Elevated Serum Free Light Chains Are Associated With Event-Free and Overall Survival in Two Independent Cohorts of Patients With Diffuse Large B-Cell Lymphoma

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ABSTRACT

Purpose

The serum free light chain (FLC) assay quantitates free kappa (κ) and free lambda (λ) immunoglobulin light chains. This assay has prognostic value in plasma cell proliferative disorders. There are limited data on serum FLC in B-cell malignancies.

Patients and Methods

The association of pretreatment FLC with event-free survival (EFS) and overall survival (OS) in diffuse large B-cell lymphoma (DLBCL) was evaluated in 76 patients from the North Central Cancer Treatment Group trial N0489 (NCT00301821) and 219 patients from the University of Iowa/Mayo Clinic Specialized Program of Research Excellence Molecular Epidemiology Resource (MER). Published reference ranges were used to define an elevated FLC or an abnormal κ : λ FLC ratio.

Results

Elevated FLC or abnormal κ : λ FLC ratio was present in 32% and 14% of patients, respectively. Patients with elevated FLC had an inferior OS and EFS in both cohorts compared with patients with normal FLC (N0489: EFS hazard ratio [HR], 3.06; OS HR, 3.16; both $P < .02$; MER: EFS HR, 2.42; OS HR, 3.40; both $P < .001$; combined EFS HR, 2.57; OS HR, 3.74; both $P < .001$). All associations remained significant for EFS and OS after adjusting for the International Prognostic Index (IPI). Abnormal κ : λ FLC ratio was modestly associated with outcome in the combined group (EFS HR, 1.61; OS HR, 1.67; both $P = .07$), but not in patients without corresponding elevated κ or λ . Elevated FLC was the strongest predictor of outcome in multivariable models with the IPI components.

Conclusion

Increased serum FLC is an independent, adverse prognostic factor for EFS and OS in DLBCL and warrants further evaluation as a biomarker in DLBCL.

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma (NHL) in the United States. Standard treatment for newly diagnosed DLBCL is rituximab and an anthracycline-based chemotherapy regimen, usually cyclophosphamide, doxorubicin, vincristine, and prednisone.^{1,2} The move to an immunochemotherapy approach has improved the event-free (EFS) and overall survival (OS) rates in DLBCL. The International Prognostic Index (IPI) is a clinical prognostic score developed for aggressive lymphoma in the chemotherapy treatment era.² Based on five clinical variables, it remains associated with outcome in the immunochemotherapy

era, although its general prognostic ability is modest.³ There is a need for additional prognostic markers in DLBCL to better identify patients who will relapse after immunochemotherapy or fail to achieve a remission. Gene-expression profiling studies have suggested that DLBCL may have several phenotypes, generally characterized by tumors expressing either a germinal center signal or activated B-cell signal.⁴ Several algorithms and gene sets have been developed to distinguish these tumor phenotypes and identify tumors with either a good or poor prognosis.^{5,6} Despite the active research in these areas, risk-adaptive treatment approaches based on tumor phenotype or other prognostics are still in the development stage at this time.

The free light chain (FLC) assay measures the concentration in the serum of immunoglobulin kappa (κ) and lambda (λ) light chains that are not attached to a heavy chain.⁷ Abnormalities in FLC are associated with plasma cell disorders.⁸ These abnormalities are monoclonal in nature and result in one of the chains being elevated, often substantially, producing an abnormal κ : λ ratio. As such, the serum FLC assay has demonstrated utility for screening, diagnosis, prognosis, and response assessment in multiple myeloma, monoclonal gammopathy of undetermined significance, and AL-amyloidosis.⁹⁻¹³ Elevation of FLC may also be secondary to a number of disease states associated with B-cell hyperplasia and immune stimulation.¹⁴ These elevations tend to be polyclonal and do not result in an abnormal κ : λ ratio. In addition, patients with renal impairment may demonstrate a polyclonal increase of serum FLC from a reduced capacity to clear the normal FLC from the blood.¹⁵

There are limited data on FLC in lymphoma and chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL). We previously reported that FLC abnormalities were present in 13% of 208 patients with lymphoma, with abnormal κ : λ FLC prevalence varying from 0% to 36% by lymphoma type.¹⁶ The prevalence of abnormal κ : λ FLC ratio was lowest in DLBCL (8%) and highest in mantle-cell lymphoma (36%) and CLL/SLL (24%); however, these results were based on only 25 patients of each NHL type. In a large series of 259 patients with CLL, Pratt et al¹⁷ reported 39% of patients had an abnormal FLC ratio and these patients had an inferior OS. This association with an adverse OS was confirmed in another cohort of patients with CLL.¹⁸ A recent study by Landgren et al¹⁹ found an association of elevated serum FLC with the risk of NHL in 291 individuals infected with HIV. In the studies of FLC in NHL by Martin¹⁶ and Landgren et al,¹⁹ no information on the role of FLC relative to outcome was presented. We report herein the largest study of serum FLC in a lymphoid malignancy to date and present the first report of FLC and outcome in DLBCL, to our knowledge, from two independent cohorts of patients.

PATIENTS AND METHODS

Study Population

This study was reviewed and approved by the human subjects institutional review board at the Mayo Clinic and the University of Iowa, and written informed consent was obtained from all participants. Patients in the first cohort were from the North Central Cancer Treatment Group clinical trial N0489 (NCT00301821); the second cohort was comprised of patients enrolled in the Molecular Epidemiology Resource (MER) of the University of Iowa/Mayo Clinic Lymphoma Specialized Program of Research Excellence (SPORE; CA97274). N0489 was a phase II trial of epratuzumab and rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone for patients with newly diagnosed, untreated DLBCL.^{20,21} Patients selected from the SPORE MER had newly diagnosed DLBCL and were managed off-study using standard treatment regimens for DLBCL.

The diagnosis of DLBCL was confirmed by the study hematopathologist (N0489: P.J.K.; MER: W.R.M. or S.I.S.) in all patients.²² Baseline clinical, laboratory, and treatment data were abstracted from medical records using a standard protocol (MER) or per the N0489 clinical trial protocol. MER participants provided an on-study peripheral blood sample for serum and DNA banking; N0489 patients had research serum drawn pretreatment, at restaging (after cycles 2 and 6), and every 3 months for 1 year. All patients were systematically followed every 6 months for the first 3 years, and then annually thereafter (MER) or every 3 months in year 1, every 4 months in

year 2, and every 6 months in years 3 to 5 (N0489). Disease progression, re-treatment, and deaths were verified through medical record review. For decedents, we obtained a copy of the death certificate as well as medical records associated with death. Tumor response was measured using the International Working Group response criteria^{23,24} on the N0489 clinical trial; in the MER response was not assessed per the clinical trial protocol, thus response was not used for this analysis.

One hundred seven patients were enrolled on North Central Cancer Treatment Group N0489 from February 2006 through August 2007; 24 were excluded for lack of serum sample and seven were excluded for not having DLBCL, leaving 76 patients for analysis. Two hundred nineteen patients with DLBCL with a pretreatment serum draw were enrolled into the MER from September 2002 through February 2008 and used in this analysis.

Serum FLC Measurements

The FREELITE assay (The Binding Site Ltd, Birmingham, United Kingdom) was used to measure serum κ and λ FLC concentrations and the κ : λ FLC ratio.^{7,25} The FLC assays were performed by the Mayo Clinic Clinical Immunology Lab using kits provided courtesy of The Binding Site. Abnormal κ : λ FLC ratio was a priori defined as a κ : λ FLC ratio outside of (0.26, 1.65) and elevated FLC as a κ concentration higher than 1.94 mg/dL or λ concentration higher than 2.63 mg/dL based on the published normal ranges for Mayo Medical Laboratories²⁵ and as used in previous studies.⁹⁻¹⁹

Statistical Analyses

The primary analysis was to assess the association of FLC concentrations and abnormal κ : λ FLC ratio with EFS and OS for DLBCL. FLC concentrations (κ , λ , and total FLC) were examined as continuous variables on the log scale. For analysis purposes, due to the biologic potential for a monoclonal increase in one light chain but not the other, elevated FLC was defined as having a λ and/or κ concentration above the established Mayo Medical Laboratories normal range. EFS was defined as the time from diagnosis to disease progression, re-treatment, or death due to any cause. 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. The local reference range was used to determine the upper limit of normal for clinically abstracted lab values. Cox proportional hazards regression models²⁶ were used to assess the association of FLC and outcome via unadjusted and IPI-adjusted hazard ratios (HR). Cox models on the combined data set were stratified by cohort. Kaplan-Meier²⁷ curves were used to graphically display the association of FLC with outcome. We also assessed the association of FLC and outcome using the continuously distributed FLC concentrations via penalized smoothing splines, or P-splines.^{27a} Logistic regression models were used to assess the association of FLC abnormalities with clinical characteristics via cohort-adjusted odds ratios. The prognostic capability of models were assessed with time dependent receiver operating characteristic curves; the area under the curve and concordance indices (C indices) were determined per the approach of Heagerty and Zheng²⁸ with bootstrap CIs used to assess the significance of differences. Analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC) and R version 2.7.1 (R Project <http://www.r-project.org/>).

RESULTS

Patient characteristics were generally similar between the two cohorts with the N0489 group having more patients with stage III/IV disease and more than two extranodal sites (Table 1). Median follow-up was 26.2 months (range, 7.5 to 40.8 months) in N0489 with 21 events and 17 deaths. Median follow-up was 34.9 months (range, 4.9 to 74.8 months) in the MER with 74 events and 57 deaths. Overall κ and λ measurements were similar in the two cohorts (Fig 1). In N0489, 34% had an elevated FLC and 12% of patients had an abnormal κ : λ FLC ratio; in the MER cohort, 31% and 15% had an elevated FLC and abnormal κ : λ FLC ratio, respectively. Across both cohorts, elevated FLC was associated with older

Table 1. Clinical Characteristics of the Patients With New, Untreated Diffuse Large B-Cell Lymphoma Enrolled Onto This Study

Characteristic	NCCTG N0489 (n = 76)		SPORE MER (n = 219)		All Patients (N = 295)	
	No.	%	No.	%	No.	%
Median age, years	62		64		62	
Range	21-82		21-93		21-93	
Male sex	43	56.6	117	53.4	160	54.2
Age > 60 years	43	56.6	131	59.8	174	59.0
Performance status 2+	11	14.5	42	19.2	53	18.0
Ann Arbor stage III/IV	61	80.3	126	58.1	187	63.8
Two or more extranodal sites	25	32.9	46	21.4	71	24.4
LDH > ULN	52	68.4	103	48.4	155	53.6
IPI						
0-1	18	23.7	79	36.1	97	23.9
2	17	22.4	59	26.9	76	25.8
3	28	36.8	49	22.4	77	26.1
4-5	13	17.1	32	14.6	45	15.3
B symptoms	29	38.2	46	21.0	75	25.4
Bulky disease	13	17.1	26	11.9	39	13.3
Bone marrow involvement	9	11.8	29	14.3	38	13.6
Creatinine > ULN	12	15.8	28	13.4	40	14.0
Immunotherapy	76	100	186	85.3	262	89.1
Median FLC						
Kappa	1.53		1.48		1.50	
Range	0.73-5.57		0.02-45.7		0.02-45.7	
Lambda	1.69		1.44		1.51	
Range	0.43-15.2		0.20-11.8		0.20-15.2	
Total FLC	3.27		2.93		3.08	
Range	1.48-19.1		0.37-51.1		0.37-51.1	
Abnormal FLC ratio	9	11.8	32	14.8	41	14.0
Elevated FLC	26	34.2	68	31.1	94	31.9
Elevated kappa	23	30.3	63	28.8	86	29.2
Elevated lambda	11	14.5	29	13.2	40	13.6

Abbreviations: NCCTG, North Central Cancer Treatment Group; SPORE, Specialized Program of Research Excellence; MER, Molecular Epidemiology Resource; LDH, lactate dehydrogenase; ULN, upper limit of normal; IPI, International Prognostic Index; FLC, free light chain.

age, worse performance status, advanced stage, and elevated creatinine, although association with the IPI was modest (Table 2).

An increase in serum FLC was univariately associated with EFS and OS in both cohorts for λ (all $P < .05$), κ (all $P < .02$), and total FLC (all $P < .002$) measurements (Appendix Table A1, Appendix Figs

Fig 1. Pretreatment serum free light chain values (mg/dL) from North Central Cancer Treatment Group N0489 and Specialized Program of Research Excellence Molecular Epidemiology Resource (MER) cohorts. Dashed box indicates normal range.

A1A-A1F, online only). Patients with elevation of either FLC had an inferior OS and EFS compared to patients with normal-range serum κ and λ FLC (N0489: EFS HR, 3.06; OS HR, 3.16; both $P < .02$; MER: EFS HR, 2.42; OS HR, 3.40; both $P < .001$; combined EFS HR, 2.57; OS HR, 3.74; both $P < .001$; Table 3; Fig 2). The results remained significant for EFS and OS after adjusting for IPI in N0489 (both $P < .02$), MER (both $P < .001$), and when both groups were combined (both $P < .001$). Furthermore, in a combined analysis, elevated FLC was associated with EFS and OS in all four IPI classification subsets (Appendix Table A2, online only; all $P < .08$).

Abnormal $\kappa:\lambda$ FLC ratio was modestly associated with outcome in the combined group (EFS HR, 1.61; OS HR, 1.67; both $P = .07$; Table 3). However, this was solely due to patients with an elevated FLC. In patients with normal range κ and λ concentrations, abnormal $\kappa:\lambda$ FLC ratio was not associated with outcome (EFS HR, 1.00; OS HR, 1.00; both $P = .99$; Appendix Table A2).

Elevated FLC was highly associated with elevated serum creatinine, with 58% of patients with an elevated creatinine having an elevated FLC compared to 29% of patients with normal creatinine having elevated FLCs. The association of FLC and outcome strengthened in the subset of 241 patients with normal creatinine (EFS HR, 2.71; OS HR, 4.09; both $P < .001$), while elevated FLC was not associated with outcome in the 40 patients with elevated creatinine

Table 2. Association of Clinical Characteristics With FLC Abnormalities

Characteristic	No.	% Elevated FLC	OR	95% CI	<i>P</i>	% Abnormal $\kappa:\lambda$ Ratio	OR	95% CI	<i>P</i>
All patients	295	31.9				14.0			
Sex									
Female	135	28.2	1.00			11.9	1.00		
Male	160	35.0	1.37	0.83 to 2.25	.21	15.7	1.39	0.71 to 2.72	.34
Age, years									
< 60	121	20.7	1.00			11.7	1.00		
60+	174	39.7	2.52	1.48 to 4.37	< .001	15.6	1.40	0.70 to 2.80	.34
Performance status									
0-1	242	26.9	1.00			13.3	1.00		
2+	53	54.7	3.29	1.79 to 6.06	< .001	17.3	1.37	0.61 to 3.07	.45
Ann Arbor stage									
I-II	106	24.5	1.00			10.4	1.00		
III-IV	187	35.8	1.72	1.01 to 2.93	.05	16.2	1.67	0.80 to 3.49	.17
Extranodal sites, No.									
0-1	220	32.3	1.00			16.5	1.00		
2+	71	28.2	0.82	0.46 to 1.48	.52	7.0	0.38	0.14 to 1.02	.05
LDH									
< ULN	134	33.6	1.00			15.7	1.00		
> ULN	155	31.0	0.89	0.54 to 1.46	.64	13.1	0.81	0.42 to 1.57	.53
B symptoms									
Absent	220	30.0	1.00			12.3	1.00		
Present	75	37.3	1.39	0.80 to 2.41	.24	18.9	1.66	0.82 to 3.67	.16
Bulky disease									
Absent	255	31.0	1.00			14.6	1.00		
Present	39	38.5	1.39	0.69 to 2.80	.35	10.3	0.67	0.22 to 1.99	.47
Bone marrow involvement									
No	241	29.5	1.00			12.5	1.00		
Yes	38	36.8	1.40	0.68 to 2.86	.36	24.3	2.25	0.67 to 5.23	.06
Creatinine									
< ULN	245	28.6	1.00			12.4	1.00		
> ULN	40	57.5	3.38	1.70 to 6.71	.005	25.0	2.37	1.05 to 5.33	.04
Treatment									
Immunotherapy	262	30.5	0.62	0.30 to 1.36	.25	13.9	0.87	0.31 to 2.40	.78
Other	32	40.6	1.00			15.6	1.00		
IPI									
0-1	97	22.7	1.00			10.3	1.00		
2	76	34.2	1.77	0.91 to 3.47	.09	19.7	2.14	0.90 to 5.08	.08
3	77	37.7	2.06	1.06 to 3.99	.03	16.0	1.66	0.67 to 4.08	.27
4-5	45	37.8	2.07	0.96 to 4.46	.06	8.9	0.85	0.25 to 2.87	.79
0-2	173	27.8	1.00			14.5	1.00		
3-5	122	37.7	1.58	0.96 to 2.59	.07	13.3	0.91	0.46 to 1.79	.79

Abbreviations: FLC, free light chain; OR, odds ratio; LDH, lactate dehydrogenase; ULN, upper limit of normal; IPI, International Prognostic Index.

($n = 40$; EFS HR, 1.24; OS HR, 1.00; both $P > .60$; Appendix Table A2). In general, the inferior EFS and OS for patients with elevated FLC was similar whether the elevated FLC was due to a monoclonal (abnormal FLC ratio) or polyclonal (normal FLC ratio with or without elevated creatinine) light chain expansion (Appendix Figs A2A and A2B, online only).

In a multivariable analysis with the five IPI components, elevated FLC was the variable most strongly associated with both EFS (HR, 2.26; $P = < .001$) and OS (HR, 2.5; $P < .001$); stage and number of extranodal sites were no longer predictive for EFS and OS in the multivariable models (all $P > .30$; Table 3). A risk model including elevated FLC in a risk model with the other IPI components resulted in a higher prognostic ability over the first 36 months compared to the IPI for both EFS (36-month C index, 0.667 ν 0.606 respectively; 95% bootstrap CI for difference, 0.019 to 0.100) and OS (36

month C index, 0.721 ν 0.658, respectively; 95% bootstrap CI for difference, 0.016 to 0.113; Appendix Figs A3A and A3B, online only).

Serum FLC was reduced significantly by treatment in the N0489 clinical trial, with reductions in total FLC during treatment observed in 100% of patients having both a pretreatment and postcycle 2 ($n = 47$) or postcycle 6 ($n = 52$) draw. Median reduction in total FLC was 42% and 57% after 2 and 6 cycles of treatment, respectively. In the 21 patients with elevated pretreatment FLC and follow-up samples, 18 (86%) achieved a normalization of their serum FLC following either cycle 2 or cycle 6.

Tumor light chain restriction status was available on the submitted N0489 on-study pathology reports for 14 patients (18%). Ten patients had κ -restricted tumors and four had λ -restricted tumors. Seven (50%) of 14 patients had an abnormal $\kappa:\lambda$ FLC ratio, of which four also had elevated FLC. Eleven patients had both pretreatment and

Table 3. EFS and OS Cox Proportional Hazards Models

Model	EFS			OS		
	HR	95% CI	P	HR	95% CI	P
Univariate						
Abnormal κ : λ ratio						
NCCTG N0489						
Unadjusted	1.87	0.63 to 5.55	.26	2.58	0.84 to 7.91	.1
Adjusted for IPI	2.22	0.76 to 7.20	.14	3.82	1.17 to 12.53	.03
MER						
Unadjusted	1.54	0.86 to 2.77	.14	1.49	0.78 to 2.81	.22
Adjusted for IPI	1.51	0.85 to 2.71	.16	1.45	0.77 to 2.74	.25
Combined						
Unadjusted	1.61	0.96 to 2.69	.07	1.67	0.96 to 2.91	.07
Adjusted for IPI	1.67	0.99 to 2.79	.05	1.75	1.00 to 3.06	.05
Elevated FLC						
NCCTG						
Unadjusted	3.06	1.28 to 7.29	.01	3.16	1.20 to 8.32	.02
Adjusted for IPI	3.00	1.26 to 7.16	.01	3.22	1.22 to 8.50	.02
MER						
Unadjusted	2.42	1.53 to 3.82	< .001	3.40	2.02 to 5.75	< .001
Adjusted for IPI	2.17	1.37 to 3.44	.001	2.84	1.67 to 4.83	< .001
Combined						
Unadjusted	2.57	1.72 to 3.85	< .001	3.74	2.13 to 5.35	< .001
Adjusted for IPI	2.37	1.58 to 3.55	< .001	2.94	1.85 to 4.77	< .001
Multivariable*						
Elevated FLC	2.26	1.44 to 5.48	< .001	2.53	1.53 to 4.24	< .001
Age > 60 years	1.56	0.97 to 2.52	.07	1.92	1.08 to 3.41	.03
PS 2+	1.64	0.98 to 2.73	.06	2.40	1.39 to 4.16	.002
Two or more extranodal sites	1.02	0.59 to 1.75	.95	1.17	0.65 to 2.09	.61
Stage III/IV	1.23	0.74 to 2.05	.42	1.37	0.76 to 2.49	.30
LDH > ULN	1.53	0.96 to 2.42	.07	1.60	0.95 to 2.71	.08

Abbreviations: EFS, event-free survival; OS, overall survival; HR, hazard ratio; NCCTG, North Central Cancer Treatment Group; IPI, International Prognostic Index; MER, Molecular Epidemiology Resource; FLC, free light chain; PS, performance status; LDH, lactate dehydrogenase; ULN, upper limit of normal.
 *Multivariable model simultaneously assessing elevated FLC with the variables for the five IPI components.

post-treatment (cycle 6) serum draws. Patients had a significantly greater reduction in the serum light chain that corresponded with the light chain restricted in the tumor (median reduction, 1.07 mg/dL; range, 0.58 to 6.03) compared with the nontumor-restricted light chain (median reduction, 0.43 mg/dL; range, -0.09 to 1.76; rank sum $P = .03$). Also, one of the patients with an identified λ -restricted tumor presented with highly elevated λ and normal κ (Appendix Fig A4, online only). The κ remained normal and unchanged during treatment, while the λ was reduced into normal range after two cycles of treatment. The patient achieved a positron emission tomography complete response after six cycles of treatment and remained in remission through 9 months of observation with stable FLC concentrations on follow-up samples. However, at 12 months post-treatment the serum λ FLC increased (with normal κ FLC) and tumor imaging and biopsy revealed tumor relapse. While based on a single patient, this case provides additional evidence that the tumor is directly influencing the corresponding light chain in the serum.

DISCUSSION

Serum FLC abnormalities are prevalent in patients with DLBCL, with 32% having elevated κ or λ concentrations and 14% of pa-

tients having an abnormal κ : λ FLC ratio. The most important parameter associated with outcome appears to be the absolute concentration of FLC rather than the κ : λ ratio. Increased serum FLC was strongly associated with an inferior outcome and remained significant after adjusting for IPI. Abnormal κ : λ FLC ratio, however, was only modestly associated with outcome across all patients, with the association only related to a concomitant elevated FLC. Patients with abnormal κ : λ FLC ratio without an elevation of either chain should be considered normal for risk. We defined elevated FLC as a concentration above the published normal range for either κ and/or λ . This definition captures patients with either a monoclonal or polyclonal FLC expansion, as outcome was similarly poor for each type of FLC expansion (Appendix Figs A2A and A2B). Examination of the hazard ratio across the continuous range of each light chain (Appendix Fig A1) shows an increase in risk for high values of κ , λ , as well as total FLC.

Strengths of this study include the large number of patients studied all with a uniform type of NHL, patients treated in the immunochemotherapy era, and replication of study results from both a cooperative group clinical trial and a clinic-based registry. FLC assessment was done in a single clinical lab with extensive experience with the assay.^{8,9,11,25} Assays were performed longitudinally on identically treated patients from the clinical trial to

Fig 2. (A) Event-free survival and (B) overall survival Kaplan-Meier survival curves by serum free light chain (FLC) in two cohorts (North Central Cancer Treatment Group trial N0489 and the Specialized Program of Research Excellence Molecular Epidemiology Resource [MER]) of patients with untreated diffuse large B-cell lymphoma.

assess the influence of treatment on FLC concentrations. Limitations of the study include the low percentage of clinical trial patients with data on tumor light chain restriction and limited follow-up time on these two cohorts of patients.

There are several plausible causes of elevated FLC in patients with DLBCL. Host effects, such as renal dysfunction,¹⁵ advanced age, or immune disruption or stimulation¹⁴ can cause a polyclonal increase in serum FLC. We observed this in our study, as elevated FLC was significantly associated with elevated creatinine and older age. Elevated FLC was observed in 57% of patients with elevated creatinine; however, the association of FLC and outcome was actually strengthened when the subset of patients with normal renal creatinine was evaluated, suggesting the association of FLC and outcome in our study was not an artifact of renal function. The tumor is another likely cause of elevated FLC in DLBCL. Most DLBCL tumors are immunoglobulin light chain restricted and we would expect that the clonal expansion of the neoplastic B-cell may cause an increase in the corresponding light chain in the serum. Because κ : λ FLC ratios in normal individuals can be either κ predominant or λ predominant, it is difficult to assess the relationship of tumor and serum light chain without knowing the patient's κ : λ FLC ratio before lymphoma. However, in the serial data on the clinical trial, there was a greater reduction in the FLC concen-

tration of the tumor-associated serum light chain during treatment, suggesting the tumor drives the excess of the residual light chain that is detected in serum.

Regardless of cause, patients with elevated FLC have similarly poor outcome. This was true in both high-risk and low-risk patients, as FLC was associated with outcome in all IPI categories. Potential reasons for this poor outcome are multiple and may vary from patient to patient. Patients with severe renal insufficiency would be expected to have both elevated polyclonal FLC and poor outcome due to nontumor-related comorbidities. We also saw an association of elevated FLC with poor performance status and elevated FLC may be a marker of general host frailty. Landgren et al¹⁹ recently showed increased FLC to be associated with an increased risk of AIDS-related lymphoma. They hypothesize this may represent a generalized disruption of B-cell function leading to increased risk of NHL development. A similar polyclonal state may result in a favorable microenvironment for the tumor or lead to a reduced host antitumor response. Elevated FLC may also be a marker of disease burden. We found an association of elevated FLC with stage in our study. More notably however, when FLC is included in a multivariable model with the IPI components, FLC is the largest single predictor of outcome. Stage and number of extranodal sites are no longer predictive of outcome in a model including FLC, and an index with FLC, performance status, age, and lactate dehydrogenase has more prognostic ability than the traditional IPI model. Finally, elevated FLC may perhaps be associated with a more aggressive tumor.

In summary, elevated FLC concentrations are associated with poor outcome in patients with DLBCL and is independent of the IPI. Our data suggest FLC may provide a better measure of disease burden for prognosis than stage and number of extranodal sites. If these results can be confirmed in additional data sets, the serum FLC assay has the potential to become a new, easily measured, serum biomarker for predicting prognosis in DLBCL. In addition, it may be used serially at times of follow-up to provide clues to relapse. Additional studies of serum FLC and outcome are warranted in this patient population and further studies are needed to better understand the mechanisms of elevated FLC in DLBCL.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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