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Serum Levels of Cytokines, and Biomarkers for Inflammation and Immune Activation, and HIV-Associated Non-Hodgkin B cell Lymphoma Risk

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Abstract

Background: HIV infection is associated with a marked increase in risk for non-Hodgkin lymphoma (AIDS-NHL). However, the mechanisms that promote the development of AIDS-NHL are not fully understood.

Methods: In this study serum levels of several cytokines and other molecules associated with immune activation were measured in specimens collected longitudinally during 1-to-5 years

preceding AIDS-NHL diagnosis, in 176 AIDS-NHL cases and 176 HIV+ controls from the Multicenter AIDS Cohort Study (MACS).

Results: Multivariate analyses revealed that serum levels of immunoglobulin free light chains (FLC), IL-6, IL-10, IP-10/CXCL10, neopterin, and TNFα were elevated in those HIV+ individuals who went on to develop AIDS-NHL. Additionally, the fraction of specimens with detectable IL-2 was increased, and the fraction with detectable IL-4 was decreased, in these subjects.

Conclusions: These results suggest that long term, chronic immune activation, possibly driven by macrophage-produced cytokines, precedes development of NHL in HIV+ individuals.

Impact: FLC, IL-6, IL-10, IP-10/CXCL10, neopterin, and TNFα may serve as biomarkers for AIDS-NHL.

Keywords

non-Hodgkin lymphoma; HIV; B lymphocyte; inflammation; cytokine

INTRODUCTION

Infection with the human immunodeficiency virus (HIV) is a well-known risk factor for several malignancies, including non-Hodgkin lymphoma (NHL) (1-3). In the past decade, several studies have helped to better define the pathogeneic mechanisms underlying the development of AIDS-associated NHL (AIDS-NHL). B cell hyperactivation is characteristically seen in HIV infection and is thought to be driven by a combination of factors, including the overproduction of B cell stimulatory molecules, as well as via the direct stimulation of B cells by HIV, through host cell immune activation molecules incorporated into the viral envelope, such as CD40 ligand (3-7). B cell activation leads to the expression of activation-induced cytidine deaminase (*AICDA*), a DNA-editing enzyme, which mediates immunoglobulin gene (*Ig*) class switch recombination and somatic hyper mutation. It has been shown that *AICDA* is overexpressed prior to the development of AIDS-NHL, consistent with a direct role for this molecule in the pathogenesis of NHL (8,9). Moreover, AICDA is responsible for the c-*MYC/IgH* recombination seen in germinal center-derived lymphomas (10), such as Burkitt lymphoma (BL), and is also responsible of DNA rearrangements in non-Ig genes, namely *BCL-6* and other oncogenes involved in NHL (11).

Several studies have shown that B cell-stimulatory cytokines, as well as biomarkers for B cell activation, are elevated in HIV+ individuals who develop NHL, including serum/plasma levels of cytokines (IL-6, IL-10, IP-10 and CXCL13), soluble forms of cytokine receptors (sCD30, sCD27), free immunoglobulin light chains (FLC), and other molecules associated with immune system activation and inflammation (CRP, sCD23, sCD44) (12-19).

Another important contributor to the genesis of AIDS-NHL is viral infection. Epstein-Barr virus (EBV) directly infects tumor cells in many AIDS-NHL. Nearly all primary central nervous system lymphomas (PCNSL) are EBV+ lymphomas. However, tumor cells in other subtypes of AIDS-NHL, such as BL or systemic diffuse large B cell lymphoma (DLBCL), are often EBV-negative (3). EBV has the potential to induce AIDS-NHL in at least two ways: via the expression of virus-encoded oncogenes in persons who have lost immunoregulatory control EBV-infection, and by inducing B cell hyperactivation, leading to the expression of *AICDA*, thereby driving the mutation of cellular oncogenes, such as *P53* or *BCL-6* (3,20).

In this study, we sought to better define the pattern of expression of immune activation-associated molecules seen in HIV+ individuals who went on to develop NHL, by measuring levels of several cytokines and immune activation-associated molecules. These molecules were measured in up to three serum specimens collected up to five years prior to NHL diagnosis, in 176 AIDS-NHL cases and 176 HIV+ controls from the Multicenter AIDS Cohort Study (MACS).

MATERIALS AND METHODS

Study population and serum specimens

This is a nested case-control study, utilizing sera from participants in the MACS. MACS participants are homosexual and bisexual men recruited from four US metropolitan areas (Baltimore, Chicago, Los Angeles, and Pittsburgh), who have study visits at six month intervals, to examine the natural and treated history of HIV infection and AIDS (www.statepi.jhsph.edu/macs/macs.html) (21). Participants in this nested study were selected from 4954 men who were enrolled in 1984-85, as well as 668 men enrolled in 1987-1991.

At the time cases were selected for this study, 179 participants were identified who had been diagnosed with AIDS-NHL for whom at least one serum sample from a time point preceding AIDS-NHL diagnosis was available in the MACS repository, and a matched HIV-infected (HIV+) control could be identified (12). 32% (n=58) of these cases were PCNSL, and 68% (n=121) systemic lymphomas. Of the systemic lymphomas, 61 (50%) were DLBCL, 21 (17%) BL or BL-like, six (5%) were other lymphoma subtypes, and 33 (27%) were not specified (12). For each case, all possible HIV+ controls were identified from among HIV-infected MACS subjects who had not developed lymphoma, matched on: 1) actual length of infection with HIV based on known date of HIV seroconversion, or date of entry ± 1 year into MACS as HIV-seroprevalent, and 2) expected sample availability at equivalent time points ± 1 year. One unique HIV+ control was randomly selected for each case. Due to sample depletion, 176 matched case:control sets were included in this study.

Longitudinal serum samples were obtained corresponding to three time points prior to NHL diagnosis in cases: >3 years pre-NHL (closest to 4 years; visit 3), 1-3 years pre-NHL (closest to 2 years; visit 2), and 0-1 year pre-NHL (closest to 0.5 year; visit 1), and at matched time-points in controls. All immune markers included in this study were measured at all three time points for cases and controls when specimen was available.

Determination of κ and λ *FLC serum levels*

Serum levels of κ and λ FLC were determined by enzyme-linked immunosorbent assay (ELISA) (Biovendor, Modrice, Czech Republic). The standard curves used to determine the levels of FLC ranged from 10 to 320 μg/l for κ chain and from 17.5 to 560 μg/l for λ chain. According to the manufacturer, the limit of detection of the assays is 6 μg/l for κ and 5 μg/l for λ.

Determination of neopterin serum levels

Serum levels of neopterin were measured by ELISA (IBL International GMBH, Hamburg, Germany). According to the manufacturer, the lower limit of detection for this assay is 0.7 nmol/L.

Determination of cytokine serum levels

Serum levels of several molecules were assessed using Luminex-based multiplexed immunoassays. The levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, GM-CSF,

IFNγ, TNFα and VEGF were determined using the Fluorokine® MAP Human Inflammation Kit (R&D Systems, Minneapolis, MN, USA), following the manufacturer's protocol. IL-17A, IL-17F, IL-20, IL-23, IL-27, IL-31, IP-10/CXCL10, LIF, sCD40L and SDF-1α were measured using the Procarta Cytokine Assay (Affymetrix, Santa Clara, CA, USA), following the manufacturer's protocol. Briefly, for both assays, Luminex microparticles, pre-coated with analyte-specific antibodies, were incubated with serum samples (diluted 1:2), followed by a biotin-antibody and by a streptavidin-phycoerythin conjugate. The fluorescence intensity of each analyte's microparticles was quantified using a Bioplex 200™ (Luminex) System Analyzer (Biorad, Hercules, CA, USA), and the data analyzed using BioPlex Manager (v 4.1.1) software.

The lower limit of detection was set either as the lowest value that the BioPlex Manager software could calculate using the standard curve, or as the lowest value of the standard curve, whichever was smaller. Taking the dilution factor into account, the limit of detection was 0.02 pg/ml for IL-2, IL-5, IL-17A and IFNγ, 0.04 pg/ml for IL-10 and GM-CSF, 0.06 pg/ml for IL-1β and IL-6, 0.08 pg/ml for LIF and IL-20, 0.1 pg/ml for IL-23 and IL-31, 0.12 pg/ml for IL-12, 0.16 pg/ml for TNFα and IL-17F, 0.58 pg/ml for IL-8, 0.82 pg/ml for IL-4, 0.84 pg/ml for VEGF, 1.62 pg/ml for sCD40L, 2.44 pg/ml for IP-10 and IL-27, and 2.70 pg/ml for SDF-1α.

Statistical Analysis

The data obtained for each of the biomarkers were natural log-transformed to approximate a normal distribution of the values in the population. Data points that were below the lower limit of detection were substituted with a value equal to half of the lower limit of detection.

For IL-5, IL-8, IL-17F, IL-20, IL-27, IFN-γ, IP-10, LIF, sCD40L, TNF-α, VEGF, κ, λ, and neopterin, the difference of the means between cases and controls were calculated using a Student t-test. Serum levels of IL-1β, IL-2, IL-4, IL-12, IL-17A, IL-23, IL-31, GM-CSF, and SDF-1α serum levels were undetectable in many subjects. The data were therefore analyzed as dichotomous variables, comparing the fraction of detectable versus undetectable between cases and controls, using a chi-squared test. Correlations studies were performed and pairwise correlation coefficients were calculated at each time point for cases and controls separately for all biomarkers, using Spearman's correlation.

Odd ratios (ORs) and 95% confidence intervals (CIs) were calculated at each time point using multivariate conditional logistic regression models, adjusting for age and CD4+T cell count at each time point. HIV disease progression was further adjusted by matching cases and controls on duration of HIV infection.

For biomarkers with continuous values (κ and λ FLC, neopterin, IL-5, IL-6, IL-8, IL-10, IL-17F, IL-20, IL-27, IFN-γ, IP-10, LIF, sCD40L, TNF-α, VEGF), ORs are in terms of one unit increase in natural log-transformed values. For IL-1β, IL-2, IL-4, IL-12, IL-17A, IL-23, IL-31, GM-CSF, SDF-1α, ORs are in terms of detectable versus undetectable.

To correct for other possible confounding factors, the aforementioned statistical analysis was also performed excluding those subjects on combination antiretroviral therapy (cART). The results obtained were essentially unchanged (Supplemental Table 1). Therefore, the results obtained from the whole study population, including those on cART (5% of cases and 5% of controls) are presented here (Table 1).

Due to the varying degree of prior evidence of association among the biomarkers investigated in this study, and expected level of correlation between closely related markers, there was no obvious method to correct for multiple testing. However, in order to prioritize

our results for interpretation and future studies, associations with p-values less than or equal to 0.01 were considered significant, while associations with p-values less than 0.05 were considered nominally significant.

RESULTS

Characteristics of AIDS-NHL cases and controls

A detailed description of the 179 cases and controls included in this study has been provided elsewhere (12). Briefly, the 179 cases and controls were similar in their age distribution (median 41 years for cases and 39 years for controls) and race/ethnicity, the majority being white, non-Hispanic (83% of cases and 87% of controls). The majority of cases and controls were cART naïve at the time of the sample collection (95% of cases and 95% controls). 100 of these cases had a clinical diagnosis of AIDS prior to their NHL diagnosis (12), with a median time from AIDS diagnosis (non-NHL) to NHL diagnosis of 1.2 years. At the visit furthest from NHL diagnosis (visit 3), four of the cases and one control had developed an opportunistic infection or other AIDS-defining illness. At the intermediate visit (visit 2), an additional 18 of the NHL cases and three of the controls had developed an opportunistic infection or other AIDS-defining illness, and at visit 1, an additional 33 of the NHL cases and seven of the controls had developed an opportunistic infection or other AIDS-defining illness.

Serum levels of κ and λ FLC are elevated preceding the diagnosis of AIDS-NHL

In univariate analysis, serum FLC mean levels were seen to be elevated in those who developed AIDS-NHL, at all three time points preceding NHL diagnosis, and were statistically significant for λ FLC at all three visits, and for κ FLC at 3-5 and 1-3 years pre-NHL (Supplemental Table 2). In the CD4– and age-adjusted models, κ and λ FLC were positively associated with AIDS-NHL risk at all three time points. Both κ and λ FLC were statistically significant at 3-5 years pre-NHL (Table 1); λ was also nominally significant at 0-1 years pre-NHL.

Serum levels of cytokines are elevated preceding the diagnosis of AIDS-NHL

In univariate analyses, neopterin, IP-10/CXCL10, IL-10, and TNFα levels were significantly increased in the serum of patients who developed AIDS-NHL, compared to the HIV+ controls, at all three time points preceding diagnosis (p<0.001, Supplemental Table 2). Similarly, IL-5, IL-6 and IFN-γ levels were seen to be significantly higher at the two time points closest to NHL diagnosis in the AIDS-NHL cases (p 0.01). IL-6 level was also higher at 3-5 years pre-NHL, with a nominally significant p-value (p=0.024). Mean IL-8 serum level was significantly higher 0-1 years pre-NHL (p=0.001) and was nominally significant at 1-3 years pre-NHL (p=0.023). There was no significant difference between the two groups in the mean serum levels of the other molecules.

The frequencies of delectable IL-2 levels were significantly higher in the AIDS-NHL case group 0-1 and 1-3 years prior to NHL diagnosis (p 0.002), and those of GM-CSF at the visit closest to diagnosis (p=0.001, Supplemental Table 2). The frequencies of detection for the other molecules analyzed did not differ between the two groups. Interestingly, the frequencies of detectable IL-4 and IL-12 were lower in AIDS-NHL cases, compared to the controls, at 3-5 and 1-3 years before diagnosis, respectively, with nominally significant p-values.

Consistent with the univariate analyses, the CD4-adjusted ORs for neopterin, and IP-10 were clearly significantly elevated at all three visits before the diagnosis of AIDS-NHL (Table 1). Serum levels of IL-10 were more readily detectable with the assay used in this

study, when compared to the IL-10 ELISA used in a prior study (12), and in contrast to that study which showed that IL-10 was associated with NHL risk only at the study visit most proximal to NHL diagnosis, showed significantly elevated ORs at all three time points pre-NHL (Table 1). As previously reported, increased ORs for IL-6 were observed at all time points, but only the visit closest to AIDS-NHL was statistically significant when using the more conservative cut-off of $p<0.01$ for determining significance. Similarly, the ORs for TNFα were elevated at all three time points preceding NHL, but were of nominal statistical significance. IFN-γ levels and detectable frequency of IL-2 showed elevated ORs of nominal significance at only one of the time points, 0-1 and 3-5 years pre-NHL respectively (Table 1). Interestingly, the OR for frequency of detectable IL-4 was decreased (with nominal significance) 3-5 years before AIDS-NHL, consistent with the results obtained with the univariate analysis (Table 1). None of the ORs for any of the other molecules analyzed were statistically significant or nominally significant.

In order to determine if cART affected these results, we analyzed these data excluding cART recipients from the analysis. No notable changes were seen in the results obtained (Supplemental Table 1).

Elevated serum levels of these biomarkers were significantly associated with the development of systemic AIDS-NHL, but not PCNSL

The stratification of AIDS-NHL cases according to CNS or systemic primary tumor location revealed that most of the biomarkers that were seen to be associated with risk for AIDS-NHL overall also were significantly associated with the development of systemic AIDS-NHL, but not PCNSL. However, the different risk estimates obtained for PCNSL vs systemic AIDS-NHL were not significant, with the exception of IL-6 and IL-27, which were seen to be associated more with systemic AIDS-NHL than PCNSL at some time points, with nominally significant p-values (data not shown).

DISCUSSION

HIV infection is characterized by an increased level of immune activation, including chronic activation of B-lymphocytes. B cell activation is believed to contribute to AIDS-NHL, by promoting errors in DNA-modifying activities, especially *IgH* SHM and CSR (3,22). Our prior results showed that the risk of developing AIDS-NHL was strongly associated with elevated pre-diagnosis levels of several B cell-stimulatory factors, including IL-6, IL-10 (12) and CXCL13 (17,23). In this study, we assessed pre-AIDS-NHL diagnosis serum levels of several additional cytokines, with the aim of better understanding potential tumorigenic mechanisms in HIV infected persons. Additionally, we quantified Ig FLC to confirm that serum levels of these molecules were elevated pre-AIDS-NHL, as reported by others (16).

Immunoglobulin κ and λ FLC are produced in excess by activated Ig-producing B lymphocytes, and are released into serum. FLCs are normally detected at low levels in healthy individuals and are measured in urine and serum as markers of plasma cell dyscrasias (24). More recently, autoimmune diseases, such as Sjögren syndrome and rheumatoid arthritis, have been associated with elevated FLCs (25), suggesting their potential role as markers for inflammation and B cell activation. In this study, we saw that FLCs were elevated pre-lymphoma, and that the risk of AIDS-NHL was associated with elevated levels of λ FLC. These results confirm and extend the observations of Landgren *et al* (16) in a larger population with longitudinal specimens, providing additional evidence that elevated B cell activation occurs preceding AIDS-NHL diagnosis.

In multivariate analyses, elevated serum levels of IL-10, IP-10, and neopterin were significantly associated with an increased risk for subsequent development of NHL, at all

three time points spanning over 5 years pre-NHL diagnosis. For IP-10 and neopterin, the increased risks ranged from 3.7-fold to more than 10-fold, with a marked increase in risk (OR=214, 95% CI 5.39-8513) seen with neopterin in the year preceding AIDS-NHL diagnosis. IL-6 levels in the year preceding NHL diagnosis also were significantly associated with an approximate 4-fold increased risk for NHL. Additionally, elevated TNFα levels were nominally associated with NHL risk at all three times pre-NHL. Interestingly, detectable serum IL-4 was seen to be associated with a decreased risk of AIDS-NHL, with nominally significant p-values. These results confirm our prior finding that elevated IL-6 and IL-10 serum levels were associated with subsequent risk for AIDS-NHL diagnosis (12). Moreover, in a prior study, using a subset of the study population utilized here, we found an association between AIDS-NHL risk and a high expression-associated IL-10 genotype, further supporting the association of IL-10 with the development of these lymphomas (26).

After stratification of AIDS-NHL cases according to tumor location (PCNSL vs systemic) it was noted that most of the biomarkers that were seen to be associated with risk for AIDS-NHL overall were not associated with risk for PCNSL. In contrast, IL-6, IL10, IP-10 and neopterin were significantly associated at one or more time points with the subsequent development of systemic AIDS-NHL. Since the different risk estimates obtained for PCNSL vs systemic AIDS-NHL were not significant for most of the biomarkers tested, the apparent differences in the association of biomarkers with these two groups of AIDS-NHL may be due to limited statistical power. However, it does appear that the elevated levels of some B cell activation-associated molecules, such as λ FLC or IL-10, with systemic NHL but not with PCNSL may reflect etiologic differences between these forms of AIDS-NHL. Perhaps B cell activation, reflected by the elevated serum levels of IL-6, plays a greater role in the genesis of systemic forms of AIDS-NHL than it does for PCNSL, which are virtually all EBV+ lymphomas, and which may result from loss of immunoregulatory control of EBV infection.

The molecules most consistently associated with NHL risk were IL-10, IP-10, neopterin (27,28), IL-6, TNFα, and FLC. It is not possible to determine from this study whether these cytokines are contributing to tumor development by creating an immune environment that promotes carcinogenesis, or if this cytokines are produced by tumor cells or tumor-reactive cells. Given that the levels of cytokines produced by these two cell subsets are elevated long before AIDS-NHL diagnosis, it seems reasonable to speculate that these molecules are likely to contribute to tumorigenesis, especially when detected several years prior to diagnosis.

Several of these cytokines (IL-6, TNFα) are associated with TH17 responses, and all of these, except FLC, are inflammatory molecules that can be produced by activated monocyte/macrophages. In contrast to recent studies by others who concluded that AIDS-NHL risk was associated with either a TH1 (15) or TH2 cytokine pattern (14) preceding NHL diagnosis, there was no strong association seen between most of the TH1 or TH2 cytokines tested and AIDS-NHL risk.

The idea that TH17 cells participate in the development of AIDS-NHL appears to be plausible. TNFα, which was elevated pre-lymphoma, is an inflammatory cytokine, which has been associated with amplification of TH17 production (29), and is directly produced by TH17 cells (30). TNFα levels have been shown to be increased in patients affected by AIDS-NHL (31), and prior work by us showed that genotypes associated with high TNFα expression are associated with the development of AIDS-NHL (32). In addition to this, in previous work we showed that CXCL13 is also elevated pre-NHL (17,23,33). IL-6 and CXCL13 are both TH17 cytokines (34,35). However, other TH17 cytokines, including IL-17A, IL-17F and IL-23, were not significantly associated with NHL risk in this study,

Interestingly, most of the molecules that were elevated pre-NHL. IL-6, IL-10, CXCL13, IP-10, neopterin, and TNFα also can be produced by activated macrophages (36-39). While initially considered to be a TH2 cytokine, IL-10 is produced by regulatory T cells (Treg) (40), and by monocyte/macrophages, and is a potent B cell-stimulatory factor (41). IP-10, which was strongly associated with risk for AIDS-NHL in this study, as well as in prior work by others (15), is produced by activated macrophages (39). Additionally we found that neopterin, which is an established biomarker for macrophage activation (27,28), is strongly associated with risk for AIDS-NHL. Therefore, our results are consistent with the notion that macrophage-produced cytokines are elevated preceding AIDS-NHL. Additionally, we found that pre-NHL levels of all these monokines correlate with each other, with statistically significant correlation coefficients seen among the following cytokines: IL-6, IL-8, IL-10, IFNγ, IP-10, neopterin, and TNFα (data not shown). Macrophages have been implicated in the pathogenesis of AIDS-NHL (42). A recent study indicates that AIDS-NHL contain macrophages that are infected with unique and distinct HIV species (43). Monocyte/ macrophage activation, in the context of HIV infection, may be driven by the translocation of bacterial products, such as lipopolysaccharide (LPS), out of the gut lumen into the peripheral circulation (44,45). In a recent study, Marks, *et al* showed that elevated serum levels of LPS, as well as of sCD14, a marker of microbial translocation, were associated with increased risk for AIDS-NHL (46). This lends support to the notion that macrophage activation driven by microbial translocation may contribute to the genesis of AIDS-NHL.

A limitation of this study is that more of the AIDS-NHL cases than the controls had a non-NHL AIDS diagnosis. Certainly, opportunistic infections or other AIDS-defining clinical conditions have the potential to lead to elevated cytokine and biomarker levels. However, it is important to note that the potential effect of AIDS-associated conditions on biomarker levels is greatest at the visit closest to NHL diagnosis (visit 1), as the number of NHL cases with a prior AIDS diagnosis was much smaller at the other two earlier visits (visits 2 and 3).

In conclusion, a pattern of cytokine production consistent with the activation of multiple immune cell types, including TH17, and monocytes/macrophages, is seen for a prolonged period of time preceding NHL diagnosis in HIV+ individuals, potentially contributing to the development and/or growth of these malignancies. More work is needed to better understand the mechanisms by which these cells act to induce immune activation in the context of HIV infection, and in particular, in those persons who develop AIDS-NHL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

The risk of developing AIDS-NHL is elevated for those individuals who have higher levels of inflammatory biomarkers prior to AIDS-NHL diagnosis.

	Visit 3 (3-5 years pre NHL)			Visit 2 (1-3 years pre NHL)			Visit 1 (0-1 years pre NHL)		
	N, sets[1]	OR[2]	95% CI	N, sets[1]	OR[2]	95% CI	N, sets[1]	OR[2]	95% CI
IL-5	125	1.29	0.93 - 1.79	117	1.27	0.81 - 1.97	81	1.14	0.74 - 1.74
IL-6	125	1.26*	1.02 - 1.57	117	1.48	0.98 - 2.26	81	**4.30****	**1.51 - 12.21**
IL-8	125	1.03	0.77 - 1.37	117	1.22	0.74 - 1.99	81	1.64	0.97 - 2.77
IL-10	125	**1.70****	**1.26 - 2.30**	117	**3.48****	**1.75 - 6.93**	81	**1.85****	**1.17 - 2.94**
IL-17F	121	0.99	0.84 - 1.17	112	0.88	0.65 - 1.19	75	1.06	0.80 - 1.41
IL-20	121	1.02	0.90 - 1.16	112	1.09	0.90 - 1.33	75	1.05	0.85 - 1.29
IL-27	121	1.04	0.69 - 1.56	112	0.84	0.37 - 1.89	75	2.15	0.80 - 5.81
IFNα	125	1.08	0.86 - 1.35	117	1.07	0.81 - 1.41	81	1.91*	1.11 - 3.16
IP-10	121	**3.77****	**1.93 - 7.35**	112	**4.10****	**1.60 - 10.51**	75	**12.5****	**2.55 - 61.94**
LIF	121	1.38	1.0 - 1.92	112	1.14	0.80 - 1.62	75	1.36	0.82 - 2.27
Neopterin	125	**3.68****	**1.55 - 8.75**	118	**13.3****	**3.20 - 55.71**	76	**214****	**5.39 – 8513**
sCD40L	121	1.05	0.86 - 1.29	112	0.98	0.71 - 1.36	75	1.06	0.77 - 1.44
TNFα	125	1.68*	1.09 - 2.59	117	2.22*	1.17 - 4.23	81	2.04*	1.10 - 3.75
VEGF	125	1.20	0.84 - 1.72	117	1.33	0.85 - 2.10	81	1.48	0.82 - 2.68
κ	126	**2.07****	**1.2 - 3.56**	119	1.60	0.88 - 2.89	79	1.41	0.83 - 2.38
λ	126	**2.77****	**1.42 - 5.40**	119	2.26	0.93 - 5.49	79	3.20*	1.06 - 9.64
IL-1β	125	1.13	0.49 - 2.60	117	1.47	0.47 - 4.62	81	1.52	0.45 - 5.18
IL-2	125	1.90*	1.01 - 3.55	117	1.84	0.78 - 4.33	81	2.97	0.89 - 9.88
IL-4	125	0.48*	0.24 - 0.97	117	0.98	0.37 - 2.55	81	1.40	0.34 - 5.81
IL-12	125	0.44	0.17 - 1.13	117	0.75	0.25 - 2.23	81	0.97	0.22 - 4.18
IL-17A	121	1.39	0.76 - 2.54	112	1.29	0.53 - 3.14	75	1.82	0.65 - 5.11
IL-23	121	1.12	0.57 - 2.22	112	1.12	0.46 - 2.68	75	2.81	0.80 - 9.82
IL-31	121	1.50	0.81 - 2.79	112	1.04	0.42 - 2.56	75	0.60	0.21 - 1.74
GM-CSF	125	1.11	0.56 - 2.20	117	1.22	0.48 - 3.10	81	2.33	0.61 - 8.91
SDF-1α	121	1.50	0.84 - 2.67	112	1.40	0.62 - 3.19	75	1.83	0.71 - 4.73

[1] Number of matched case-control sets,

[2] Odds ratios and 95% confidence intervals were calculated at three different time points pre-NHL using a multivariate matched analysis, controlling for age and CD4+ T cell count. For IL-6, IL-8, IL-10, IL-17F, IL-20, IL-27, IFN-α, IP-10, LIF, neopterin, sCD40L, TNF-α, VEGF, κ and λ FLC ORs are in terms of one unit increase in natural log-transformed values. For IL-1β, IL-2, IL-4, IL-12, IL-17A, IL-23, IL-31, GM-CSF, SDF-1α, the ORs are in terms of detectable versus undetectable (shaded area).

* p<0.05 (nominally significant)

** p<0.01(statistically significant, represented in bold)