Elevated Monoclonal and Polyclonal Serum Immunoglobulin Free Light Chain (FLC) as Prognostic Factors in B- and T-cell Non-Hodgkin Lymphoma

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Abstract

The serum immunoglobulin free light chain (FLC) assay quantitates free kappa (κ) and lambda (λ) light chains. FLC elevations in patients with diffuse large B-cell lymphoma (DLBCL), Hodgkin lymphoma (HL), and chronic lymphocytic leukemia (CLL) are associated with an inferior survival. These increases in FLC can be monoclonal (as in myeloma) or polyclonal. The goal was to estimate the frequency of these elevations within distinct types of B-cell and T-cell non-Hodgkin lymphoma (NHL) and whether the FLC measurements are associated with event-free survival (EFS). We studied serum for FLC abnormalities using normal laboratory reference ranges to define an elevated κ or λ FLC. Elevations were further classified as polyclonal or monoclonal. 492 patients were studied: 453 B-cell and 34 T-cell NHL patients. 29% (142/453) had an elevated FLC of which 10% were monoclonal elevations. Within B-cell NHL, FLC abnormalities were most common in lymphoplasmacytic lymphoma (79%), MCL (68%) and MALT (31%); they were least common in FL (15%). The hazard ratio (HR) for EFS in all patients was 1.41 (95% CI; 1.11–1.81); in all B-cell NHL the HR was 1.44 (95% CI 1.11–1.96); in all T-cell NHL the HR was 1.17 (95% CI 0.55–2.49). FLC abnormalities predicted an inferior OS (HR = 2.75, 95% CI: 1.93–3.90, p<0.0001). The serum FLC assay is useful for prognosis in both B-cell and T-cell types of NHL. In B-cell NHL further discrimination between a monoclonal and polyclonal elevation may be helpful and should be analyzed in prospective clinical trials.

Keywords

free light chains; lymphoma; T-cell lymphoma; B-cell lymphoma
INTRODUCTION

Intact human immunoglobulins contain a heavy chain (Igγ, α, μ, δ, or ε) and either a kappa (κ) or lambda (λ) light chain. Quantitation of heavy and light chains is important to diagnosis and management of a wide variety of diseases. Small amounts of κ and λ immunoglobulin light chains circulate in normal human serum and can be quantified by the free light chain (FLC) assay. In patients with illnesses that involve systemic inflammation or renal insufficiency the elevations of these FLC are polyclonal (increases in one or both light chains with a normal κ λ ratio) and are associated with increased all-cause mortality. In contrast, in B-cell malignancies such as multiple myeloma and AL-amyloidosis the elevation is typically monoclonal, defined as elevation of one FLC resulting in an abnormal ratio.

We have applied the serum FLC assay to lymphoid malignancies other than myeloma or amyloid and found monoclonal FLC in 13% of patients with a variety of lymphoma types. The prevalence of monoclonal FLC was highest in mantle cell (36%) and small lymphocytic (24%) lymphomas; it was found in 8% of patients with DLBCL. In this initial publication the prevalence and prognostic relevance of polyclonal increases in FLC were not studied. However, in a more recent study of 295 patients with new, untreated DLBCL we found 32% of patients with an elevated FLC of which 18% were polyclonal elevations. In CLL/SLL we reported that 49% (165/339) of patients had an abnormal FLC with 15% polyclonal elevations. Both monoclonal and polyclonal FLC elevations were associated with an adverse prognosis in both CLL and DLBCL, independent of established clinical prognostic factors. In Hodgkin lymphoma (HL) 30% had elevated FLC (all polyclonal) and this was an adverse prognostic factor. In addition to elevated FLC values we have also studied ratio-only FLC abnormalities, defined as an abnormal κ λ ratio without elevation of either κ or λ FLC. In DLBCL ratio-only FLC abnormalities were not prognostic but they were in CLL.

In this report we expand our studies of the prognostic impact of serum FLC to T-cell NHL and types of B-cell NHL other than CLL/SLL and DLBCL. The goal was to understand not only the prevalence of FLC abnormalities in these diseases but also to evaluate the clinical importance of monoclonal or polyclonal elevations in a new, independent population of patients from the Lymphoma SPORE Molecular Epidemiology Research (MER) data set.

Patients and Methods

Patients

Newly diagnosed patients with lymphoma were prospectively enrolled in the University of Iowa/Mayo Clinic SPORE Molecular Epidemiology Resource (MER). This study was approved by the Institutional Review Board and all patients signed informed consent to have their samples used for research. This report does not include FLC results from any of our previously reported patient groups.
Free light chain assay

Serum FLC was quantitated from enrollment research serum using the FREELITE assay (The Binding Site, Ltd., Birmingham, UK). The FLC assays were performed by the Mayo Clinic Clinical Immunology Lab using kits provided courtesy of The Binding Site (Birmingham, UK). Abnormal κ/λ FLC ratio was *a priori* defined as a κ/λ FLC ratio outside of the published normal range for the test (0.26 – 1.65). Elevated FLC was defined as a κ concentration higher than 1.94 mg/dL or a λ concentration higher than 2.63 mg/dL (the published normal ranges for Mayo Medical Laboratories).\(^\text{14}\) The definition of a monoclonal elevation of FLC was defined as an elevated FLC with the corresponding FLC ratio outside the reference range (0.26–1.65). Polyclonal elevation of FLC was defined as an elevation of either or both κ or λ FLC outside the laboratory normal range but with a normal ratio.\(^\text{10}\) Abnormal ratios without elevation of either FLC were considered normal based on our previous studies in DLBCL that indicated these values were not prognostic.\(^\text{9}\) Event-free survival (EFS) was defined as the time from diagnosis to relapse, retreatment, or death due to any cause. Overall survival (OS) was defined as the time from diagnosis to death due to any cause. Patients without an event or death were censored at time of last known follow-up. Due to the infrequency of missing data, analyses were performed on complete case sets for the variables involved in each test or model. Cox proportional hazards models and Kaplan Meier curves were used to assess associations of FLC with EFS and OS when there were sufficient numbers (9 or more) of events or deaths within the subtype to evaluate. All models were unadjusted for clinical factors unless specified.

RESULTS

All Patients

492 patients were enrolled in the study and had a pretreatment sample evaluated for FLC. 453 (92%) had B-cell lymphoma with the most common subtypes being follicular lymphoma (n=237), MALT lymphoma (n=62), and mantle cell lymphoma (n=50); 34 had T-cell lymphoma and 5 patients had lymphoma not otherwise specified. Across all patients, 142 (29%) had an elevated FLC with 10% being monoclonal and 19% polyclonal (Table 1). An additional 35 patients (7%) had an abnormal FLC ratio without elevation of κ or λ. Compared to patients with normal FLC, those with elevated FLC were older (43% vs. 70% > age 60, p<0.0001), had worse performance status (5% vs. 12% ECOG PS ≥2, p=0.0048), more advanced stage disease (60% vs. 78% stage III/IV, p=0.0002), higher LDH (20% vs. 33% with LDH >ULN, p=0.0041), higher creatinine (mean creatinine 0.98 +/− 0.22 vs 1.14 +/− 0.51, p=0.0009), and more frequent B-symptoms (8% vs 16%, p=0.01).

At a median follow-up of 83 months (range, 1–121), 58% of patients had an event and 26% of patients had died. In all subtypes combined, patients with elevated FLC had an inferior EFS (HR = 1.41, 95% CI: 1.11–1.81, p=0.0058) and OS (HR = 2.75, 95% CI: 1.93–3.90, p<0.0001) compared to patients with normal FLC at diagnosis (Figure 1A, B). These associations remained significant after adjusting for age (≤60 vs. >60), stage (I/II vs. III/IV), ECOG PS (0–1 vs. 2–4), and LDH (≤ULN vs. >ULN) (EFS HR=1.33, 95% CI: 1.01–1.73, p=0.040; OS HR = 2.24, 95% CI: 1.52–3.30, p<0.0001). Outcomes were similar whether the elevation was polyclonal (EFS HR=1.34, 95% CI: 1.00–1.79, p=0.050; OS HR=3.01, 95%
CI: 2.04–4.46, p<0.0001) or monoclonal (EFS HR=1.56, 95% CI: 1.09–2.32, p=0.014; OS HR=2.31, 95% CI: 1.40–3.82, p=0.0011) (Figure 1C, 1D). The difference between monoclonal and polyclonal elevation was not significant (p=0.33).

**FLC Elevation by Lymphoma Type**

**B-cell Lymphoma**—In the 453 B-cell NHL, 27.8% had an elevated FLC with 10.4% monoclonal and 17.4% polyclonal. The FLC results in the different types of B-cell NHL are summarized in Table 1. The tumor types most likely to have elevated FLC were MCL (68%) and post-follicular NHL (41%); the least likely were FL (15%). In each type of NHL except lymphoplasmacytic lymphoma (LPL), there were more cases of polyclonal FLC elevations than monoclonal. Both monoclonal and polyclonal FLC elevations were associated with inferior EFS in most B-cell NHL types where there were adequate numbers of patients that allowed assessment of risk. Monoclonal elevations were associated with higher HR for shorter EFS than polyclonal elevations in MCL (EFS HR = 1.58, 95% CI: 0.79–3.19, p=0.20) and MALT NHL (EFS HR = 1.82, 95% CI: 0.84–3.93, p=0.13, figure 2c). Notably, elevated FLC was not associated with EFS in FL grades 1–2 (EFS HR=0.89, 95% CI: 0.53–1.49, p=0.65) (Figure 2c). With respect to OS, FLC elevations were associated with a poor outcome in FL grades 1–2 (HR=2.86, 95% CI: 1.35–6.09, p=0.0063, Figure 2b) and MALT (HR=3.85, 95% CI: 0.92–16.16, p=0.065, Figure 2c).

**T-Cell Lymphoma**—FLC elevations were found in 41% (14/34) of patients with T-cell NHL. As expected, all but one of these cases was a polyclonal FLC elevation. While T-cell NHL patients with elevated FLC showed no association with EFS (HR 1.17, 95% CI 0.55–2.49), FLC elevation was associated with inferior EFS in PTCL-NOS (HR 2.00, 95% CI 0.52–7.61), although that estimate was based on 14 cases and was not statistically significant (p=0.31).

**Discussion**

The secretion of immunoglobulin FLC is common in untreated NHL but varies substantially by types of FLC secretion – monoclonal vs polyclonal, and by the NHL subtypes. In our previous work using pre-treatment serum in 339 patients with untreated CLL/SLL, 49% had FLC abnormalities (17% monoclonal) and in 295 patients with untreated DLBCL, 32% had an elevated FLC (14% monoclonal). The data in the current study extends these findings to other types of B- and T-cell NHL. The highest frequencies of FLC abnormalities were found in LPL at 79%, a result similar to the reports of others. The next most common B-cell NHL type was MCL at 68% of patients. Furtado et al. reported that 52% (18/34) of relapsed MCL patients had abnormal FLC, and these patients had a shortened OS compared to those with a normal FLC ratio. In contrast to MCL, the patients with the lowest frequency of FLC abnormalities were those with FL at 15% (35/237). While FLC elevation showed no association with EFS in grade I-II FL, it was associated with inferior EFS in grade 3 FL. In addition, FLC abnormalities in FL patients did predict an inferior OS. This relationship between FLC elevation and OS in FL is likely a manifestation of patient co-morbidities, as well as correlation with other adverse clinical factors that impact OS. Thus, although FLC abnormalities do not predict...
progression of FL they may be an important predictor of OS that can be used in management of these patients. In the current study we included patients with two less common types of DLBCL- PMBCL and PCNSL. In PMBCL, none of the 8 patients had an elevated FLC; in PCNSL, only 1 patient of 12 was found to have an elevation. We did not have any patients in this study with transformed lymphoma, and this important group merits additional study.

The results in peripheral T-cell NOS were also interesting. Although FLC abnormalities were quite common (41%), they were virtually all polyclonal elevations and these patients had an inferior EFS. We previously have published\textsuperscript{12} that polyclonal elevation is the only type of FLC elevation found in HL and these elevations are associated with an adverse prognosis. The polyclonal FLC elevation in T-cell NHL is most likely due to a polyclonal B cell immune response to the T cell lymphoma. It will be important to include serum FLC in large prospective clinical trials of new agents that are ongoing for T-cell NHL to validate our initial findings.

The pathophysiology of FLC secretion in lymphoma cells most likely relates to the differentiation state and genetic defect of the cell when it becomes malignant.\textsuperscript{18} The frequency of FLC abnormalities increases in lymphomas that arise after the B-cell has traversed the germinal center. For example, PMBCL originates in a thymic B-cell\textsuperscript{18} and we found no PMBCL cases with elevated FLC. In the germinal center lymphomas such as FL the frequency was 15%, 31% in post-follicular MALT NHL, 32% of DLBCL\textsuperscript{9}, 44% of splenic marginal zone, 49% of CLL/SLL,\textsuperscript{10} 68% of MCL, and 79% of LPL. It is to be remembered that in untreated multiple myeloma, 95% of patients have an abnormal FLC.\textsuperscript{19} It is evident that FLC abnormalities become more frequent as tumors develop across the spectrum of B-cell differentiation to mature plasma cells. Within DLBCL we have previously reported that patients with monoclonal FLC abnormalities are more likely to have ABC-type DLBCL and also have increased serum levels of IL-12, sIL-2Ra, IL-1R, and IP-10 (all p<0.001).\textsuperscript{11} In contrast, DLBCL patients with polyclonal FLC elevations had higher levels of IL-6 (P= 0.033), IL-8 (P=0.025), sIL2Ra (P=0.011), and IL-1R1 (P= 0.041). These pro-inflammatory cytokines likely drive B-cell proliferation resulting in increased FLC secretion. In the T-cell NHL and Hodgkin lymphoma FLC secretion is all polyclonal and relates to inflammatory cytokines that produce the prominent symptoms of fevers, sweats, and fatigue that often affect these patients.

Serum FLC is a valuable marker for the plasma cell proliferative disorders and is commonly incorporated into clinical practice. The studies reported herein, taken together with our previous work\textsuperscript{8-10,12} and that of others\textsuperscript{17,20} indicate that FLC may be a valuable marker for prognosis in all types of Hodgkin and non-Hodgkin lymphoma. It is our recommendation that analysis of this serum biomarker be further studied in large randomized trials of lymphoma to validate our findings, learn if abnormal FLC measurements are independent of other biomarkers, and to assess their overall clinical utility.

Acknowledgment

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References


Figure 1.
Figure 2.

2a) Event-free Survival by Elevated FLC
Follicular Grade 1-2

2b) Overall Survival by Elevated FLC
Follicular Grade 1-2

2c) Event-free Survival by Elevated FLC
MALT

2d) Overall Survival by Elevated FLC
MALT
Table 1

Monoclonal versus polyclonal serum free light chain results by tumor type and subtype.

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<th>Total N</th>
<th>Sex, male</th>
<th>Age&gt;60</th>
<th>ECOG PS 2-4</th>
<th>Stage III/IV</th>
<th>LDH &gt; ULN</th>
<th>B Symptoms</th>
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<td>6%</td>
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Abbreviation: NHL, non-Hodgkin lymphoma; FL, follicular lymphoma; DLBCL, diffuse large B cell lymphoma; PMBCL, primary mediastinal B cell lymphoma; PCNSL, primary CNS lymphoma; PTLD, post transplant lymphoproliferative disorder; MALT, mucosa associated lymphoid tissue; LPL, lymphoplasmacytic lymphoma, PS, performance status, LDH, lactate dehydrogenase; ULN, upper limit of normal; NOS, not otherwise specified, FLC; free light chain.