Research Article

Cancer Epidemiology, Biomarkers & Prevention

Serum Biomarkers of Immune Activation and Subsequent Risk of Non-Hodgkin B-Cell Lymphoma among HIV-Infected Women

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Abstract

Background: There is increasing evidence that chronic immune activation predisposes to non-Hodgkin lymphoma (NHL). Whether this association exists among women representative of the current HIV epidemic in the United States who are at high risk of HIV-associated NHL (AIDS-NHL), remains to be determined.

Methods: We conducted a nested case–control study within the Women's Interagency HIV Study with longitudinally collected risk factor data and sera. Cases were HIV-infected women with stored sera collected at three time-windows 3 to 5 years, 1 to 3 years, and 0 to 1 year before AIDS-NHL diagnosis ($n = 22$). Three to six HIV-infected controls, without AIDS-NHL, were matched to each case on age, race, $CD4^+$ T-cell count, and study follow-up time ($n = 78$). ORs and 95% confidence intervals (CI) for the association between one unit increase in log-transformed biomarker levels and AIDS-NHL were computed using random effect multivariate logistic regression models.

Results: Elevated levels of sCD27 (OR = 7.21; 95% CI, 2.62–19.88), sCD30 (OR = 2.64; 95% CI, 1.24–5.64), and CXCL13 (OR = 2.56; 95% CI, 1.32–4.96) were associated with subsequent diagnosis of AIDS-NHL overall. Elevated sCD23 was associated with a two to three-fold increased risk of AIDS-NHL in certain subgroups, whereas elevated interleukin 6 was associated with a two-fold increased risk in the 0 to 1 year time-window, only.

Conclusions: These findings support the hypothesis that chronic B-cell activation contributes to the development of AIDS-NHL in women.

Impact: Soluble CD23 (sCD23), sCD27, sCD30, and CXCL13 may serve as biomarkers for AIDS-NHL. *Cancer Epidemiol Biomarkers Prev; 22(11); 2084–93. ©2013 AACR.*

Introduction

AIDS-associated B-cell non-Hodgkin lymphoma (AIDS-NHL) is the most common malignancy among people with HIV infection in some regions where highly active antiretroviral therapy (HAART) is readily available, including the United States, Europe, and Australia (1). Prolonged infection with HIV causes immune dysfunction including chronic immune suppression and B-cell hyperactivation. The depletion of $CD4^+$ T-cells contributes to the development of AIDS-NHL, particularly primary central nervous system lymphoma (PCNSL), through the loss of immunoregulatory control over Epstein–Barr virus (EBV)-infected B-cells (2). Some of the more common subtypes of non-PCNSL (or systemic) AIDS-NHL, including diffuse large B-cell lymphoma (DLBCL), occur in the setting of chronic B-cell activation (3), which may be an indirect response to gut microbial translocation or other factors (4). The downstream effects of chronic B-cell activation, with ongoing engagement of the B-cell receptor complex on lymphomagenesis, are numerous, and include the accumulation of oncogene mutations and translocations resulting from aberrant expression and gene targeting of the DNA-mutating enzyme, activation-induced cytidine deaminase (AID; refs. 5, 6).

Several recent studies have reported significant associations between the elevated levels of B-cell activation biomarkers and subsequent risk of AIDS-NHL,

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doi: 10.1158/1055-9965.EPI-13-0614

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including numerous cytokines, chemokines, soluble receptors, kappa- and lambda-free light chains, and AID (7–12). These studies have been conducted in populations predominantly composed of men who were not treated with HAART before the diagnosis of AIDS-NHL. HAART results in suppression of HIV replication and partial restoration of immune competence, although the markers of inflammation remain elevated. Thus, although several immune biomarkers have been found to be reduced following HAART initiation, these markers have not normalized (13). It has also been suggested that HAART exposure modifies the relationship between the biomarkers of B-cell activation and AIDS-NHL risk (9).

To examine the relationship between the markers of B-cell activation and subsequent development of AIDS-NHL, we examined the longitudinal circulating levels of B-cell activation and immune stimulatory molecules [soluble CD23 (sCD23), sCD27, sCD30, CXCL13/BCA1, interleukin-6 (IL-6), and complement-reactive protein (CRP)] in relation to AIDS-NHL risk in a cohort of HIV-infected women, about 40% of whom had been treated with HAART before the development of AIDS-NHL.

Materials and Methods

Study design and population

A nested case–control study was conducted within an ongoing prospective study of HIV infection among women, the Women's Interagency HIV Study (WIHS; refs. 14, 15). The WIHS includes 3,768 adult women from five metropolitan areas (the San Francisco Bay Area, Los Angeles, Chicago, Washington D.C., and New York City) enrolled during two recruitment periods (1994–1995 and 2001–2002). WIHS participants are seen semiannually for an in-person interview, physical exam, and specimen collection. Baseline and follow-up interviews elicited a wide range of detailed information, including demographic data, medications taken, and clinical events. Self-reported antiretroviral use was ascertained at each visit, with the aid of photo-medication cards, and was summarized according to the Department of Health and Human Services/Kaiser Panel to define HAART usage (16). Venous blood samples were drawn at each 6-month study visit and processed; sera were stored in a central repository. HIV plasma load and $CD4^+$ T-cell counts were measured at each 6-month study visit.

Case and control definitions

AIDS-NHL cases were ascertained through six monthly self-reports and confirmed by pathology records and state cancer registries. Cases included all HIV-infected WIHS participants with AIDS-NHL diagnosed after their baseline visit and before 2006 who had at least one available pre-AIDS-NHL diagnosis serum specimen ($n = 22$). The median follow-up time for cases was 3.0 years. On the basis of classification of International Classification of Diseases codes and InterLymph recommendation for epidemiologic research (17), 17 cases were systemic and five were central nervous system lymphomas (Table 1). Of the systemic cases, 11 were DLBCL, two were B-cell precursor lymphoblastic leukemia/lymphoma, one was Burkitt lymphoma, and three B-cell lymphoid neoplasms, not otherwise specified. Up to three serum samples were obtained for each case, collected 2.7 to 4.7 years pre-NHL (median = 4.0 years, $n = 10$), 1.0 to 2.9 years pre-NHL (median = 1.9 years, $n = 14$), and 0.1 to 0.9 years pre-NHL (median = 0.5 years, $n = 19$). At least three, and up to six (if available), HIV-infected WIHS participants without a diagnosis of AIDS-NHL were individually matched to each case on (i) time since first HIV-positive study visit (±1.0 year), (ii) age (±2.5 years), (iii) $CD4^+$ T-cell count at first HIV-positive study visit (±50 cells/mm^3), and (iv) race/ethnicity. Reference date for controls was a date matched on HIV-positive follow-up time to the case of each matched set at time of AIDS-NHL diagnosis. Specimens for controls were obtained from three time-windows with a median of 3.9, 2.1, and 0.4 years before the reference date.

Serum biomarker determination

Immune biomarkers were measured in archived sera by ELISA. Assays were carried out according to the manufacturer's protocol for sCD23 and sCD30 (Bender MedSystems), sCD27 (PeliKine-compact ELISA kit, CLB/Sanquin, with 1:20 dilutions), CXCL13 (R&D Systems), IL-6 (Biosource/Invitrogen, with color development time extended to 40 minutes to ensure consistent low-level detection), and CRP (high-sensitivity protocol, Virgo CRP 150, Hemagen). All samples from each case and matched controls were tested together as a set on the same assay plate; laboratory personnel were blinded to the identity of the samples within each set. All immune markers were detectable in more than 98% of samples tested, except for sCD23 (90.9%, Table 2). All of the immune markers had intra-assay coefficient of variations (CV) $\leq 7\%$. The inter-assay CVs were comparatively higher, particularly for IL-6 and CRP, which also exhibited low concentrations in tested samples.

Statistical analysis

We calculated frequencies for select covariates and χ^2 P values to test for differences between cases and controls. Samples with biomarker levels below the detection limit were set equal to half the value of the lower detection limit. Unmatched comparisons of mean natural log-transformed ($\log_e$) biomarker levels at each time-window for cases compared with controls were conducted using t tests, and the means were reported as back-transformed geometric means. Geometric means were also calculated for categories of select covariates among HIV-infected control women. One-way ANOVAs were used to compare means across categories for each covariate, and a $P \leq 0.05$ was considered evidence for statistically significant variation. ORs and 95% confidence intervals (CI) for the association between $\log_e$ immune biomarker levels and AIDS-NHL were computed using random effects multivariate logistic

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Table 1. Select characteristics of AIDS-NHL cases and HIV-infected controls from the WIHS

	AIDS-NHL cases ($n = 22$)	HIV-infected controls ($n = 78$)	
	N (%)	*N* (%)	*P*
Baseline characteristics			
WIHS Site			
New York	9 (40)	24 (31)	
DC	1 (5)	9 (12)	
California	11 (50)	28 (35)	
Chicago	1 (5)	17 (22)	0.16
Race/ethnicity			
African-American, non-Hispanic	11 (50)	47 (60)	
White, non-Hispanic	8 (36)	20 (26)	
Latina/Hispanic	2 (9)	11 (14)	
Other	1 (5)	0 (0)	0.18
Education			
Less than high school	7 (32)	28 (36)	
High school graduate	6 (27)	23 (30)	
More than high school	9 (41)	26 (34)	0.82
HCV positive[a]			
No	18 (82)	58 (75)	
Yes	4 (18)	19 (25)	0.53
Characteristics at AIDS-NHL diagnosis or reference date			
Reference year			
1995–2001	16 (73)	53 (68)	
2002–2008	6 (27)	25 (32)	0.67
Age, y			
<30	4 (18)	8 (10)	
30–39	10 (45)	41 (53)	
40–49	7 (32)	24 (31)	
≥50	1 (5)	5 (6)	0.76
Cigarette smoking[b]			
Never	5 (24)	26 (34)	
Current	8 (38)	38 (50)	
Former	8 (38)	12 (16)	0.08
$CD4^+$ T-cells/mm^3[c]			
<200	13 (72)	28 (41)	
200–400	3 (17)	12 (18)	
>400	2 (11)	28 (41)	0.04
HIV RNA copies[d]			
<4,000	2 (11)	25 (36)	
4,000–20,000	4 (21)	15 (22)	
>20,000	13 (68)	29 (42)	0.07
Prior AIDS diagnosis			
No	9 (41)	34 (44)	
Yes	13 (59)	44 (56)	0.82
Prior HAART exposure[e]			
No	13 (59)	41 (53)	
Yes	9 (41)	37 (47)	0.66

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Table 1. Select characteristics of AIDS-NHL cases and HIV-infected controls from the WIHS (Cont'd)

	AIDS-NHL cases ($n = 22$)	HIV-infected controls ($n = 78$)	
	N (%)	*N* (%)	*P*
Tumor subtype[f]			
Systemic	17 (77)		
DLBCL	11		
Precursor lymphoblastic leukemia/lymphoma, B-cell	2		
Burkitt lymphoma	1		
B-cell lymphoid neoplasm, NOS	3		
Central nervous system	5 (23)		

[a]RNA positive or antibody positive at baseline study visit.
[b]Missing values for three participants.
[c]Missing values for 14 participants.
[d]Missing values for 12 participants.
[e]"No" indicates unexposed at all three serum sampling time points, "Yes" indicates exposed at least one time point.
[f]Classified according to the InterLymph recommendation for epidemiologic research (17).

regression with the GLIMMIX procedure in SAS (18). The ORs represent risk of AIDS-NHL associated with one unit increase in $\log_e$ biomarker levels. The matching by design within each matched case–control set and correlation between samples from the same individual were incorporated into the models by adding a random effect term, with the following equation: logit $P(D = 1|\mathbf{X}) = \beta_0 + \beta_1 \text{Biomarker} + \beta_{2-7} \text{Covariates} + \rho_i \text{Match}$, where logit $P(D = 1|X)$ is the log odds of being a case versus control, and all effects are fixed effects except for ρ_iMatch, which is a random effect variable (mean zero, constant variance) that has the same value for all visits belonging to the *i*th matched case–control set (19). Covariates were selected *a priori* if they were plausibly related to AIDS-NHL risk, HIV disease progression, and/or immune biomarker levels. Covariates included in the final multivariate models were education status and hepatitis C virus (HCV) positivity (RNA or antibody) at baseline, absolute $CD4^+$ T-cell counts, HIV RNA levels, and HAART exposure ascertained at the study visit which the biomarkers were measured, and cigarette smoking status (never, former, or current) at the study visit closest to and preceding AIDS-NHL diagnosis date in the cases and reference date in the controls. Multiple imputations were used to estimate missing covariate data in the multivariate models with the MIANALYZE procedure in SAS (20).

Results

Cases and controls were similarly distributed by race, reference year and age (reflective of the matching criteria), and education (Table 1). The large percentage of Black, non-Hispanic women in this case–control study is representative of the WIHS cohort at large, and of the HIV epidemic in women in the United States (15). More cases than controls were from New York and California, although this difference was not statistically significant. Less than one third of cases and controls were HCV positive at study entry. Most cases and controls were current or former smokers, although fewer cases were never smokers compared with controls ($P = 0.08$). Cases had a lower $CD4^+$ T-cell count ($P = 0.04$) and higher HIV

Table 2. Descriptive and quality control data for immune markers

	Lower limit of detection	% detectable (# undetectable/total)	Intra-assay CV%	Interassay CV%
sCD23	13 U/mL	90.9 (19/208)	2.6%	19.8%
sCD27	32 U/mL	100 (0/208)	2.6%	4.4%
sCD30	6 U/mL	100 (0/208)	6.1%	20.8%
CXCL13	7.8 pg/mL	100 (0/208)	4.1%	15.7%
IL-6	0.16 pg/mL	98.6 (3/208)	3.1%	25.5%
CRP	0.25 μg/mL	98.1 (4/208)	7.0%	29.2%

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Table 3. Geometric means for immune markers at visit 0 to 1 year before reference date among HIV-infected control women by select characteristics

	sCD23 (U/mL)	sCD27 (U/mL)	sCD30 (U/mL)	CXCL13 (pg/mL)	IL-6 (pg/mL)	CRP (μg/mL)
Baseline characteristics						
WIHS Site						
New York	49	494	115	213	2.7	3.3
DC	56	361	103	220	1.5	1.5
California	39	342	76	153	1.2	2.0
Chicago	64	406	78	198	2.0	2.3
P	0.338	0.075	0.052	0.230	0.044	0.470
Race/ethnicity						
African-American, non-Hispanic	47	409	92	211	2.0	2.3
White, non-Hispanic	52	422	92	173	1.4	2.7
Latina/Hispanic	54	339	76	113	1.3	2.0
P	0.877	0.557	0.698	0.018	0.372	0.872
Education						
Less than high school	59	420	97	197	1.7	1.7
High school graduate	40	409	83	194	1.9	3.1
More than high school	49	381	90	175	1.7	2.6
P	0.342	0.785	0.670	0.760	0.894	0.263
HCV positive[a]						
No	49	376	87	177	1.7	3.0
Yes	49	519	103	233	2.1	1.0
P	0.977	0.022	0.324	0.116	0.469	0.005
Characteristics at AIDS-NHL diagnosis or reference date						
Reference year						
1995–2001	51	425	103	210	2.0	2.1
2002–2008	46	366	71	154	1.5	2.8
P	0.633	0.224	0.008	0.039	0.216	0.404
Age, y						
<30	35	397	130	241	1.8	1.9
30–39	55	399	94	196	1.9	3.1
40–49	47	433	90	177	1.5	1.6
≥50	31	322	44	141	1.7	1.8
P	0.427	0.683	0.016	0.502	0.896	0.318
Cigarette smoking						
Never	53	340	79	158	1.1	2.2
Current	51	504	105	208	2.2	2.1
Former	43	336	83	209	2.6	3.0
P	0.781	0.001	0.129	0.200	0.025	0.726
$CD4^+$ T-cells/mm^3						
<200	44	450	123	243	2.6	3.0
200–400	45	427	95	189	1.1	1.1
>400	52	354	65	142	1.5	2.4
P	0.731	0.171	<0.001	0.002	0.019	0.065
HIV RNA copies						
<4,000	45	299	55	128	1.2	2.2
4,000–20,000	43	429	106	200	1.4	2.1
>20,000	56	504	126	253	2.8	2.6
P	0.544	<0.001	<0.001	<0.001	0.005	0.837

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Table 3. Geometric means for immune markers at visit 0 to 1 year before reference date among HIV-infected control women by select characteristics (Cont'd)

	sCD23 (U/mL)	sCD27 (U/mL)	sCD30 (U/mL)	CXCL13 (pg/mL)	IL-6 (pg/mL)	CRP (μg/mL)
Prior AIDS diagnosis						
No	51	426	94	179	1.8	2.5
Yes	47	387	87	195	1.8	2.2
P	0.710	0.434	0.570	0.560	0.945	0.682
Prior HAART exposure[b]						
No	50	447	110	207	2.2	2.0
Yes	48	366	75	172	1.5	2.8
P	0.836	0.091	0.005	0.203	0.094	0.284

[a]RNA positive or antibody positive at baseline study visit.

[b]"No" indicates unexposed at all three serum sampling time points, "Yes" indicates exposed at least one time point.

RNA load ($P = 0.07$) compared with controls. The majority of cases and controls had a prior AIDS diagnosis, whereas 41% of cases and 46% of controls had been exposed to HAART. A larger proportion of lymphomas were systemic tumors ($n = 17, 77\%$) compared with PCNSL tumors ($n = 5, 23\%$), and a majority of systemic tumors were of the DLBCL subtype.

Biomarkers were explored among the HIV-infected control women to understand the factors related to biomarker levels in absence of NHL (Table 3). All of the biomarkers showed significant or borderline significant variation by categories of $CD4^+$ T-cell count or HIV RNA, except for sCD23. Women with the most advanced HIV disease (i.e., $CD4^+$ T-cell count <200 or HIV RNA copies >20,000) had the highest mean biomarker levels; the only exception was sCD23, which had the highest mean level in women with $CD4^+$ T-cell more than 400. In addition, there were significant variations across the categories of WIHS site for IL-6 ($P = 0.044$), race for CXCL13 ($P = 0.018$), age for sCD30 ($P = 0.016$), and cigarette smoking for sCD27 ($P = 0.001$) and IL-6 ($P = 0.025$). Furthermore, sCD27 was higher ($P = 0.022$) and CRP lower ($P = 0.005$) among HCV-positive controls. Women with a reference year between 1995 and 2001 (early HAART period) had higher levels of sCD30 ($P = 0.008$) and CXCL13 ($P = 0.039$), and sCD30 was significantly higher among women unexposed to HAART ($P = 0.005$).

Next, biomarker levels were compared between the case and control groups. In univariate analyses, mean levels of sCD27 were significantly higher in cases compared with controls at all the three time-windows, whereas sCD30 and CXCL13 showed increased levels of borderline significance at more than 3 years, and clearly significant increased levels at the 1 to 3 and 0 to 1 years time-windows (Fig. 1). IL-6 was elevated in cases at the 0 to 1 year time-window only. Levels of sCD23 and CRP were not significantly different between cases and controls at any time-window.

In multivariate models including serum measurements from all available time-windows combined, sCD27 was strongly associated with AIDS-NHL risk across all subgroups (Table 4). Elevations of sCD23, sCD30, and CXCL13 were statistically associated with an increased risk of systemic AIDS-NHL and in those who were HAART unexposed. IL-6 and CRP were generally not associated with AIDS-NHL risk. When the biomarker data were analyzed according to sampling time-window, there were no clear trends with respect to biomarker associations increasing or decreasing with time to NHL diagnosis (Table 5). However, sCD27 seemed to be more strongly associated with AIDS-NHL at the time-window closest to AIDS-NHL diagnosis date, as was IL-6, although the latter was not statistically significant overall.

Discussion

Elevated serum levels of sCD23, sCD27, sCD30, CXCL13, and IL-6 were associated with an increased risk of AIDS-NHL overall or in specific subgroups in our study of racially and ethnically diverse HIV-infected women. Of particular interest were the strong and consistent associations observed for sCD27, sCD30, and CXCL13, which are consistent with previous studies. In the Multicenter AIDS Cohort Study (MACS), among the eight immune biomarkers examined, sCD27, sCD30, and CXCL13 exhibited the strongest (3- to 8-fold) and most consistent (over three time-windows up to 5 years before an AIDS-NHL diagnosis) associations with increased risk of AIDS-NHL (7, 11). In addition, among HIV-uninfected study populations, sCD27, sCD30, and CXCL13 have been reported increased up to 13 years before B-cell NHL; these associations were the most pronounced for the DLBCL subtype which is also the main subtype observed in the setting of HIV (12, 21). Replication of these biomarker associations in the HIV-infected women of the WIHS, in light of the major differences between this and previous study populations evaluated (in terms of gender, race, and other factors), adds support to the hypothesis that prolonged chronic B-cell activation is

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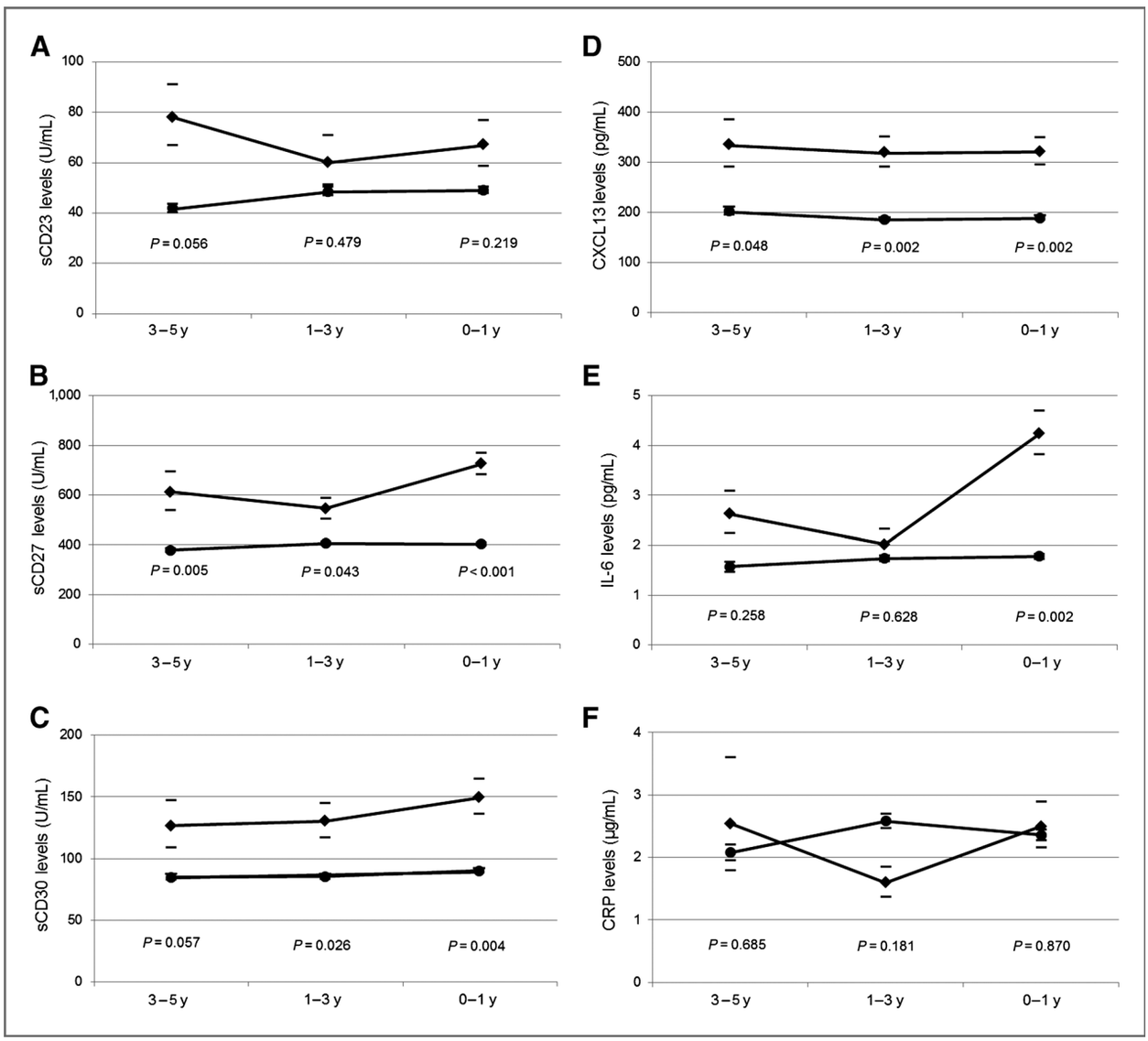

Figure 1. Mean serum biomarker levels in AIDS-NHL cases and matched HIV-infected controls. Geometric means for cases (black diamonds) and controls (black circles) and 95% CIs (bars) at three time-windows preceding an AIDS-NHL diagnosis in cases and reference date in controls for (A) sCD23, (B) sCD27, (C) sCD30, (D) CXCL13, (E) IL-6, and (F) CRP. *P* values represent the significance level for the difference in mean biomarker values between cases and matched controls.

important in the etiology of B-cell NHL. Furthermore, we and others have observed that these immune marker associations are not limited to the time-window closest to NHL diagnosis date, and can be observed up to 13 years before NHL diagnosis, which suggests that these elevations in immune marker levels do not result from an already existing, though not yet clinically diagnosed, case of AIDS-NHL.

CD27 and CD30 are receptors for TNF-like immune stimulatory molecules. CD27 is expressed on the surface of B-cells following activation, and is a marker for memory B-cells (22). CD30 is expressed on activated T- and B-cells, and is considered a marker for cells producing Th2 cytokines that support B-cell activation and differentiation into antibody-secreting plasma cells (23). Soluble forms of these receptors (sCD27 and sCD30), cleaved from the cell surface following cellular activation, are found in relatively low levels in the blood of healthy individuals, whereas high levels are associated with a number of viral infections and immune-related disorders dominated by a Th2 immune response (24–27). CXCL13 is a chemokine produced by follicular dendritic and T-helper cells, which plays a central role in homeostatic trafficking of antigen-naïve B-cells into and within follicles of secondary lymphoid organs, an essential step in the development and structure of secondary lymphoid organs and the differentiation of B-cells into antibody-producing plasma cells (28–30).

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Table 4. Association between immune markers and AIDS-NHL risk overall and in select subgroups

	All samples 22 cases/78 controls	Systemic 17 cases/62 controls	HAART Unexposed[b] 13 cases/41 controls	HAART Exposed 9 cases/37 controls
	OR[a] (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
sCD23	1.45 (0.96–2.19)	1.91 (1.14–3.23)	2.00 (1.07–3.74)	1.18 (0.61–2.31)
sCD27	7.21 (2.62–19.88)	29.07 (6.72–125)	6.99 (1.94–25.3)	13.07 (1.87–91.7)
sCD30	2.64 (1.24–5.64)	4.30 (1.70–10.91)	3.61 (1.36–9.63)	1.55 (0.46–5.30)
CXCL13	2.56 (1.32–4.96)	5.52 (2.29–13.32)	2.42 (1.05–5.58)	2.15 (0.67–6.91)
IL-6	1.26 (0.88–1.81)	1.57 (1.05–2.37)	1.17 (0.69–2.00)	1.37 (0.75–2.53)
CRP	0.96 (0.71–1.31)	1.21 (0.86–1.73)	1.26 (0.86–1.87)	0.64 (0.37–1.10)

[a]The ORs represent risk of AIDS-NHL associated with one unit increase in the biomarker on the natural log scale. ORs were adjusted for $CD4^+$ T-cell count, HIV load, and HAART exposure at each visit where the biomarker was measured, cigarette smoking, HCV infection, and education, in addition to the matching factors (time since first HIV-positive study visit, age, $CD4^+$ T-cell count at first HIV-positive study visit, and race/ethnicity).

[b]HAART exposure at the time of the blood draw for the sample used in the immune marker assays.

In previous studies, higher serum levels of sCD27 and CXCL13 were observed among HIV-infected men when compared with HIV-uninfected controls (27, 31), and elevated sCD30 levels predicted faster progression to AIDS in HIV-infected patients (32). Serum levels for sCD27, CXCL13, and sCD30 were significantly reduced in HIV-infected men following HAART exposure compared with their pre-HAART levels in a recent study, yet levels still remained higher when compared with HIV-uninfected controls (13). In our study, we also observed important associations between HIV disease progression and HAART exposure and biomarker levels among HIV-infected control women. After controlling for indicators of HIV disease progression and immune status ($CD4^+$ T-cell count, HIV RNA, and HAART exposure), sCD27, sCD30, and CXCL13 remained significantly associated with AIDS-NHL risk.

Interestingly, similar to what was observed recently in the MACS, sCD23 was associated with an increased risk of AIDS-NHL at more than 3 years prediagnosis but not at visits closer to the time of diagnosis (11). Somewhat paradoxically, among HIV-positive controls without AIDS-NHL, slightly higher mean levels of sCD23 were seen in the most immunocompetent women ($CD4^+$ T-cells >400 cells/mm^3) as well as in those with the highest HIV viral loads. CD23 is a receptor for immunoglobulin E that is upregulated on activated B-cells, and in its soluble form, has B-cell stimulatory properties including enhancement of immunoglobulin class switch recombination. Prior observations that sCD23 levels are higher among HIV-uninfected versus HIV-infected individuals (33, 34), plus the observations noted above, allow for the possibility that there is a complex interaction between the competence of the immune system (number and/or function of $CD4^+$ T-helper cells), B-cell activation due to high HIV viral load, and lymphomagenesis that cannot yet be teased apart. Furthermore, our observation that sCD23 was significantly elevated in the systemic subgroup but not overall (after inclusion of PCNSL cases, which are almost uniformly EBV positive) is consistent with this possibility, as well as

Table 5. Association between immune markers and AIDS-NHL risk at three sampling time-windows

	3–5 years 10 cases/42 controls	1–3 years 14 cases/54 controls	0–1 year 19 cases/69 controls
	OR[a] (95% CI)	OR (95% CI)	OR (95% CI)
sCD23	4.56 (1.14–18.35)	1.24 (0.63–2.5)	1.45 (0.82–2.58)
sCD27	3.44 (0.42–28.27)	3.41 (0.65–18.01)	16.43 (2.93–92.19)
sCD30	1.80 (0.32–10.41)	2.37 (0.68–8.35)	2.90 (0.95–8.94)
CXCL13	1.25 (0.38–4.18)	4.40 (1.25–15.61)	2.70 (0.91–8.08)
IL-6	1.94 (0.85–4.47)	0.88 (0.43–1.82)	2.11 (1.17–3.83)
CRP	1.48 (0.76–2.92)	0.54 (0.29–1.04)	1.01 (0.66–1.56)

[a]The ORs represent risk of AIDS-NHL associated with one unit increase in the biomarker on the natural log scale. ORs were adjusted for $CD4^+$ T-cell count, HIV load, and HAART exposure at each visit where the biomarker was measured, cigarette smoking, HCV infection, and education, in addition to the matching factors (time since first HIV-positive study visit, age, $CD4^+$ T-cell count at first HIV-positive study visit, and race/ethnicity).

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with prior data showing an association between sCD23 and EBV tumor negativity (35).

IL-6 and CRP were not strongly associated with AIDS-NHL in our study. IL-6 is a pluripotent cytokine produced by many different cell types whose major functions include driving acute and chronic inflammation, participation in B-cell maturation and activation, and involvement in tumor initiation and growth. One previous study in the MACS found that elevated serum IL-6 levels were associated with subsequent development of Burkitt lymphoma, but not DLBCL, which represents majority of the cases in our study (33). However, a subsequent larger study in the MACS found that IL-6 levels were associated with AIDS-NHL overall (11). CRP, which is produced by hepatocytes in response to IL-6, participates in the clearance of necrotic and apoptotic cells and is considered a marker for the bioactivity of IL-6. Our observation that the ORs for CRP were low in all subgroups is similar to what was observed in the MACS where ORs for CRP were significant, but low (11). The high interassay CVs for IL-6 and CRP and the fact that these immune markers exhibited levels at the lower end of the detection curve in our samples may contribute to our inability to detect significant associations with AIDS-NHL risk, as may the relatively small number of AIDS-NHL cases in the current study.

In subgroup analyses, the ORs for all immune markers except CRP were significantly increased when the analysis was restricted to cases with systemic AIDS-NHL and their matched controls. Although we could not estimate the associations for the PCNL subgroup due to sparse data, our observations are consistent with the hypothesis that chronic B-cell activation is a primary pathway for development of systemic AIDS-NHL. An alternative explanation may be that the contribution of B-cell dysfunction to lymphomagenesis is obscured among people who have severely suppressed T-cell immunity, which is characteristic of PCNSL. Furthermore, the ORs in the HAART-exposed subgroup seemed to be slightly attenuated for sCD23, sCD30, and CXCL13, and strengthened for sCD27, compared with the HAART unexposed. The variability in ORs may be due to the small sample size, or may also reflect an effect of HAART on immune marker levels (13). In addition, there are a number of factors influencing HAART initiation and continuation, which may confound the association (36).

Among the study's limitations, we did not have an adequate sample size to reliably assess immune marker associations in all desired subgroups. In addition, we could not address the role of EBV in our immune marker associations because we lacked data on EBV status of the tumors. The main strength of this study is the inclusion of HIV-infected women from a large prospective study, including detailed longitudinal data and stored serum specimens. This study population has several unique aspects compared with the populations investigated in previous AIDS-NHL biomarker studies. The WIHS participants were predominantly African-American or Latina, in comparison with the White, non-Hispanic male populations studied previously (8, 9, 11), and are representative of the HIV epidemic in women in the United States (15). Furthermore, the large percentage of women exposed to HAART allowed us to conduct stratified analyses by HAART exposure.

In the current era of HAART, HIV-infected individuals are living longer in the setting of chronic B-cell activation, and will continue to be at increased risk of developing AIDS-NHL. We have added evidence to support previous reports that biomarkers of B-cell activation are important predictors of AIDS-NHL in diverse populations including African and Latina women, and those who are receiving HAART.

Disclosure of Potential Conflicts of Interest

K. Anastos has honoraria from speakers' bureaus of Miriam Hospital Providence and St Luke's Hospital, and is a consultant/advisory board member for Bristol Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

The authors thank the women who consented to be part of this study, Mr. Larry Magpantay for technical support in the performance of the immune assays, and Dr. Jeffrey Gornbein for statistical consultation. Data in this article were collected by the WIHS Collaborative Study Group with centers (Principal Investigators) at New York City/Bronx Consortium (K. Anastos); Brooklyn, New York (H. Minkoff); Washington DC Metropolitan Consortium (M. Young); The Connie Wofsy Study Consortium of Northern California (R. Greenblatt); Los Angeles County/Southern California Consortium (A. Levine); Chicago Consortium (M. Cohen); and Data Coordinating Center (S. Gange).

Grant Support

This work was supported by grants from the NIH (K07-CA-140360 to S.K. Hussain, an NCI supplement to U01-AI-035040 to O. Martínez-Maza, R01-CA-121195 to O. Martínez-Maza, and R01-CA-168482 to O. Martínez-Maza). This work was carried out in the facilities of the UCLA AIDS Institute, which were supported, in part, by funds from the James B. Pendleton Charitable Trust and the McCarthy Family Foundation, and by NIH grant AI-028697: UCLA Center for AIDS Research (CFAR). The WIHS is funded by the National Institute of Allergy and Infectious Diseases (UO1-AI-35004, UO1-AI-31834, UO1-AI-34994, UO1-AI-34989, UO1-AI-34993, and UO1-AI-42590) and by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (UO1-HD-32632). The study is co-funded by the National Cancer Institute, the National Institute on Drug Abuse, and the National Institute on Deafness and Other Communication Disorders. Funding is also provided by the National Center for Research Resources (UCSF-CTSI Grant Number UL1 RR024131).

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Received June 17, 2013; revised August 20, 2013; accepted September 2, 2013; published OnlineFirst September 17, 2013.

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