups in LGG and KIRC respectively.

sion molecules, proteoglycans in cancer, bacterial invasion of epithelial cells, platelet activation, and phagosome (Fig. 2D).

Finally, we used the Tumor Immune Dysfunction and Exclusion (TIDE) framework (tide.dfci.harvard.edu) to evaluate tumor immune dysfunction and exclusion in order to predict the impact of CD24 on immune checkpoint blockade (ICB) treatment.[10] Consequently, there was no difference in ICB response prediction between the CD24-high and CD24-low groups in LGG and KIRC. This demonstrated that CD24 treatment may be administered to COVID-19-infected LGG or KIRC patients, either alone or in combination with ICB, without compromising the immunotherapy's efficacy.

In conclusion, owing to the effective implementation of CD24-Fc in clinical trails, our findings have contributed the understanding of the relationship between CD24 in COVID-19 and malignancies. We proposed that CD24-Fc should not be administered to COVID-19 individuals with the BRCA, CESC, LIHC, or MESO cancers, but that it could be appropriate for LGG and KIRC patients. We hoped that our results might hasten the therapies in cancer patients during the COVID-19 pandemic.

Declaration of competing interest

All the authors declare that there are no conflicts of interest.

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Transmission of monkeypox virus through sexual contact – A novel route of infection

Dear Editor,

In this Journal, Green and Cladi highlighted the need for international surveillance of zoonoses[1]. With increasing travel and given the long incubation period, it is unsurprising that Monkey Pox (MPX) cases have been reported in over ten countries including the UK, however, the current outbreak is atypical in that the majority of cases are unrelated to travel with increasing prevalence in men who have sex with men (MSM). MPX is an *Orthopox* virus, endemic in West and Central Africa [2]. On the 7th May 2022, the UK Health Security Agency (UKHSA) reported a classic case of imported MPX in an individual who had recently returned from Nigeria. Subsequently, many UK cases have been identified who were symptomatic before this individual was identified to have MPX. Between 2018 and April 2022, seven cases of MPX have been reported in the UK. At the time of writing, there have been 20 cases reported in the month of May 2022 alone[3].

MPX is classically transmitted through bodily fluids and close skin-to-skin contact from active lesions. While transmission during sexual intercourse has been suggested as a viable route, we report what we believe to be the first case of MPX infection with documented transmission through sex.

Our case involves two white British men (patients 1 and 2) who report no recent travel outside of the UK and no history of travel to regions with endemic MPX. They were both fit and well, with Patient 2 having a history of well controlled HIV on antiretroviral treatment. At the time of condomless sexual contact at the end of April 2022, Patient 1 was the receptive partner and Patient 2 the insertive partner during both anal and oro-anal sex. Of note, ten days prior to sexual contact, Patient 1 reported kissing an unrelated individual who had a crusted oral lesion.

Twenty-four hours after sexual contact, Patient 1 developed perioral white spots and painful perianal blistering lesions. Forty-eight hours later, Patient 2 had symptoms of perioral papules which blistered and ulcerated, with subsequent papules on the mons pubis and penile shaft which evolved into painful ulcers.

Neither individual reported prodromal symptoms. Skin lesions at the point of sexual contact were likely to be the herald signs of infection and (atypically) followed by systemic features of lymphadenopathy, fever, headache and diarrhoea.

Following the appearance of skin lesions, both patients presented separately to different sexual health clinics where the initial diagnoses of severe infection with herpes simplex virus (HSV) or varicella zoster virus (VZV) with superimposed bacterial infection were made. Both individuals were commenced on high-dose oral antiviral and antibacterial medication and managed in the outpatient setting.

Patient 1′s cutaneous lesions were painful and remained localised to the perioral and perianal areas, with the latter having a mixed morphology- some displaying umbilication, others deroofed with exudate (see Fig. 1). There were no genital lesions. Patient 2′s symptoms persisted at the genital and pubic regions and the facial ulcers extended to involve the tongue, oral and buccal mucosae (see Fig. 2). The lesions mirrored the points of skin-to-skin sexual contact with very few papules appearing outside of these anatomical sites. Both patients deteriorated and required admission to hospitals in Newcastle and London. With patient consent, communication channels were opened between the two sites to allow for information sharing, ensuring concurrent treatment.

The temporal association of symptoms to sexual intercourse and the location of primary lesion sites matching those of sexual contact, led us to consider a sexually transmissible infection. Lesions

Fig. 1. Perioral and perianal lesions on Patient 1.

Fig. 2. Genital and perioral lesions on Patient 2.

from both patients were negative for HSV and VZV on RT-PCR and syphilis serology showed no active infection. Virological screening through respiratory RT-PCR, serology and in the case of Patient 2 who presented with a headache, CSF, was negative for all pathogens tested. The short incubation period, along with the presence of pustulo-nodular lesions and systemic symptoms, made disseminated gonococcal infection (DGI) a probable diagnosis at the time. In view of this, on admission, both patients were commenced on treatment with intravenous ceftriaxone. While Patient 1 showed significant improvement, Patient 2 continued to develop new lesions despite antibiotic therapy. Results from nucleic acid amplification testing and culture at oral, pharyngeal, rectal, and skin lesion sites were negative for *Neisseria gonorrhoeae*, leading to questioning of the DGI diagnosis.

On the 14th of May, UKHSA published images of cutaneous MPX lesions which were recognised as being morphologically similar to those seen on the London patient. The admitting team liaised with UKHSA and the Rare and Imported Pathogens Laboratory and serum, lesion and urine samples were sent for MPX testing. Within 24 h, lesion swabs from both individuals were confirmed to be positive for MPX, and Patient 2 was transferred to a High Consequence Infectious Disease Unit. Both patients remained systemically well throughout their hospital stay. There were no recorded fevers following admission, white cell counts remained within normal limits and inflammatory markers were only mildly raised (CRP $<$100 g/L).

As a non-endemic infection, all MPX cases in the UK are of significant interest, however, our cases raise additional concerns around the involvement of previously unaffected communities and subsequently exposed healthcare workers (HCW). With transmission through sexual intercourse potentially becoming a more common transmission route than previously recorded, it is likely that sexually active individuals of all demographics will be affected. As we describe, these presentations may not fall into the expected 'fever in a returning traveller' scenario with ulcerating lesions and hence MPX should be considered in the differential diagnosis of any at risk individual with a rash, further characterisation of the clinical features is vital to inform clinicians and aid rapid diagnosis. Given that MPX virus is a category 3 pathogen, it is imperative that HCW utilise appropriate PPE, receive relevant support and education with clinical pathways instigated to manage possible MPX cases. Rapid communication and diagnostics will be the greatest tools to ensure constructive collaborative efforts amongst clinicians to benefit staff, services and patients, and sensitive community engagement/education will be crucial to avoid stigmatising groups with higher incidence rates.

Ethics and consent

Both patients provided written consent for the use of their case details and medical images in this publication, and we are sincerely grateful to them for allowing us to do so.

Authors' contributions

All listed authors made substantial contributions to the conception or design of the work; or to the acquisition and analysis of data for the work; and drafting the work or revising it critically ahead of submission for publication.

All authors had full access to the data involved in the study and accept responsibility to submit for publication. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

CRediT roles

All authors contributed to this manuscript.

JH – conceptualisation, Writing - original draft, Writing - review & editing; AB – conceptualisation, Writing - original draft, Writing - review & editing; CM – conceptualisation, Writing - original draft, Writing - review & editing; NB – Writing - review & editing; YW – Writing - review & editing; CS – Writing - review & editing; MB – Writing - review & editing; LSPM – Writing - review & editing; NM – Writing - review & editing; TR – Writing - review & editing; AW – Writing - review & editing; MN – Writing - review & editing; SD – Writing - review & editing; RJ – conceptualisation, Writing - original draft, Writing - review & editing, supervision; DAP – conceptualisation, Writing - original draft, Writing - review & editing, supervision; BMP – conceptualisation, Writing - original draft, Writing - review & editing, Supervision.

Conflict of interest

There are no known conflicts of interest associated with this work.

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SARS-CoV-2 humoral and cellular immune responses in COVID-19 convalescent individuals with HIV ☆[1]

Dear Editor,

Recently, Venturas et al. had reported in this Journal that HIV infection is neither a risk factor for moderate or severe COVID-19 nor for mortality.[1] Moreover, Martin-Vicente described that people with HIV (PWH) presented similar levels of anti-SARS-Cov-2 antibodies compared to HIV-negative individuals.[2]

The outbreak of SARS-CoV-2, etiologic agent of COVID-19, has raised concerns around the world and Argentina was not the exception.[3] PWH were, *a priori*, considered an at-risk population for severe COVID-19. As clinical and immunological data was generated, controversies emerged mainly because different studies included PWH with different progression rates and PWH on and off antiretrovirals (ART). Reports in line with Venturas et al. indicate that HIV infection is not a risk factor for severe COVID-19 as long as individuals are on-ART and have a preserved CD4+T cell count.[4] In agreement with Martin-Vicente, others have shown that similar antibody responses can be achieved by PWH on ART with complete HIV suppression compared to HIV-uninfected individuals.[5] However, lower conversion rates in PWH, compared to HIV-negative persons, were also reported by other researchers.[6,7] In addition, memory cellular immunity could be assessed after SARS-CoV-2 infection in PWH[7]. Studies such as those of Venturas et al.[1] and Martin-Vicente et al.,[2] are key to have a deeper and more comprehensive understanding of SARS-CoV-2 immunity in PWH, which is fundamental to apply health care strategies (including optimal vaccination strategies) tailored for this population.

In this line, we aimed to study the immune landscape that occurs after COVID-19 in PWH. To that, we performed a cross-sectional study. Between April and December 2020 peripheral blood samples from donors to the Argentinean Biobank of Infectious Diseases (BBEI) with confirmed COVID-19 diagnosis were collected at convalescence: 29 PWH with preserved CD4+T-cell counts on ART and 29 HIV-negative individuals (HIVneg) were included. Written informed consent was obtained from all partici-

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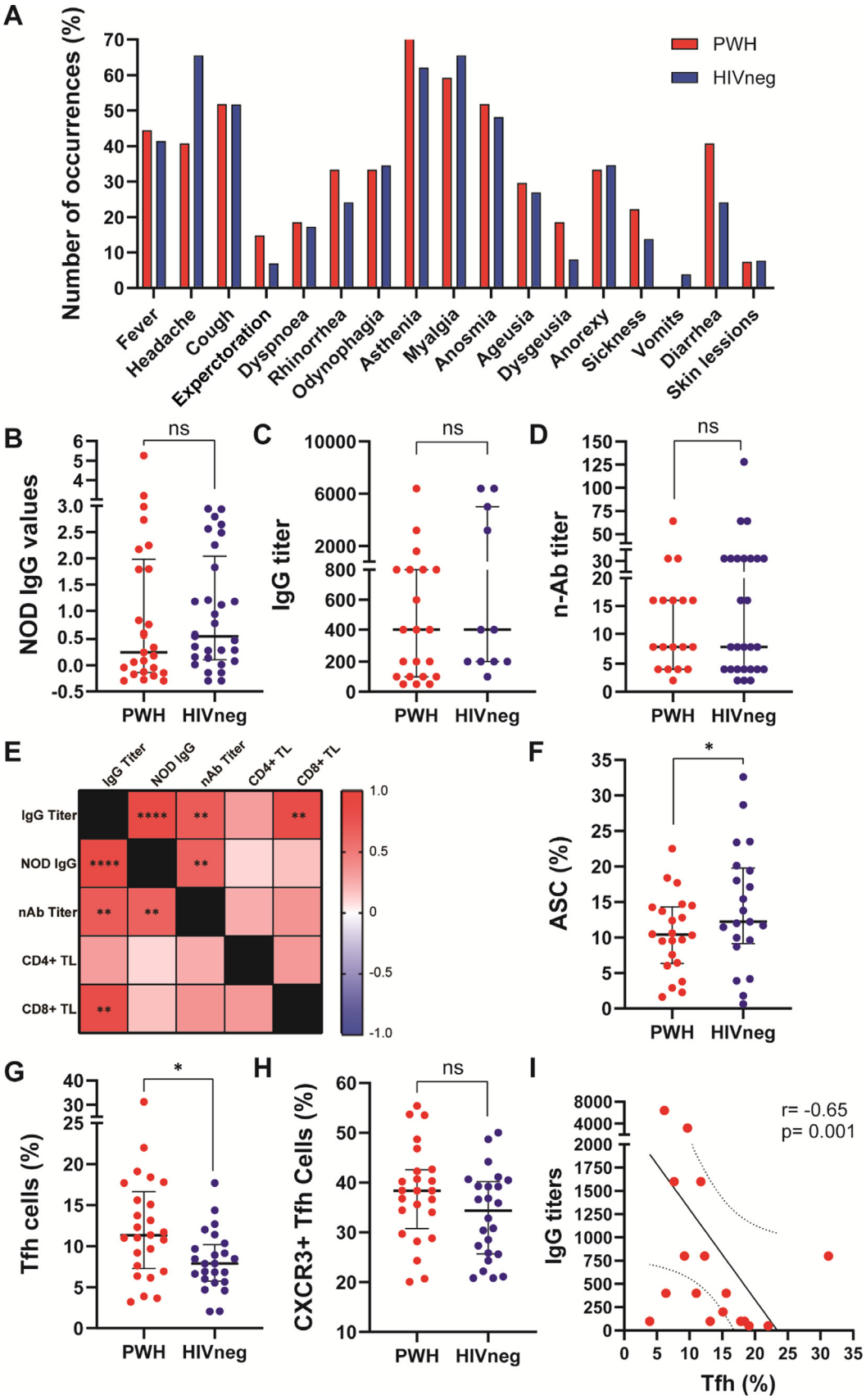

Figure 1. Clinical manifestations, anti-SARS-CoV-2 antibody responses, B and T cell phenotype in PWH and HIVneg individuals. (A) Frequency of occurrence of the depicted symptoms in PWH (red) and HIVneg (blue) cohorts. (B) IgG NOD values, (C) IgG titers, and (D) neutralizing Anti-SARS-CoV-2 antibodies were determined in plasma from PWH and HIVneg individuals (COVIDAR kit, Laboratorio Lemos S.R.L., Argentina). Normalized optical density (NOD) values were calculated by subtracting the cut-off value to each donor sample OD value, and the resulting value was divided by the mean positive control OD value. Mann-Whitney test was used. $p < 0.05$ were considered significant. Data are expressed as median and interquartile range. (E) Heatmap depicting from red (+1) to blue (-1) Spearman Rank correlation values between each parameter. p values per correlation are shown in those boxes where statistics were significant. $^{**}p < 0.01$; $^{****}p < 0.0001$. Frequency of (F) antibody-secreting cells (ASC), (G) Tfh, and (H) CXCR3+ Tfh cells in SARS-CoV-2 convalescent PWH (n =25) and HIV-negative (n =24) individuals via traditional gating flow cytometric analysis. Each dot represents an individual donor. Data are expressed as median and interquartile range. (I) Spearman test (two-tailed) showing a negative correlation between IgG titters and frequency of Tfh in PWH.

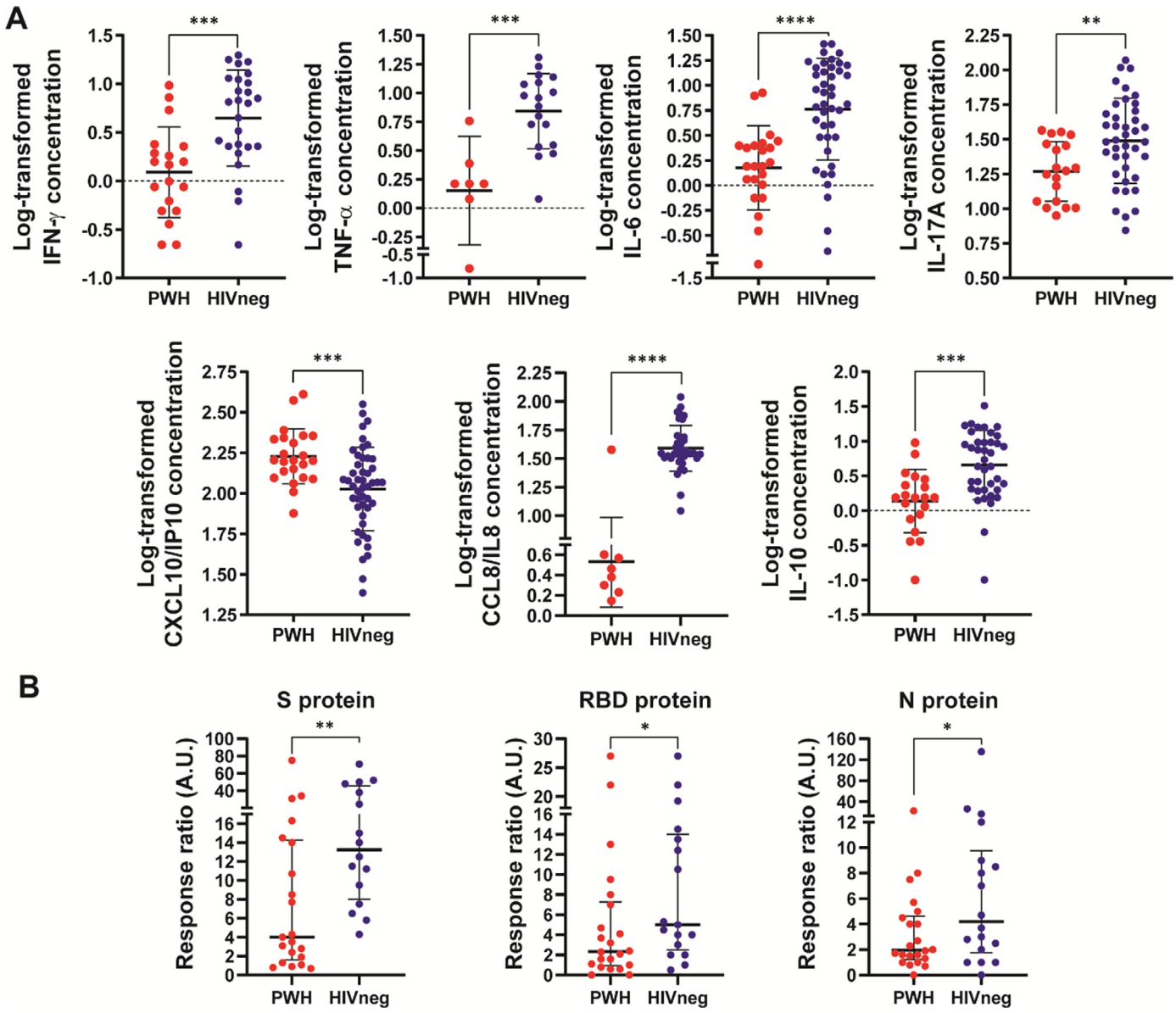

Figure 2. Assessment of serum cytokines and chemokines and cellular immunity in PWH and HIVneg donors. (A) The concentrations of CCL8/IL8; CXCL10/IP10; IFN-γ; TNF-α; IL-17A; IL-10 and IL-6 were determined by a multiplex assay and flow cytometry. Data are depicted as the log-transformed concentration values (pg/mL). Each point represents an individual donor. Data are expressed as median and interquartile range. Significance was determined by two-tailed Mann–Whitney *U* test, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. (B) IFN-γ ELISpot assays were performed to determine the frequency of Ag-experienced T cells in peripheral blood from the individuals enrolled. Stimulation of PBMCs with Spike (S) protein, RBD protein or Nucleocapside (N) protein was performed. Afterwards, IFN-γ producing cells were determinined as illustrated in the *Supplementary Materials* section. In order to compare group differences, data were normalized to media levels. Each dot represents an individual donor. Data are expressed as median and interquartile range. Significance was determined by two-tailed Mann–Whitney *U* test, $^{*}p < 0.05$; $^{**}p < 0.01$. A.U.: arbitrary units.

pants. Research was conducted according to protocols approved by the institutional review board of Fundación Huésped, Argentina.

Serum, plasma and PBMCs were fractioned from blood samples. Levels (normalized optical density, NOD) and titers (2-fold bases) of SARS-CoV-2-specific IgG antibodies were evaluated by ELISA. Viral neutralization assays were performed as described in *Supplemental methods*. Flow cytometry was performed to determine the frequency of CD4+ and CD8+T-lymphocytes, antibody-secreting cells (ASC), follicular helper T-cells (Tfh) and soluble plasma mediators (CBA assays). Finally, SARS-CoV-2-specific T-cell responses were determined by ELISpot assays using viral proteins and peptide pools as stimuli (*Supplemental materials*).

The median age of HIV-negative donors was 41 years (IQR: 35-51.5) and 11/29 (37.9%) were male. In PWH, median age was 44 years (IQR: 34.7-53.5) and 22/29 individuals (75.8%) were male. Median CD4+ T-cell count was 513 cells/μL (IQR: 351-873). All individuals had confirmed COVID-19 infection before enrolment (positive SARS-CoV-2 RT-PCR). In both groups 23/29 individuals showed a mild to moderate COVID-19 presentation, whereas the remaining individuals presented a severe disease. The mean time from symptoms onset to sampling was 69 days (IQR: 31-93) for PWH and 41 days (IQR: 18.5-55) for HIVneg. Both groups displayed similar symptom patterns with no statistical differences (Chi square test, Yates correction; significance level: 0.05; not significant for any symptom analyzed, Fig. 1A). We observed similar levels of SARS-CoV-2-specific IgG, IgG titers and neutralizing antibodies between groups (Fig. 1 B, C and D respectively). Within PWH, neutralization capacity correlated with IgG titers (r:0.70, p<0.001) and NOD values (r:0.65, p<0.001). Additionally, IgG titers were associated with NOD values (r:0.87, p:0.0001) and CD8+TL

counts (r:0.82, p<0.001) (Fig. 1E), as was also reported previously by our group in HIVneg donors.[3]

Regarding relevant lymphocytic populations involved in the immune response against SARS-CoV-2, diminished percentages of circulating ASC were observed among PWH compared to HIVneg (Fig. 1F). Although PWH displayed augmented percentages of Tfh $CD4^+$ cells, the proportion of CXCR3-expressing Tfh subset, a population involved in antibody responses against viral infections and vaccination,[8] were similar between groups (Fig. 1G and H). Importantly, we observed a negative correlation between IgG titers and Tfh proportions in PWH (Fig. 1I), therefore highlighting that the dysregulation previously reported of TFh among PWH play an important role in the capacity to exert specific humoral responses against SARS-CoV-2.[9]

Regarding plasma concentration of cytokines and chemokines, a marked decrease of IL-8/CCL8 and increased IP-10/CXCL10 levels were observed in PWH compared to HIV-neg (Fig. 2A). Moreover, significantly diminished levels of IFN-γ, TNF-α, IL-17A, IL-6 and IL-10 plasma concentrations were noticed in PWH compared to HIVneg (Fig. 2A). Notably, IL-8/CCL8 was not detected in 65.2% of PWH, whereas in HIVneg it was undetectable only in 22.9% of individuals ($p = 0{,}0005$, *Chi* square test, Yates correction).

Advancing on our studies, we determined T-cell responses against SARS-CoV-2 proteins and peptide pools. Our data shows an overall diminished response against SARS-CoV-2 antigens, specifically against Spike, RBD and Nucleocapside whole proteins in PWH (Fig. 2B), with no differences in T-cell responses against Spike or Nucleocapside peptide pools (data not shown). These data show that although PWH presented lower memory T-cell responses against SARS-CoV-2 compared to HIV-negative donors and a dysregulated Tfh population, that is enough to generate a T-B collaboration that allows to elicit a detectable humoral response against the pathogen.

Untreated HIV infection has been proposed as a serious comorbidity for COVID-19, but it is increasingly clear that suppressive ART and conserved CD4+ T-cell levels provide a proper environment for the generation of an effective immunity against SARS-CoV-2, not different to HIV-negative individuals.[10] Our data support the landscape of reduced cellular responses and altered plasma cytokines concurrent with effective antibodies responses against SARS-CoV-2 in PWH on-ART, which reinforces the idea of a significant impact of ART not only in HIV control but also in reducing overall morbi-mortality by, for instance, helping to restrict other infections.

Authorship

NL and MFQ conceived and designed experiments; DG, MBV, NL and MFQ analyzed and interpreted the data and wrote the manuscript. DG, MBV, AC, MLP and LC processed samples and performed experiments. SB, BWG, NL and YL recruited donors, collected samples and obtained clinical data. YL performed serological studies. VGP performed and analyzed flow cytometry data. GT, NL and YG contributed reagents/materials and analyzed and interpreted the data. All authors contributed to the refinement of the report and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.05.026.

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Lymphocyte subset changes in neonates with respiratory syncytial virus pneumonia

Dear Editor,

We read with great interest the study by Luo et al., which reported respiratory syncytial virus (RSV) infection mainly occurred in children (<5 years old) and the elderly (≥60 years old).[1] RSV is one of the main pathogens causing serious lower respiratory tract infections (LRTI) in infants and children. At least 17.3% of infants under 23 months of age are infected more than twice with RSV.[2] According to a global meta-analysis of causes of death by age group in 2010, LRTI caused by RSV accounted for approximately 2–3% of all neonatal deaths.[3] Severe RSV infection can increase the incidence of asthma and chronic wheezing in older children and adults, which is thought to be related to the TH2 cellular immune response.[4] However, most studies have focused on infants and elderly adults, and less on neonates, especially regarding immunological changes before and after RSV infection. The immune system of neonates is very different from that of older children and adults. In this letter, we report variations in immune cells (T, B, NK) and cytokines (IFN-γ and IL-4) in peripheral blood samples collected from neonates with RSV pneumonia.

The study group consisted of 23 randomly selected neonates with RSV pneumonia and was defined as the acute stage within 48 h after admission. The control group consisted of 20 neonates with convalescence of simple neonatal hyperbilirubinemia. After 5–10 days of hospitalization, the convalescent stage was defined as the time when the patient's temperature became normal, with no dyspnea or cyanosis, the cough was reduced or gone, and the lung signs had disappeared. There were no significant differences in birth weight, gestational age, and male to female ratio between the study group and the control group (Table 1 in Supplementary Material). The main clinical manifestations in this study were sneezing, runny nose, coughing, shortness of breath, foaming choking milk, and moist pulmonary rales. Only two babies had wheezing rales. Routine blood examination showed low leucocyte levels, the lymphatic classification being the most important, C-reactive protein not increasing, and chest radiographs revealing patchy shadows in the lung.

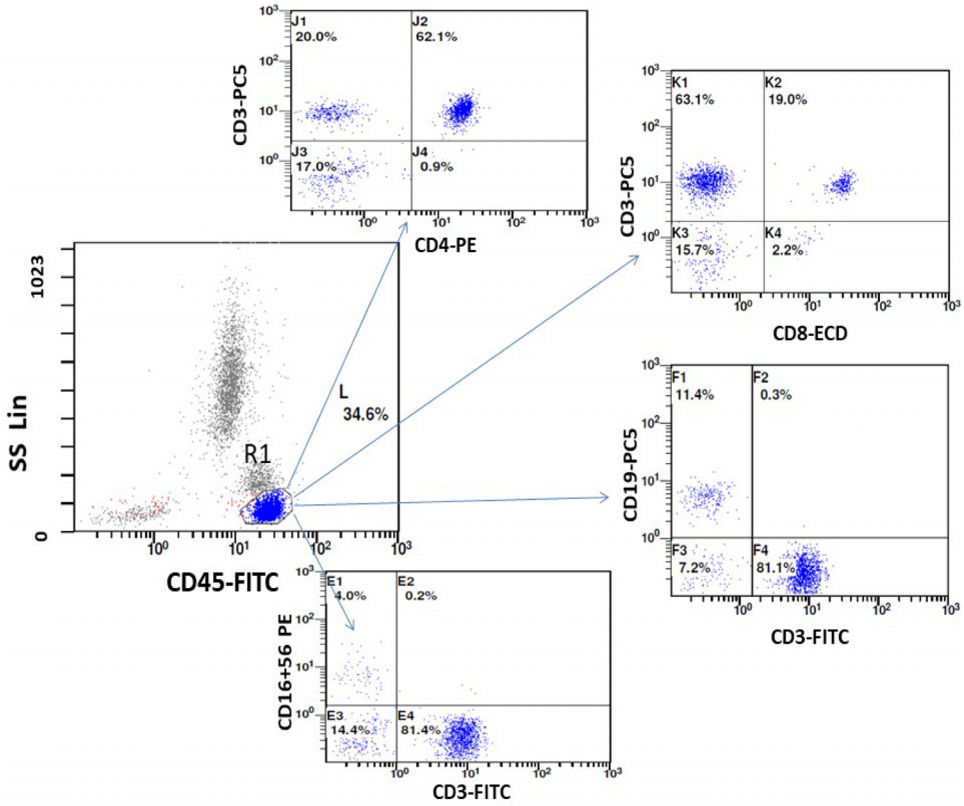

Fig. 1. Detected results of lymphocyte subsets by flow cytometry. Gate R1 represented lymphocytes collected. On the right are isolated CD4+ T cells (J2), CD8+ T cells (K2), B cells (F1), and NK cells (E1).

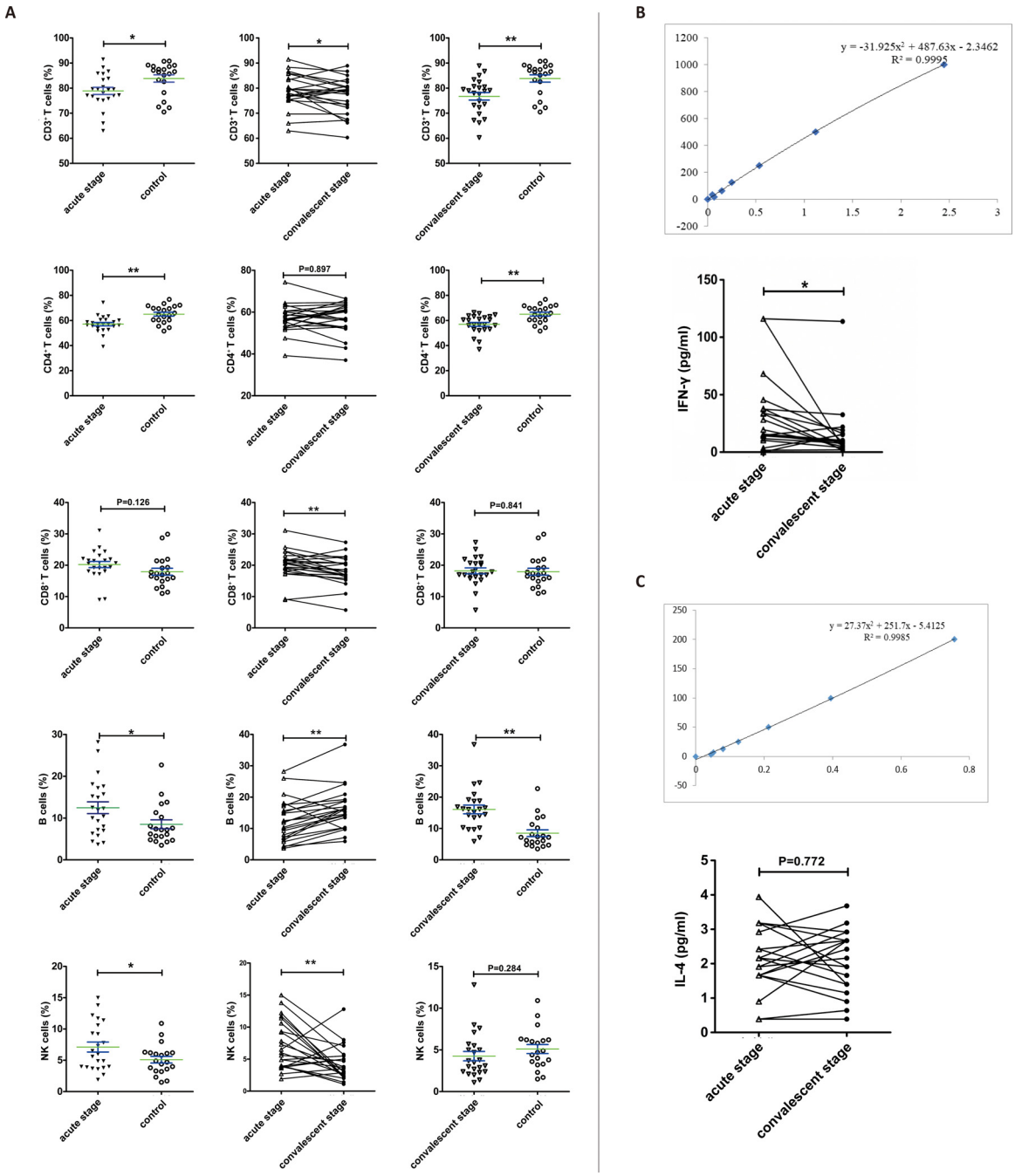

Fig. 2. (A). Comparison of lymphocyte subsets between the acute and convalescent stages of RSV pneumonia and the control group. (B, C) The standard curve of IFN-γ and IL-4, and comparison of IFN-γ between the acute stage and the convalescent stages of respiratory syncytial virus (RSV) pneumonia. *, $P < 0.05$; **, $P < 0.01$.

Peripheral blood samples were drawn from the study group at the acute and convalescent stages and from the control group simultaneously. Flow cytometry was used to detect the proportion of peripheral blood lymphocyte subsets (Fig. 1). After RSV infection, the number of CD3+ T cells decreased in both the acute and convalescent stages (acute stage vs. control group: 78.9% ± 6.8% vs. 83.8% ± 6.6%, P<0.05; convalescent stage vs. control group: 76.7% ±7.1% vs. 83.8% ± 6.6%, P<0.01), and the convalescent stage was lower than the acute stage (76.7% ± 7.1% vs. 78.9% ± 6.8%, P<0.05). Likewise, the level of CD4+ T cells was reduced in both acute and convalescent stages (57.1% ± 6.6%, 56.9 ± 7.5% vs 65.0% ± 6.9%, P<0.01), but their proportions were not statistically different (57.1% ± 6.6% vs 56.9% ±7.5%, $P = 0.897$). In contrast to CD3^+T and CD4^+T cells, the number of CD8^+T cells increased slightly after RSV infection but was not statistically significant (acute stage vs. control group: 20.2% ± 4.7% vs. 17.9% ± 5.0%, $P = 0.126$; convalescent stage vs. control group 18.3% ± 4.6% vs. 17.9% ± 5.0%, $P = 0.841$). However, the proportion of CD8^+T cells in the convalescent stage of the study group was significantly lower than that in the acute stage (18.3% ± 4.6% vs. 20.2% ± 4.7%, P<0.01). We found that the ratio of CD4+/CD8+ cells decreased after RSV infection (P<0.01) and increased with disease recovery (P<0.05). The proportion of B cells increased significantly after RSV infection and was higher in the convalescent stage than in the acute stage (acute stage vs. control

group: 12.5% ± 6.7% vs. 8.5% ± 4.8%, $P<0.05$; convalescent stage vs. acute stage: 16.1% ± 6.6% vs. 12.5% ± 6.7%, $P<0.01$). The proportion of NK cells in the peripheral blood of neonates increased after RSV infection and the convalescent stage could be reduced to normal (acute stage vs. control group: 7.1% ± 3.8% vs. 5.1% ± 2.4%, $P<0.05$; convalescent stage vs. control group: 4.3% ± 2.7% vs. 5.1% ± 2.4%, $P = 0.284$). (Fig. 2A, Table 2 in Supplementary Material)

The concentration of IFN-γ and IL-4 cytokines in serum were determined by an enzyme-linked immunosorbent assay (Fig. 2B&C). In our study, IFN-γ concentration in serum increased in neonates with acute RSV pneumonia and decreased with the convalescent stage of the disease (convalescent stage vs. acute stage: 15.6 pg/ml ± 24.4 pg/ml vs. 31.9 pg/ml ± 33.2 pg/ml, $P<0.05$). The level of IL-4 showed little change, and its concentration was low. The IFN-γ/ IL-4 ratio increased in the acute stage of infection but returned to normal levels in the convalescent stage (Table 2 in Supplementary Material).

This study showed that immune cells and cytokines were involved in anti-RSV infection. RSV can stimulate the production of asthma-related factors by heightening allergen sensitization and inducing Th1 and Th2 responses.[5] Neonatal immunity was characterized by low Th1 and Th2 levels.[6] After RSV infection, the imbalance of Th1/Th2 response represented by IFN-γ/IL-4 was involved in the pathogenesis process; however, the results of different studies were inconsistent as to whether the body presents Th1 or Th2 dominance after RSV infection.[7,8] Our study proved that the levels of CD8+ T cells, B cells, NK cells, and cytokine IFN-γ of Th1 were elevated in the acute stage of neonatal RSV pneumonia, which may play a role in anti-RSV infections. CD8+ T cells, NK cells, and IFN-γ levels could be reduced back to normal during the convalescent stage of neonatal RSV pneumonia, suggesting that when neonates were infected with RSV, the dominant response was from Th1. However, the role of Th2 cells in the disease remained unclear. In addition, antibodies produced by B cells directly neutralized free viruses or exerted antiviral effects through immune conditioning of extracellular viruses or infected cells.[9] The number of B cells in the study increased further, and humoral immunity continued to be maintained. Understanding the immune signature of neonatal RSV infection is key to addressing this issue.[10]

Credit authorship contribution statement

Peicen Zou: Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Guigui Li:** Data curation, Formal analysis, Investigation, Validation, Writing – original draft. **Xiaoling Ge:** Formal analysis, Investigation, Validation. **Jie Wang:** Formal analysis, Validation. **Xiaolin Wang:** Investigation, Resources. **Ying Li:** Supervision. **Ying Liu:** Resources, Supervision. **Jinjing Zhang:** Data curation, Resources, Validation. **Jingang Gui:** Conceptualization, Writing – review & editing. **Yajuan Wang:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Writing – review & editing.

Ethics approval

This study was reviewed and approved by the Ethics Committee of the Beijing Children's Hospital. All methods were performed by relevant guidelines and regulations.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

Not applicable.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.05.025.

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A Mendelian randomization cytokine screen reveals IL-13 as causal factor in risk of severe COVID-19

Dear Editor,

We read a recent paper by Li and colleagues with interest, who reported association of genetic polymorphisms in *IFITM3* with COVID-19 susceptibility and severity with implicated conclusions for vaccination or therapeutic goals.[1] In the current study we leveraged the previously discovered association of single-nucleotide polymorphisms (SNPs) for circulating cytokine levels to investigate their causal role in severe COVID-19. To this end, we used the promising methodology of Mendelian randomization (MR) to infer potential causality of risk factor-disease associations. This method uses genetic variants as instrumental variables to test for the effect of exposures on outcomes while minimizing confounding bias. We selected a recently introduced, powerful MR approach, i.e., Generalized Summary-data-based MR (GSMR),[2] to detect any potentially causal links between baseline blood cytokine levels and severe COVID-19 disease. We then confirmed the observed links with MR sensitivity analyses and also validated the findings using replication in three independent datasets: 1) replication in an independent East Asian GWAS for severe COVID-19, 2) internal replication using subsets of the discovery GWAS for severe COVID-19, and 3) replication at transcript level using an independent set of SNPs as instrumental variables. Subsequently, for any cytokine showing a significant effect on severe COVID-19 we performed MR analyses with other cytokine levels as outcomes, to examine their regulatory role.

Our study is based on the GWAS results of 4336 very severe COVID-19 cases and 623,902 controls (Supplementary Table 1), conducted by the Host Genetics Initiative consortium [3] with severe COVID-19 disease (or death due to COVID-19) as the outcome. For the exposures, we used GWAS results of 41 cytokines and growth factors from an independent population of 8293 individuals.[4] For independent replication, we used GWAS results of 65 East Asian severe COVID-19 cases versus 138 ancestry-matched controls [5] and for replication at transcript level, we used expression quantitative trait loci data from blood T cells by Chen et al. [6]Detailed description of the data and methods is given in Supplementary Materials.

We were able to test 13 cytokines having at least 3 independent SNPs for GSMR of which our analyses suggested a causal role for IL-13 in severe COVID-19 with each standard deviation (SD) increase in plasma levels of IL-13 leading to about 20% higher odds of developing severe COVID-19 (OR [CI 95%]=1.23 [1.05–1.44], $P = 0.0087$) (Table 1 and Fig. 1A). The full results of all tested cytokines are represented in Supplementary Table 2.

Leave-one-SNP-out sensitivity analysis showed that none of the SNPs used is driving the association. However, in one case, i.e., when excluding rs9472168, the association becomes stronger (OR [CI 95%] = 1.51 [1.09–2.10]; per standard deviation increase in IL-13 levels) (Fig. 1B). This could be an influential, potentially pleiotropic SNP, the inclusion of which may have resulted in underestimation of the MR effect. Reverse MR analysis of IL-13 did not provide evidence for reverse causation (Beta[se] = −0.05[0.04]; p-value = 0.25). Neither the heterogeneity nor the pleiotropy tests were significant for IL-13 (Q[df] = 1.37[3], Egger intercept[se] = 0.07[0.05]; p-values = 0.71, and 0.23, respectively). Furthermore, except for the MR Egger method all other four MR approaches confirmed the observed causal association (Table 1).

The observed causal role of IL-13 in severe COVID-19 was consistently validated and confirmed by all three validation strategies. First, independent replication in an East Asian GWAS of severe COVID-19 returned a significant effect (OR [CI95%] = 6.17 [1.42–26.84]; $P = 0.01$) (Supplementary Fig. 1). Second, internal replication in the GenOMICC study confirmed the observed significant results (OR [CI 95%] = 1.23 [1.01–1.50], $P = 0.04$). Finally, also replication at transcript level yielded a significant association (OR [CI95%] = 1.27 [1.15–1.41]; $P = 4.09 \times 10^{-6}$) (Supplementary Fig. 2).

We also identified 13 nominally significant causal associations for IL-13 against blood levels of other cytokines, of which six passed a Bonferroni threshold of $P < 0.00125$ i.e., 0.05/40. These include VEGF, IL12p70, IL10, IL7, IL5, and IFNg (Supplementary Table 3).

In summary, we found an OR of about 1.2 for severe COVID-19 per 1 SD increase in IL-13 levels and validated this finding in three independent datasets. IL-13 has previously been studied in asthma with its contribution to IgE production, histamine release and inflammation,[7] as well as barrier damage in the airways.[8] An important observation supporting its role in severe COVID-19 is provided by a recent study reporting that COVID-19 patients receiving Dupilumab, a monoclonal antibody which blocks IL-13/IL-4 sig-

Table 1
Results of IL-13 association with severe COVID-19 from different MR approaches.

Method	N_SNPs	Effect estimate	SE	P	OR	CI 95%
Primary MR analysis						
GSMR	5	0.21	0.08	0.008752	1.23	1.08–1.39
MR sensitivity analysis						
Inverse variance weighted	5	0.21	0.08	0.008422	1.23	1.08–1.39
Weighted median	5	0.17	0.08	0.042155	1.19	1.03–1.34
MR Egger[#]	5	−0.02	0.17	0.921923	0.98	0.65–1.31
MR-PRESSO[*]	5	0.21	0.07	0.049244	1.23	1.10–1.37

[#] As the directional pleiotropy test is not significant, the MR Egger result is not informative.
[*] P-value of global test (pleiotropy hypothesis) = 0.5. P-values of heterogeneity and pleiotropy tests for IL-13 are 0.71 and 0.23, respectively.
SE: Standard error of estimate; N_SNPs: the number of genome-wide significant SNPs for IL-13 that are used as instrumental variable in GSMR analysis.

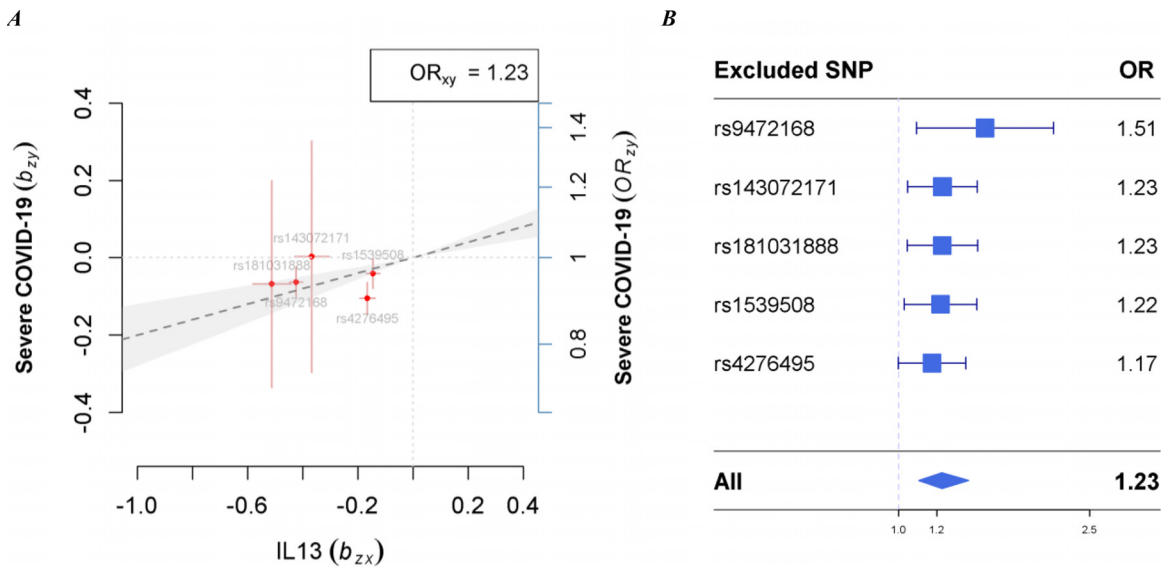

Fig. 1. (A) The independent ($r^2 < 0.05$) SNPs associated with IL-13 (p-value $< 5 \times 10^{-8}$) and their associations with severe COVID-19. (B) MR leave-one-out sensitivity analysis of IL-13 levels on severe COVID-19, using the five significant, independent IL-13 SNPs ($r^2 < 0.05$, p-value $< 5 \times 10^{-8}$). b_{zx} indicates the effect of SNP (z) on IL-13 level as exposure (x); OR_{zy} and b_{zy} indicate the effect of SNP (z) on severe COVID-19 as outcome (y), in odds ratio and logodds scale, respectively. Red lines indicate standard error of effect estimates. These five SNPs explain ~9% of IL-13 variance.[4] The dashed line shows the overall estimated effect of IL-13 levels on severe COVID-19 based on all SNPs through a generalized least square approach (GSMR). GSMR effect size (se): 0.2 (0.08), OR (CI95%) = 1.23 (1.05–1.44), $P_{GSMR} = 0.0087$; i.e. one SD increase in IL-13 levels will lead to a 23% higher chance of developing severe COVID-19 symptoms.

naling, had less severe disease.[9] Warranting further investigations, a study in SARS-CoV-2-infected mice has shown that IL-13 inhibition leads to reduced hyaluronan expression, a polysaccharide which deposits in lungs of severe COVID-19 patients, as well as reduced mortality and disease severity.[9] Our observation of a positive causal effect of IL-13 on a number of other cytokine levels is in line with a previously described key regulatory role of IL-13 in the cytokine storm of COVID-19 patients, where IL-13 recruits inflammatory cells (such as neutrophils, macrophages, eosinophils, and lymphocytes) to the lung mucosae, leading to hyper-production of various pro-inflammatory cytokines.[10]

Despite successful identification (and consistent independent validation) of a causal relationship for IL-13 on severe COVID-19, our study also suffers from a number of limitations: First, the causal effect of IL-13 on severe COVID-19 did not survive Bonferroni correction ($P < 0.0038$ i.e., 0.05/13; with 13 as the number of tested cytokines). This can be due to the limited power of a modest number of suitable genetic instruments available for MR. Second, cytokines without sufficiently strong genetic instruments were not included in our MR analyses and therefore, our results do not reject potential causal associations for these cytokines. Third, the majority of study populations for our analyses are of European descent, which limits the generalizability of the results to other ancestries. While we independently replicated our findings using GWAS results of severe COVID-19 in an East Asian population ($n = 203$), we didn't have IL-13 GWAS in the same ancestry. Larger efforts using exposure and outcome GWASs from the same/similar populations are needed to unravel trans-ancestry portability of this finding.

To conclude, our study provides evidence for a causal effect of IL-13 on severe COVID-19. As such, further investigation is warranted exploring IL-13 as a potential therapeutic target for patients with severe COVID-19 or for those that are at risk of developing severe symptoms.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.05.024.

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