Circulating Serum Free Light Chains As Predictive Markers of AIDS-Related Lymphoma

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ABSTRACT

Purpose

HIV-infected persons have an elevated risk of developing non-Hodgkin's lymphoma (NHL); this risk remains increased in the era of effective HIV therapy. We evaluated serum immunoglobulin (Ig) proteins as predictors of NHL risk among HIV-infected individuals.

Patients and Methods

By using three cohorts of HIV-infected persons (from 1982 to 2005), we identified 66 individuals who developed NHL and 225 matched (by cohort, sex, ethnicity, age, and CD4 count), HIV-infected, lymphoma-free controls who had available stored prediagnostic blood samples. Serum/plasma samples obtained 0 to 2 years and 2 to 5 years before diagnosis/selection were assayed for IgG, IgM, and IgA levels; monoclonal (M) Igs; and κ and λ free light chain (FLC) levels. Patients and matched controls were compared by using conditional logistic regression.

Results

The κ and λ FLCs were both significantly higher in patients (eg, in 2- to 5-year window: median κ , 4.24 v 3.43 mg/dL; median λ , 4.04 v 3.09 mg/dL) and strongly predicted NHL in a dose-response manner up to 2 to 5 years before diagnosis/selection (eg, NHL risk 3.76-fold higher with κ concentration at least 2.00 times the upper limit of normal, and 8.13-fold higher with λ concentration at least 2.00 times the upper limit of normal compared with normal levels). In contrast, IgG, IgM, and IgA levels were similar in patients and controls. M proteins were detected in only two patients with NHL (3%) and in nine controls (4%), and they were not significantly associated with NHL risk.

Conclusion

Elevated FLCs may represent sensitive markers of polyclonal B-cell activation and dysfunction and could be useful for identifying HIV-infected persons at increased NHL risk.

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INTRODUCTION

Chronic HIV infection leads to progressive immunosuppression (ie, AIDS). As a result, HIV-infected individuals have a markedly increased risk for certain cancers, including non-Hodgkin's lymphoma (NHL),¹⁻³ which is considered an AIDS-defining malignancy. The NHL subtypes that are most often encountered in the setting of HIV infection are diffuse large B-cell lymphoma (DLBCL); its variant, primary CNS lymphoma (PCNSL); and, to a lesser extent, Burkitt lymphoma (BL). Though the pathogenesis of NHL in the setting of HIV is poorly understood and has not yet been elucidated, immune dysregulation leading to loss of control of viruses, such as Epstein Barr virus (EBV), is thought to play an important role; indeed, in the setting of HIV infection, all people with PCNSL, as well as a high percentage of other people with DLBCL, are

EBV positive.^{3,4} For people with PCNSL in particular, but also for those with other types of DLBCL, the risk of developing lymphoma in the setting of HIV-infection increases directly with declining CD4 T-lymphocyte counts.⁵ Though somewhat variably for different subtypes, NHL incidence has declined substantially with the widespread use of highly active antiretroviral therapy (HAART), beginning in 1996; nonetheless the risk remains substantially elevated compared with HIV-negative counterparts.⁶

Although the hallmark of HIV infection is progressive loss of CD4 lymphocytes, HIV-infected individuals at all stages of progression also manifest abnormalities in B-cell function.⁷ B-cell dysfunction is characterized by abnormally low levels of antibodies to specific pathogens and poor immune responses to vaccines. Paradoxically, total serum levels of immunoglobulin (Ig; mostly of Ig isotype G) are elevated, reflecting nonspecific

polyclonal B-cell activation.^{8,9} Potential causes of B-cell dysfunction include dysregulated T-cell function, high levels of interleukin-6 or interleukin-10, and direct interaction of HIV with B cells.⁷

In a previous Australian study that was based on 219 patients with AIDS-related NHL and 219 matched HIV-infected controls without NHL, high levels of serum globulins (mostly Ig) were predictive of the development of NHL.¹⁰ Also, there was evidence of a dose-response increase in NHL risk with increasing globulin level. However, a recent study that was based at an HIV clinic found that, although serum globulin levels were elevated compared with the general population, NHL risk was unrelated to this marker of B-cell activation.^{10a} Additionally, there has been growing interest in monoclonal gammopathy of undetermined significance (MGUS) among HIV-infected persons. Indeed, a few small studies have suggested that MGUS prevalence may be elevated among HIV-infected people.¹¹⁻¹⁵ MGUS is associated with substantial risk of multiple myeloma and other lymphoproliferative disorders in the general population,^{16,17} and it is thus possible that MGUS could predict AIDS NHL among HIV-infected people.

By taking advantage of three established cohorts of HIV-infected people, we evaluated the role of B-cell dysfunction in the etiology of AIDS-related NHL by directly measuring serum-based markers of B-cell stimulation.¹⁸⁻²⁰ Among approximately 5,000 HIV-infected people, we identified 66 individuals who developed AIDS NHL and for whom serially collected blood samples collected before NHL diagnosis were available. We applied protein electrophoresis, immunofixation, and assays for κ and λ free light chains (FLCs) to define patterns of serum protein abnormalities before NHL diagnosis. For comparison, we also evaluated 225 HIV-infected matched controls who were free from NHL. Our study tested the hypothesis that the risk of lymphomagenesis is related to an altered immunoregulatory state, reflected in circulating polyclonal or monoclonal Ig levels.

PATIENTS AND METHODS

We conducted a case-control study of NHL, nested in three cohorts of HIV-positive individuals: the Washington and New York Men's Research Study (also known as the DC Gay Men's Study, or DCG)²¹; the Multicenter Hemophilia Cohort Study^{22,23}; and the AIDS Cancer Cohort (ACC) Study²⁴ (Table 1). Institutional review boards at the National Cancer Institute (NCI) and collaborating institutions approved each cohort study, and all participants gave written informed consent.

Study participants contributed blood samples at 6- to 12-month intervals. Information on incident cancers was obtained prospectively by using standardized questionnaires and medical record review. Eligible patients with NHL selected from these cohorts were HIV infected at the time of NHL diagnosis and had available serum or plasma samples obtained 0.01 to 2.00 and/or 2.01 to 5.00 years before diagnosis (referred to below as 0- to 2-year and 2- to 5-year periods). For each patient, we selected up to four controls who were HIV infected and lymphoma free on the patient's diagnosis date and who had similar availability as the patient for serum or plasma (same material type) in the two antecedent time periods. Additional matching criteria were cohort, sex, ethnicity (white *v* nonwhite), age (within 5 years), and CD4 count at diagnosis/selection (strata: 0 to 99, 100 to 199, 200 to 499, and ≥ 500 cells/mm³). Of 79 identified patients with NHL, 13 were excluded, because we could not identify appropriate controls. All study patients were allowed to contribute no more than one blood sample within the 0- to 2-year and 2- to 5-year time frames; if more than one specimen was available, we selected the specimen closest in time to the midpoint of the interval. For all patients, serum or plasma samples stored at -70°C were thawed and analyzed in the Mayo Clinic Protein Immunology Laboratory (Rochester, MN; Table 2).^{18-20,25,26}

Table 1. Characteristics of Patients With NHL and Controls Among HIV-Infected People

Characteristic	Patients With NHL (n = 66)		Controls (n = 225)	
	No.	%	No.	%
Male sex	63	95	213	95
Age, years*				
Median	38		38	
Interquartile range	29-44		32-44	
White ethnicity	55	83	181	80
CD4 count in cells/mm ³ *				
Median	79		71	
Interquartile range	31-230		16-252	
Year of NHL diagnosis/control selection				
Median	1994		1995	
Interquartile range	1991-1998		1991-1999	
Cohort				
ACC	19	29	74	33
DCG	11	17	27	12
MHCS I and II	36	55	124	55
NHL subtype				
DLBCL	27	41	—	—
PCNSL	10	15	—	—
Burkitt lymphoma	3	5	—	—
Other/unknown	26	39	—	—
Patients with available sample				
0-2 years prior to NHL diagnosis/selection	59	89	207	92
2-5 years prior to NHL diagnosis/selection	40	61	129	57
Both 0-2 and 2-5 years prior to NHL diagnosis/selection	33	50	111	49
HIV treatment in the 0-2-year period prior to NHL diagnosis/selection				
HAART	7	11	26	12
Other treatment	5	8	13	6
No treatment	47	71	168	75
Unknown	7	11	18	8

NOTE. The Washington and New York Men's Research Study (also known as the DC Gay Men's Study, or DCG) enrolled male homosexual patients from two clinical practices.²² This study included 133 HIV-positive men observed from 1982 to 2000. The Multicenter Hemophilia Cohort Study enrolled patients with hemophilia or another congenital coagulation deficiency from multiple sites in North America, Europe, and South America. The study had two successive phases (ie, MHCS-I and MHCS-II) and encompassed 2126 HIV-infected individuals (99% men) who were observed from 1983 to 2005.^{23,24} The AIDS Cancer Cohort (ACC) Study enrolled 2,803 patients with AIDS (82% men) at multiple treatment and clinical trial sites in the United States during 1997, and these individuals were observed through 2000.²⁵

Abbreviations: HIV, human immunodeficiency virus; NHL, non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; PCNSL, primary central nervous system lymphoma; ACC, AIDS Cancer Cohort study; DCG, DC Gay Men's Study; MHCS I and II, Multi-center Hemophilia Cohort Study I and II; HAART, highly active antiretroviral therapy.

*Age and CD4 count refer to measures at NHL diagnosis for patients and at selection date for matched controls.

Statistical Analysis

We compared Ig proteins and MGUS prevalence in patients and matched controls using conditional logistic regression. As a result of matching, the analyses were adjusted for cohort, sex, ethnicity, age, and CD4 count at diagnosis/selection. We tested for a trend of increasing NHL risk across Ig protein levels by using the quartile distribution in controls. For FLC proteins, we also calculated odds ratios (ORs) that related to NHL risk to multiples of the upper limit of normal (ULN).²⁶ We also calculated ORs

Table 2. Serum Ig, κ and λ Free Light Chains, and Monoclonal Proteins in Patients With NHL and Controls

Ig Marker, mg/dL	NHL	Control	P*	ULN†
0-2 years prior to NHL diagnosis/selection				
IgG			.38	1,500
Median	1,760	1,694		
% greater than ULN	61.0	56.6		
IgM			.18	300
Median	156	152		
% greater than ULN	22.0	11.2		
IgA			.64	400
Median	268	301		
% greater than ULN	30.5	34.6		
κ FLC			.03	1.94
Median	4.19	3.25		
% greater than ULN	88.1	83.4		
λ FLC			.003	2.63
Median	4.33	3.58		
% greater than ULN	84.8	67.3		
M protein			.53	
Prevalence	2	4		
Total No.	59	207		
%	3.4	1.9		
Abnormal κ : λ FLC ratio			.57	
Prevalence	8	28		
Total No.	59	205		
%	13.6	13.7		
2-5 years prior to NHL diagnosis/selection				
IgG			.25	1,500
Median	1,815	1,815		
% greater than ULN	72.5	62.5		
IgM			.03	300
Median	166	144		
% greater than ULN	15.0	9.4		
IgA			.54	400
Median	267	245		
% greater than ULN	25.0	25.0		
κ FLC			.002	1.94
Median	4.24	3.43		
% greater than ULN	95.0	85.9		
λ FLC			.001	2.63
Median	4.04	3.09		
% greater than ULN	77.5	60.9		
M protein			.20	
Prevalence	0	7		
Total No.	40	129		
%	0	5.4		
Abnormal κ : λ FLC ratio			.65	
Prevalence	8	30		
Total No.	40	128		
%	20.0	23.4		

NOTE. Serum protein electrophoresis was performed on agarose gels (REP; Helena Laboratories, Beaumont, TX). By using a 5-antiserum mixture of anti- γ , μ , α , κ , and λ (Pentafix; Sebia, Norcross, GA), all samples with a discrete or suspicious localized band on protein electrophoresis were then tested by immunofixation electrophoresis (Hydrasys and Hydragel; Sebia). This was the same method used in the monoclonal gammopathy of undetermined significance prevalence study in Olmsted County.²⁶ IgG, IgM, and IgA were quantified on a Dade-Behring BNII Nephelometer (Deerfield, IL). FLC concentrations were determined by using the quantitative FLC assay (Freelite; The Binding Site, Birmingham, United Kingdom) performed on a Dade-Behring BNII Nephelometer.^{19,21} The assay separately measures κ FLCs (normal range, 0.33 to 1.94 mg/dL) and λ FLCs (normal range, 0.57 to 2.63 mg/dL). In addition, the assay provides the κ : λ FLC ratio (normal range, 0.26 to 1.65); abnormal ratios reflect an immunoglobulin imbalance indicative of monoclonality.²⁷

Abbreviations: NHL, non-Hodgkin's lymphoma; ULN, upper limit of normal; Ig, immunoglobulin; FLC, free light chain; M-protein, monoclonal protein.

*P values are derived from conditional logistic regression, except for the comparison of the prevalence of M proteins in the 2- to 5-year time period, for which the Fisher exact test was used. For continuous variables, P values correspond to trend across quartiles.

†Upper limit of normal refers to reference ranges for general population provided in references.^{27,28}

associated with elevated FLC levels (ie, greater than ULN) for NHL overall and according to CD4 count at diagnosis/selection.

RESULTS

Sixty-six patients with NHL (95% men; median age, 38 years) were included (Table 1). The 66 patients with NHL comprised 27 with DLBCL, 10 with PCNSL, three with Burkitt lymphoma, and 26 with other/unknown NHL subtypes (mostly unspecified and likely predominantly DLBCL). Eighty-nine percent of the patients with NHL had available blood samples in the 0- to 2-year period before diagnosis/selection, and 61% had samples in the 2- to 5-year period. We included 225 controls who closely resembled patients according to demographic characteristics and blood sample availability (Table 1). Most patients and controls were not receiving HIV therapy in the 0- to 2-year period (Table 1). Treatment data were limited for the 2- to 5-year period (not shown), but a great majority would not have been receiving HAART on the basis of the calendar year of selection.

Markers of B-Cell Activation and NHL Risk

In both patients with NHL and controls, IgG levels were higher than in the general population (Table 2).^{26,27} IgG, IgA, and IgM levels in prediagnostic blood were similar in patients and controls, which reflected an absence of association between Ig levels and NHL risk (Table 2). Similar patterns were present in blood obtained 0 to 2 and 2 to 5 years before diagnosis/selection.

In contrast, patients with NHL had significantly higher levels than controls of κ and λ FLCs for both time periods before diagnosis/selection (Table 2). The κ and λ FLC concentrations also were higher than observed in the general population (Table 2).

As shown in Figure 1, differences between patients and controls in FLC concentrations translated into strong difference with regard to NHL risk. For example, increasing κ FLC levels in the 2 to 5 years before diagnosis/selection were associated with a steadily increasing risk (*P* for trend = .002). NHL risk was 3.76-fold (95% CI, 0.76 to 18.59) elevated in individuals with a concentration of κ at least 2.00 times ULN compared with κ levels less than or equal to ULN. Similarly, increasing λ levels in the 2- to 5-year period were associated with increasing NHL risk (*P* for trend = .001), and NHL risk was 8.13-fold (95% CI 2.06 to 32.03) elevated in individuals with a concentration of λ at least two times ULN compared with λ levels less than or equal to ULN. Similar associations were present when using measurements in the 0 to 2 years before diagnosis/selection (Fig 1). When we assessed associations for systemic NHLs alone (excluding PCNSL), the results were similar (not shown). There were too few patients with PCNSL to examine this subgroup separately.

We found strong correlations between κ and λ FLCs within each time period (Spearman *r* = 0.65 to 0.76; *P* < .0001). In addition, κ FLC levels measured 0 to 2 and 2 to 5 years before diagnosis/selection were strongly correlated (Spearman *r* = 0.68; *P* < .0001). A similar correlation was seen for λ FLCs in the two time periods (Spearman *r* = 0.77; *P* < .0001). NHL risk was not associated with the change in FLC concentration between the two time periods (not shown).

Among individuals with a CD4 count 100 cells/mm³ or higher, associations between FLC levels and NHL risk were present in blood collected at both time periods (Fig 2). In contrast, for individuals with

Fig 1. Non-Hodgkin's lymphoma (NHL) risk as a function of serum free light chain (FLC) levels among HIV-infected people. Odds ratios (ORs) and associated 95% CIs are presented for NHL in relation to FLC levels. FLC levels are categorized as normal or as a multiple of the upper limit of normal (ULN). ULNs are 1.94 mg/dL for κ and 2.63 mg/dL for λ . The panels correspond to results for (A) κ FLC levels measured 0 to 2 years prior to NHL diagnosis/control selection; (B) κ FLC levels measured 2 to 5 years prior to NHL diagnosis/control selection; (C) λ FLC levels measured 0 to 2 years prior to NHL diagnosis/control selection; and (D) λ FLC levels measured 2 to 5 years prior to NHL diagnosis/control selection.

CD4 counts less than 100 cells/mm³, associations with FLC levels were more modest and were most apparent in blood obtained 2 to 5 years before diagnosis/selection. Among controls, κ FLC levels 0 to 2 years before selection were inversely associated with CD4 counts (median κ FLC levels, 3.97 v 2.75 mg/dL for CD4 counts less than 100 cells/mm³ or greater than 100 cells/mm³, respectively; $P = .0009$). A similar inverse association with CD4 count was seen for λ FLC levels (median, 3.91 v 2.80 mg/dL; $P < .0001$).

We also conducted analyses restricted to patients not on HAART (HIV treatment data were available only for the 0- to 2-year window). As shown in Figure 2, the associations between FLC levels and NHL risk were stronger in these analyses (ie, risk was 2.71-fold elevated with κ FLCs greater than ULN compared with 1.42-fold elevation for all patients and was 4.60-fold elevated for λ FLC levels greater than ULN compared with 3.35-fold elevation for all patients). CD4 counts in the 0- to 2-year window were lower among patients on HAART than among patients not receiving HAART or with unknown treatment status (median, 33 v 101 cells/mm³; $P = .0008$).

Prevalence of MGUS and an Abnormal FLC Ratio Before NHL Diagnosis

MGUS was detected in only 3.4% and 0% of patients with NHL at 0 to 2 years and 2 to 5 years before NHL, respectively. One patient with an M-protein detectable at 0 to 2 years before DLBCL diagnosis had an IgG MGUS, and the other patient (NHL subtype unknown) had an IgM MGUS; neither had a blood sample 2 to 5 years before diagnosis.

MGUS prevalence was similar in controls (4.0% of controls overall; specifically, 1.9% and 5.4% at 0 to 2 years and 2 to 5 years before selection, respectively; Table 2). In the 2- to 5-year period, MGUS was characterized as IgG ($n =$ four controls), IgM ($n =$ one), and biclonal ($n =$ two). Of the five controls with IgG or IgM MGUS in the 2- to 5-year period, one had persistent MGUS in the 0- to 2-year period, two no longer had MGUS, one developed biclonal MGUS, and one had no blood sample. The two controls with biclonal MGUS in the 2- to 5-year period became negative in the 0- to 2-year period. Finally, two additional controls had IgG MGUS

Fig 2. Non-Hodgkin's lymphoma (NHL) risk associated with elevated free light chain levels among HIV-infected people according to CD4 count. Odds ratios (ORs) and 95% CIs associated with elevated serum free light chain levels (ie, greater than the upper limit of normal) are presented. Results are shown separately for all patients, for patients with CD4 counts 0 to 99 cells/mm³ at NHL diagnosis/control selection, for patients with CD4 counts $\geq$ 100 cells/mm³ at NHL diagnosis/control selection, and for patients not receiving highly active antiretroviral therapy (HAART) in the 0 to 2 years before NHL diagnosis/control selection. ORs correspond to measurements obtained in the 0-to 2-year period and 2- to 5-year period before diagnosis/control selection. Results are for (solid circles) κ free light chains and (open circles) λ free light chains.

detected in the 0- to 2-year period, neither of whom had blood from the 2- to 5-year period.

We also evaluated the prevalence of abnormal κ -to- λ FLC ratios (Table 2). In the 0- to 2-year and 2- to 5-year time periods before NHL diagnosis, abnormal ratios were present in 13.6% and 20.0% of patients with NHL, respectively. Corresponding prevalence estimates in controls were similar (13.7% and 23.4%, respectively).

DISCUSSION

We were intrigued that clinic-based case series have produced conflicting results regarding whether the serum globulin level, a marker of a dysregulated B-cell function, might predict NHL development among HIV-infected persons.^{10,10a} By taking advantage of sensitive serum/plasma Ig assays (ie, protein electrophoresis confirmed with immunofixation, and κ and λ FLC assays), we tested whether AIDS lymphomas are typically preceded by a precursor state manifested in elevated markers of B-cell activation.

Notably, we found that the presence of elevated free κ and λ FLC levels strongly predicted NHL among HIV-infected individuals, and NHL risk increased directly in relation to FLC level. In contrast, although IgG levels were higher among our HIV-infected patients than in the general population,²⁷ levels of IgG, IgA, and IgM were not associated with NHL risk. Serum FLC levels may be more sensitive indicators of B-cell activation than IgG, IgA, and IgM,^{26,28} and abnormally elevated levels have been reported in patients with hepatitis C virus infection, rheumatoid arthritis, and Sjögren syndrome,^{29,30} which are themselves associated with B-cell immune dysfunction and increased NHL risk. In addition, Breen et al³¹ previously reported that elevated serum levels of soluble CD30,

also a marker of B-cell activation, were predictive of subsequent NHL among HIV-infected patients.

AIDS NHL risk increases with declining CD4 counts, which reflects advancing immunosuppression.⁵ In our investigation, we observed higher FLC levels among HIV-infected individuals with lower CD4 counts, and the association between FLC levels and NHL varied depending on the CD4 count. Specifically, for individuals with a CD4 count at or greater than 100 cells/mm³, the association was present for FLCs measured in both prediagnostic time periods, but for individuals with CD4 counts less than 100 cells/mm³, the association was present only for FLCs measured in the earlier 2- to 5-year period. We hypothesized that, as AIDS progresses, B-cell dysfunction becomes more common; however, because the profound effects of T-cell immunosuppression become increasingly important, the contribution of B-cell dysfunction to NHL pathogenesis becomes harder to discern. Thus, the predictive value of FLCs in relation to lymphomagenesis is most apparent at relatively preserved CD4 counts. Furthermore, we found stronger associations between FLCs and NHL when we excluded individuals on HAART therapy (Fig 2). Patients on HAART had lower CD4 counts than those not receiving HAART, which likely reflects both physician choices regarding whom to treat and the selection criteria for our study (ie, we selected NHL patients and matched controls who would be expected to have had low CD4 counts even if HAART treated). HAART has profound effects on the immune system that may mitigate the utility of FLCs as a prognostic marker, although our study did not include sufficient number of patients receiving HAART to look at that group separately. Taken together, these observations suggest that abnormal B-cell activation is a risk factor for development of NHL in HIV-infected individuals, particularly those not receiving effective HIV treatment. It is unknown how such a pathway might relate to loss of immune control of EBV and other viruses implicated in lymphomagenesis and how it may have variable roles in the individual pathogenesis of different NHL subtypes. EBV replication, particularly, is poorly regulated in HIV-infected patients, and this virus is detectable in many, but not all, patients with AIDS NHLs.^{3,4}

Importantly, we did not find that MGUS or an abnormal FLC ratio predicted NHL risk among HIV-infected persons. MGUS was uncommon and appeared to be somewhat transient (ie, some patients with MGUS in the 2- to 5-year period no longer had a detectable M-proteins in the 0- to 2-year period). The prevalence of MGUS is similar to that described in prior reports (3% to 4%),^{12,32} and one study likewise observed that M-proteins may be transient in HIV-infected individuals.³² Our report is the first, to our knowledge, to define the prevalence of abnormal FLC ratios in HIV-infected persons before NHL diagnosis (20.0% at 2 to 5 years, and 13.6% at 0 to 2 years). The higher prevalence of abnormal FLC ratio than for detectable M-protein reflects that this ratio is a more sensitive measure of monoclonality than protein electrophoresis.²⁸ Nonetheless, the prevalence of abnormal FLC ratio was virtually the same in controls. These findings suggest that the generation of M-proteins and abnormal FLC ratios, presumably by dysregulated plasma cells, is not strongly driven by HIV-related immunosuppression, and that this process is not related to AIDS lymphomagenesis. HIV-infected individuals have an approximately two- to three-fold elevated risk for multiple myeloma compared with the general population,³³ but this risk does not increase steeply with onset of AIDS.³⁴

Strengths of our study include its prospective design, available prediagnostic blood samples, and the application of assays for the determination of B-cell stimulation and clonal proliferation. Limitations also should be noted. Although the size of this study was sufficient to study NHL risk overall, we had insufficient statistical power to evaluate associations with FLC levels in strata defined by NHL subtypes. Other limitations were the lack of information regarding tumor EBV status and the lack of central blinded pathology review, which was impossible to implement in a retrospective study.

We speculate that FLC measurement may have clinical utility in assessing risk for NHL and perhaps other AIDS-related outcomes. At present, decisions regarding initiation of HAART are guided mainly by the CD4 count and, to a secondary degree, the HIV viral load.³⁵ Commonly, a threshold CD4 count of 350 cells/mm³ is used, but recent evidence suggests that earlier initiation of HAART may be beneficial to prevent HIV-related complications.³⁶ One possibility is that clinicians could utilize FLC levels to help determine which patients might benefit most in starting HAART. Although this is a preliminary suggestion, this approach would find support from our observations that FLCs were especially predictive of NHL risk among patients not on HAART who had a relatively high CD4 count (≥ 100 cells/mm³) and that this predictive relationship was obtained over a prolonged period of 2 to 5 years after FLC measurement.

Another possibility is that the B-cell abnormalities identified by elevated FLC levels may not be specific to the development of NHL but may instead indicate a generalized disruption of B-cell function. If that is the case, then this marker also may reflect risk for HIV-related complications other than NHL. Future studies are needed to explore these topics and to better define the biologic mechanisms underlying the observed associations.

In conclusion, we found that the presence of elevated FLC levels, a marker of polyclonal B-cell activation, is a strong risk factor for AIDS-related NHL among HIV-infected individuals. Although NHL

risk has declined substantially in the HAART era, lymphoma still remains a considerably important morbidity in the setting of HIV infection.⁶ On the basis of our findings presented here, additional elucidation of the pathogenetic mechanisms of AIDS-related NHL should continue to be a priority in epidemiologic studies and clinical trials, and this elucidation may help in the development of improved prevention strategies and the identification of novel therapeutic targets.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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