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Longitudinal Changes in Immune Activation Serum Biomarkers Prior to Diagnosis and Risk of B-Cell NHL Subtypes

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Abstract

Background: To examine the contribution of B-cell activation molecules to B-cell follicular lymphoma (FL) and diffuse large B cell lymphoma (DLBCL), a prospective study was conducted using prediagnosis serial serum samples from the US Department of Defense Serum Repository.

Methods: Each case (n=142 FL, n=211 DLBCL) was matched to two controls on age, gender, race, military branch, and blood collection dates. Immune activation molecules (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, CXCL13, IL-8, TNF-α, IFN-γ, GM-CSF, VEGF, sCD30, IgE) were quantified using ELISA or multiplex immunometric (Luminex) assay. Longitudinal data were analyzed using linear mixed modeling. As serial specimens were collected over several years before diagnosis, we evaluated the temporal dynamics of these markers.

Results: Increased serum levels of sCD30, CXCL13, and to a lesser extent IL-10 were associated with both FL and DLBCL in cases compared with controls, with a median follow-up of 5.5

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Authors' Disclosures

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years from the earliest specimen collection to diagnosis date. Significant increasing sCD30 and CXCL13 trajectories for FL and DLBCL subtypes were noted starting at the earliest time points and with IL-10 levels increasing significantly at time points closer to diagnosis.

Conclusions: These results suggest that sCD30, CXCL13, and IL-10 may contribute to the etiology of FL and DLBCL and are potential biomarkers for these NHL subtypes.

Impact: The increasing trajectories of the B-cell activation molecules, sCD30, CXCL13, and to a lesser extent IL-10, may indicate early disease-induced effects or reflect the chronic stimulation of B-cells that promotes the development of FL and DLBCL subtypes.

Keywords

follicular lymphoma; diffuse large B cell lymphoma; epidemiology; immune biomarker trajectories

INTRODUCTION

Errors in DNA modifying activities that are associated with the activation of B lymphocytes, including immunoglobulin heavy chain gene (*IgH*) class-switch recombination (CSR) and somatic hypermutation (SHM) of Ig genes, can contribute to the development of mature B-cell malignancies, including several histologic subtypes of non-Hodgkin lymphoma (NHL) of B cell origin. [1, 2] In fact, there is ample evidence that chronic stimulation of B cells by infection (e.g., HIV, *H. pylori*, hepatitis C virus), or by forms of autoimmunity that involve autoantibody production (e.g., Sjögren's), [3-7] is associated with an increased risk of B-cell NHL. Therefore, factors that promote B-cell activation, including cytokines and other immune stimulatory molecules, would be expected to be associated with an increased risk for the development of B-cell NHL. [8] The overall goal of this investigation is to determine whether an immune system environment that promotes chronic B-cell stimulation precedes the development of NHL of B-cell origin, by determining if the rate of change in serum levels of B-cell-stimulatory cytokines and molecules that are associated with B-cell activation precede the development of follicular lymphoma (FL) and/or diffuse large B-cell lymphoma (DLBCL).

There are several reports in the literature, in study populations worldwide, which have examined the association between prediagnosis B-cell activation markers and NHL risk by quantifying serum/plasma levels of various cytokines and other immune molecules; [9-27] levels of circulating (serum/plasma) sCD30, CXCL13 and IL-10 have been associated with NHL risk in several studies. [12, 14-23, 25-27] In most of these studies, however, single serum samples were available for analysis at varying time points prior to NHL diagnosis, resulting in cross-sectional examinations of serum cytokine levels in relation to NHL risk. Three of these cohort studies measured a limited number of cytokines in two serum samples per participant. [25-27] In all three studies, there was limited ability to examine the changes of these immune markers over time in FL and DLBCL subtypes because of small sample sizes.

Larger studies of NHL utilizing several serial samples within individuals to assess a panel of B-cell activation molecules would allow for a more robust evaluation of

immune marker trajectories prior to diagnosis, especially by NHL subtypes given the heterogeneity in B-cell NHL and the likelihood that the trajectories of immune activation molecules differ by histologic subtypes. For these reasons, we conducted a prospective study, with independent review of pathology results, in an ethnically-diverse, apparently immunocompetent population without HIV infection to determine whether the risk of B-cell NHL subtypes is associated with prediagnosis serum levels of 15 cytokines, chemokines, and other immune-related biomarkers: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, CXCL13, IL-8, TNF-α, IFN-γ, GM-CSF, VEGF, sCD30, IgE. In order to better characterize the underlying cytokine immune milieu prior to diagnosis, we constructed several traditional statistical models such as linear mixed equations and Generalized Estimating Equations (GEE) models as well as a machine learning algorithm based on random forests to simultaneously assess cytokine levels variable importance. [28] We also used more traditional regression methods to assess the relationship of several variables to diagnosis.

As we were able to retrieve up to three serial serum samples collected over several years prior to B-cell diagnosis in this study cohort, we also evaluated the longitudinal changes in levels of these molecules and the subsequent development of FL and DLBCL, two common histologic subtypes of NHL that emerge from germinal center B cells.

MATERIALS AND METHODS

Study population

Incident cases of NHL of the follicular lymphoma (FL) or diffuse large B cell lymphoma (DLBCL) histologic subtypes, identified from 1992 through 2008 among relatively young, ethnically-diverse active-duty military personnel with an archived specimen in the DoD Serum Repository (DoDSR) drawn prior to the diagnosis date, were eligible for inclusion in the study. Cases were identified from the Armed Forces Institute of Pathology (AFIP) National Pathology Repository and the military Automated Centralized Tumor Registry (ACTUR). Pathology reports were obtained and reviewed by study pathologist for over 90% of FL and DLBCL cases, to confirm the diagnosis and date of diagnosis. Histologic determination was based on the WHO 2008 Classification. [29] Each case was matched whenever possible with two controls on age (± 1 year), gender, race, branch of service, and dates of blood collection (± 90 days), via the Defense Medical Surveillance System (DMSS), the capabilities of which include linking individuals identified from various demographic and medical databases to serum specimens archived in the DoD Serum Repository. [30] All study subjects were HIV seronegative, and had no previous cancer diagnosis, other than non-melanoma skin cancer.

Serum collection

The DoDSR, which is managed by the Armed Forces Health Surveillance Center, has archived over 40 million serum specimens from active-duty and reserve personnel with more than 2 million new samples added yearly. Residual serum is stored from routine HIV-1 screening and from HIV-1 testing of pre- and post-deployment specimens. During handling outside the freezers, the specimens are kept on beds of dry ice to preclude freeze-thaw cycles and then are stored at −30°C.

Laboratory analysis

Serum levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-8, TNF-α, IFN-γ, GM-CSF, VEGF were quantified by multiplexed immunometric assay (Luminex platform) 12-plex bead array immunoassay (High Sensitivity Human Cytokine Panel, R & D Systems). Briefly, Luminex microparticles pre-coated with analyte-specific antibodies were incubated with diluted serum samples, followed by a biotin–antibody and by a streptavidin–phycoerythrin conjugate. The fluorescence intensity of each analyte's microparticles was quantified using a Bioplex 200 (Luminex) System Analyzer (Bio-Rad), and the data analyzed using BioPlex Manager (v 4.1.1) software, using a five parameter logistic curve fit, with assay results expressed in pg/ml. Serum levels of sCD30 (Bender MedSystems), CXCL13 (R & D Systems), and total IgE were determined by enzyme-linked immunosorbent assay (ELISA) and also expressed as pg/ml. Total serum IgE was determined utilizing the CIA-7.12 and CIA-4.15 monoclonal antibodies as previously described. [31, 32]

In many subjects, serum levels of IL-2, IL-4, and IL-12 were undetectable. For these markers as well as other markers with undetectable results, we first assigned the value of zero to the undetectable level and then we added the value of 1.0 for log transformation.

Laboratory personnel were blinded to samples' case–control status. We randomly ordered case and control samples within matched sets and tested all samples of a given set on the same Luminex or ELISA plate. We interspersed blinded quality control aliquots of pooled sera from the control population and observed good to excellent within-plate and between-plate assay coefficients of variation (CV). Intra-plate CVs for IL-8, IL-10, TNFα, VEGF, sCD30, IgE and CXCL13 were 6.2%, 18.4%, 8.8%, 9.3%, 10.8%, 10.7%, and 9.5%, respectively. Inter-plate CVs for IL-8, IL-10, TNFα, VEGF, sCD30, IgE and CXCL13 were 8.5%, 25.1%, 11.1%, 11.6%, 11.6%, 17.0%, and 11.4%, respectively. Serum levels of several of the cytokines measured (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-12, GM-CSF, IFNγ) were either below or near the level of detection in most determinations, precluding the calculation of meaningful CV estimates for those molecules.

Statistical analysis

Prior to analysis, data quality was explored by assessing outliers and skewness. Groups were compared using the t-test or Wilcoxon Rank Sum test as appropriate. All of the immune marker variables had non-normal distributions with right-skewing, thus immune-biomarker results were natural log-transformed to meet model assumptions. The immune markers were analyzed as continuous variables as well as categorical variables with cut points for each assay set at the quartiles of the control distributions. For immune markers with very limited data above the level of detection, data were analyzed as dichotomous variables, comparing the fraction of detectable versus undetectable between cases and controls.

Longitudinal data were analyzed by using linear mixed modeling with both fixed and random effects and odds ratios (OR) and 95% confidence intervals (95% CI) between cases and controls were estimated. This allowed each individual to deviate randomly from the population probability distribution. To assess nonlinearity, a spline-based lowess regression model also was fitted. An interaction term was included to assess the relation between time

to diagnosis and FL or DLBCL. GEE (GEE) analyses were also conducted to allow for the examination of repeated measures data and to determine the trajectory of the immune marker measurements as time to diagnosis approached. All p-values were 2-tailed. Data were analyzed using SAS version 9.2.

Missing data were imputed using rfimpute in the randomForest package in R. We also used Fuzzy Forests, a machine learning algorithm that extends Random Forests in the case of correlation. [28] Fuzzy Forests works in stages, first with an unsupervised learning part that creates weighted correlation networks on all potential predictor variables. The second stage consists of a supervised learning part that ranks the variable importance in terms of prediction. Fuzzy Forests is freely available from CRAN and was implemented using Fuzzy Forest package using R Studio version 1.1.463.

The institutional review boards of the Walter Reed Army Institute of Research and UCLA approved the research protocol.

Data Availability Statement

The datasets generated during the current study are available from the corresponding author on reasonable request.

RESULTS

The study population was comprised of 500 NHL cases and 985 controls, all of whom were on active duty on the case diagnosis date (Table 1). Comparison of the distribution among study subjects according to age, gender, race, or branch of service did not reveal any notable differences between the case and control groups as expected, as the controls were matched to cases on these factors (Table 1). The median age of cases was 36 years, 92% of cases were male, and 67% were white. Pathology review of all cases determined that 211 were diffuse large B-cell lymphoma (DLBCL) and 142 were of the follicular lymphoma (FL) subtype (Table 2).

Two or more serial pre-NHL diagnosis serum samples were available from the DoDSR for 86.2% of NHL cases and 86.0% of controls. Three serial pre-NHL diagnosis serum samples were available for 71% of NHL cases and 70.7% of controls. The majority of the specimens tested came from the time period most proximal to NHL diagnosis, with 61% of the serum specimens tested obtained during the time window 0-5 years preceding NHL diagnosis, and 11% of specimens collected >10 years prediagnosis.

We conducted the analyses separately for DLBCL and FL. We modeled serum levels of sCD30, CXCL13, IL-10, IL-5, IFN-γ, TNF-α, IL-6, GM-CSF, IgE, IL-8, IL-1β, and VEGF as continuous variables and estimated an Odds Ratio per unit increase in the immune marker on the natural log scale with adjustment for the matching factors (Table 3). When all of the biomarkers were entered into the same model, only sCD30, CXCL13, IL-10 and VEGF remained significant among DLBCL cases compared with controls. When the B-cell activation molecules were mutually adjusted for the other biomarkers, only sCD30 and CXCL13 remained significant for FL.

Changes in mean levels of sCD30, CXCL13, IL-10, TNF-α and IFN-γ from the earliest to most recent measurement before diagnosis in DLBCL cases compared with controls are shown in Table 4 and Supplemental Figure 1 for DLBCL, and Table 5 and Supplemental Figure 2 for FL. P-values were calculated for the delta between the earliest and the newest mean of the serum sample for each of these markers using the t- test (Table 4 for DLBCL and Table 5 for FL, respectively). Longitudinal changes in biomarkers prior to diagnosis showed increases in serum levels of sCD30, CXCL13, IL-10, TNF-α, and IFN-γ in cases compared with controls in DLBCL. For FL, longitudinal changes prior to diagnoses showed increases in serum levels of sCD30, CXCL13, and IL-10. In both DLBCL and FL subtypes, a strong, statistically significant increase in serum levels from the earliest to the most recent specimens was noted for sCD30 and CXCL13. In contrast, a significant rate of change in IL-10 serum levels in FL and DLBCL subtypes was observed in specimens collected closer to diagnosis. Serum levels of TNF-α and IFN-γ were elevated only in specimens closest to diagnosis, comparing cases to controls in DLBCL subtypes.

The results of the lowess analysis for DLBCL and FL for sCD30, CXCL13, and IL-10 are shown in Figure 1, with individual biomarker levels plotted on the y axis versus years to diagnosis on the x axis. sCD30 and CXCL13 levels appeared to be elevated as early as 5 years prior to diagnosis for FL cases compared with controls. CXCL13 levels were seen to be higher in cases than in controls even earlier in DLBCL subtypes. IL-10 trajectories were noted closer to diagnosis for both FL and DLBCL.

Fuzzy forest analysis confirmed sCD30, CXCL13 and IL-10 as the most important variables in predicting cases from controls for both FL and DLBCL. In fact, the two most predictive markers were CXCL13 at time point 3 (closest to diagnosis) and sCD30 at time point 3, and clustered together in the weighted correlation network in the forest part of the algorithm. It is important to point out that the module formation is an unsupervised learning technique where the outcomes are not known to the algorithm. It is also interesting to note that it grouped the time point 3 biomarkers separately from time points 1 and 2 for the most cases. This is most likely because there is a dramatic increase in these activation markers shortly before diagnosis.

DISCUSSION

The availability of serum samples collected longitudinally in the DoDSR allowed the prospective assessment of the association between circulating serum levels of 15 cytokines, chemokines and other immune-related biomarkers and the risk of FL and DLBCL, two common histologic subtypes of NHL, over a period of nearly twenty years preceding the diagnosis of these B-cell NHL. These results indicate that sCD30, CXCL13, and IL-10 levels are elevated with increasing serum concentrations over time preceding the clinical recognition of DLBCL and FL in presumably immunocompetent persons, and suggest that B-cell-stimulatory cytokines may contribute to the etiology of these NHL subtypes. Additionally, these three biomarkers have the potential to serve as a biomarker for NHL. These findings are in general agreement with the findings of several prior studies examining prediagnostic B-cell-activation associated molecules in single samples per participant, both in immunocompetent persons, [12, 14-23] and in the setting of HIV infection, [33-35] and

further characterize the role of immune markers in lymphomagenesis by examining immune marker trajectories. Additionally, several studies have noted that polymorphisms in the IL-10 gene, as well as in the gene encoding the receptor for CXCL13, are associated with risk for the development of FL and/or DLBCL. [36-38]

CD30 is a member of the TNF receptor superfamily and the receptor for CD30 ligand (CD153 or TNFSF8) a TNF-like immune stimulatory molecule. [39] CD30 is expressed on Hodgkin lymphoma (HL) Reed-Sternberg (HRS) cells, [40, 41] where it has the potential to serve as a receptor for signals that may enhance their proliferation. The results presented here indicate that sCD30 levels in circulation are elevated for several years preceding NHL diagnosis in FL and DLBCL subtypes and are markedly increased as time to diagnosis approaches.

Elevated serum levels of sCD30 have been reported to precede the diagnosis of AIDS-associated B-cell NHL [31, 34] and B-cell NHL in immunocompetent subjects, [12, 14, 15, 18, 19, 22, 23] neither of which typically express CD30, as well as HL in immunocompetent persons. [32] In a nested case-control study of a subset of participants enrolled in two cohorts, CLUE-1 and CLUE-11, sCD30 levels were measured in two serum samples [26]; however, this study did not contain sufficient numbers of FL and DLBCL cases with repeated samples to allow for analyses by subtype. Spath *et al* [25] examined the trajectory of sCD30 and CXCL13 in two serum samples from 30 FL and 42 DLBCL cases that had specimens stored in The Northern Sweden Health and Disease Study (NSHDS). Consistent with our findings, increasing levels of sCD30 over time towards diagnosis was associated with FL risk. Purdue and colleagues, [27], however, reported elevated but stable sCD30 levels, with no evidence of an increasing trajectory, in two samples from the Janus Serum Bank in Norway for 39 cases of FL and 66 cases of DLBCL. It is noteworthy that we observed a significant trajectory for sCD30 levels and risk of DLBCL. Neither Spath et al. [25] nor Purdue et al. [27] reported similar findings. One possible explanation is that our sample size was much larger with 211 DLBCL cases matched 1:2 to controls. We also had access to three specimens per individual for 71% of our study population, which provided a more stable estimate of serial immune marker levels. The median time from the earliest specimen collection to diagnosis was shorter, however, compared with the prior two studies, [25] [27] which may be the reason for the differences in the findings. As DLBCL is considered an aggressive NHL subtype, with a relatively short latency, these findings suggest that the observed sCD30 trajectory is likely indicative of the presence of an emerging DLBCL.

IL-10 is a B-cell stimulatory cytokine that also can suppress T cell and monocyte responses, including anti-viral cell-mediated responses. [42] IL-10 can enhance *BCL2* expression, thereby promoting B-cell viability.[43] IL-10 has the potential to contribute to the pathogenesis of NHL in several ways; IL-10 can directly stimulate B cells, and also can inhibit anti-viral T cell-mediated responses, such as those involved in the immunoregulation of EBV infection, so may also promote B-cell activation driven by EBV. IL-10 is produced by various immune cell subsets, including regulatory T and B cells and monocytes. [44, 45] DLBCL tumor cells also can produce IL-10, allowing cell-autonomous growth and immune escape by these tumor cells [46]. Elevated levels of IL-10 in circulation have been seen to

be associated with an increased risk for NHL, both in HIV-infected [33] and in presumably immunocompetent persons. [16, 20, 21]. Additionally, elevated levels of regulatory B cells (IL-10-producing cells) in circulation were seen to precede the diagnosis of AIDS-associated NHL. [47] Genetic polymorphisms in the IL-10 gene also have been seen to be associated with risk for NHL in several studies (reviewed in [37, 38]). To our knowledge, the current study is the first to report on the trajectory of IL-10 serum levels prior to FL and DLBCL diagnosis, finding that the rate of change in IL-10 levels increased closer to diagnosis compared with results for sCD30 and CXCL13.

CXCL13 is a chemokine produced by follicular dendritic cells and helper T cells in secondary lymphoid organs. [48] The receptor for CXCL13 is CXCR5 (BLR1). CXCL13 plays a central role in the trafficking of B cells into and within lymphoid follicles, that is necessary for antigen exposure and activation within germinal centers, leading to the differentiation of B cells into antibody-producing plasma cells. [49-51] Aberrant CXCL13 and CXCR5 participate in the pathogenesis of multiple subtypes of lymphomas, [52-55] and elevated levels of human CXCL13 were seen in a NOD-SCID xenograft model of AIDS-associated Burkitt lymphoma. [56] Elevated levels of circulating CXCL13 have been seen to be associated with an increased risk for NHL in both HIV-infected persons [35, 57] and in presumably immunocompetent persons. [17, 19, 23] Additionally, the gene encoding the receptor for CXCL15, CXCR5/BLR1, was seen to be associated with risk for FL in a large GWAS study carried out in the Interlymph consortium, [36] suggesting an important role for CXCL13 and its receptor in the pathobiology of NHL. Spath *et al* [25] also examined changes in CXCL13 serum levels in repeated samples. A significant slope was found for DLBCL, comparing cases with controls, but no trajectory was found for FL. In contrast, we observed a significant trajectory of CXCL13 levels with both FL and DLBCL subtypes.

Our study had some limitations. The study population was composed mainly of young men, with a median age of diagnosis of 36 years, which is younger than the age distribution of the cohorts in other similar investigations. [25-27] Our results, therefore, may not apply to women or be extrapolated to all cases of FL or DLBCL. In addition, the median follow-up time from the earliest serum sample to incident disease was 5.5 years, which is a relatively short window compared with other studies, and hampered our ability to interpret whether the observed immune markers trajectories may reflect early disease processes. The study also had considerable strengths. The availability of three serum samples for 71% of the study population, collected longitudinally over several years before the onset of B-cell NHL, allowed the examination of the temporal dynamics of these markers. Pathology reports were retrieved for over 90% of the cases and each report was reviewed by a study pathologist to determine NHL histologic subtypes. We controlled for potential confounding by matching on known demographic risk factors for B-cell NHL. The present study also improves on evidence from previous similar investigations as the sample size was relatively large allowing a more robust assessment of the rate of change prior to diagnosis for a wide range of B-cell activation molecules

The use of machine learning techniques in immunological studies has been limited until recently. In this study, machine learning indicated that three biomarkers, namely sCD30, CXCL13, and IL-10 are important markers for predicting lymphoma. While machine

learning identified the same three markers that have already been identified using classical modeling approaches, and three markers that are already well-established in the literature, obtaining the same finding using an alternative, unrelated analytical approach enhances the strengths of this finding, and points to common biological pathways leading to NHL.

Ongoing stimulation by B-cell activation molecules, including sCD30, CXCL13 and IL-10 may lead to B-cell activation, promote the viability of these cells, and drive B cells toward a germinal center, where they undergo interactions with other immune cells that lead to *IgH* CSR and SHM, DNA-modifying activities that are mediated by activation-induced cytidine deaminase (AICDA). This can place B cells at higher risk for the development of the many forms of NHL that emerge from germinal center B cells, as errors that occur during *IgH* CSR and SHM can result in lymphomagenic lesions, such as oncogene translocation and/or mutation. As the serum levels of sCD30, CXCL13 and IL-10 increased progressively over time to NHL diagnosis, the markedly elevated levels closer to diagnosis likely reflect the presence of the nascent lymphoma, systemic inflammation associated with the presence of NHL, and/or the infiltration of reactive lymphocytes into the affected lymph nodes. The stable elevation in B-cell NHL risk with elevated levels of sCD30, CXCL13, and IL-10 across several years prior to diagnosis may also indicate the presence of a B-cell-stimulatory environment that promotes the genesis of these cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Data from the Defense Medical Surveillance System, The Armed Forces Health Surveillance Branch, Defense Health Agency, U.S. Department of Defense, Silver Spring, Maryland were obtained for study subjects from 1990 to 1999. Serum specimens from the Department of Defense Serum Repository, The Armed Forces Health Surveillance Branch, Defense Health Agency, U.S. Department of Defense, Silver Spring, Maryland were retrieved from study subjects from 1988 to 1999.

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REFERENCES

1. Pasqualucci L, Neumeister P, Goossens T, Nanjangud G, Chaganti RS, Kuppers R, et al. : Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. Nature 2001, 412(6844):341–346. [PubMed: 11460166]
2. Kuppers R, Klein U, Hansmann ML, Rajewsky K: Cellular origin of human B-cell lymphomas. N Engl J Med 1999, 341(20):1520–1529. [PubMed: 10559454]
3. Wang SS, Slager SL, Brennan P, Holly EA, De Sanjose S, Bernstein L, et al. : Family history of hematopoietic malignancies and risk of non-Hodgkin lymphoma (NHL): a pooled analysis of 10 211 cases and 11 905 controls from the International Lymphoma Epidemiology Consortium (InterLymph). Blood 2007, 109(8):3479–3488. [PubMed: 17185468]
4. de Sanjose S, Benavente Y, Vajdic CM, Engels EA, Morton LM, Bracci PM, et al. : Hepatitis C and non-Hodgkin lymphoma among 4784 cases and 6269 controls from the International Lymphoma Epidemiology Consortium. Clin Gastroenterol Hepatol 2008, 6(4):451–458. [PubMed: 18387498]
5. Hjalgrim H, Engels EA: Infectious aetiology of Hodgkin and non-Hodgkin lymphomas: a review of the epidemiological evidence. J Intern Med 2008, 264(6):537–548. [PubMed: 19017178]
6. Engels EA, Chatterjee N, Cerhan JR, Davis S, Cozen W, Severson RK, et al. : Hepatitis C virus infection and non-Hodgkin lymphoma: results of the NCI-SEER multi-center case-control study. Int J Cancer 2004, 111(1):76–80. [PubMed: 15185346]
7. Giordano TP, Henderson L, Landgren O, Chiao EY, Kramer JR, El-Serag H, et al. : Risk of non-Hodgkin lymphoma and lymphoproliferative precursor diseases in US veterans with hepatitis C virus. JAMA 2007, 297(18):2010–2017. [PubMed: 17488966]
8. Smedby KE, Hjalgrim H, Askling J, Chang ET, Gregersen H, Porwit-MacDonald A, et al. : Autoimmune and chronic inflammatory disorders and risk of non-Hodgkin lymphoma by subtype. J Natl Cancer Inst 2006, 98(1):51–60. [PubMed: 16391371]
9. Vendrame E, Martinez-Maza O: Assessment of pre-diagnosis biomarkers of immune activation and inflammation: insights on the etiology of lymphoma. J Proteome Res 2011, 10(1):113–119. [PubMed: 20886858]
10. Makgoeng SB, Bolanos RS, Jeon CY, Weiss RE, Arah OA, Breen EC, et al. : Markers of Immune Activation and Inflammation, and Non-Hodgkin Lymphoma: A Meta-Analysis of Prospective Studies. JNCI Cancer Spectr 2018, 2(4):pky082. [PubMed: 30873511]
11. Saberi Hosnijeh F, Krop EJ, Scoccianti C, Krogh V, Palli D, Panico S, et al. : Plasma cytokines and future risk of non-Hodgkin lymphoma (NHL): a case-control study nested in the Italian European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol Biomarkers Prev 2010, 19(6):1577–1584. [PubMed: 20501772]
12. Hosnijeh FS, Portengen L, Spath F, Bergdahl IA, Melin B, Mattiello A, et al. : Soluble B-cell activation marker of sCD27 and sCD30 and future risk of B-cell lymphomas: A nested case-control study and meta-analyses. Int J Cancer 2016, 138(10):2357–2367. [PubMed: 26684261]
13. Gu Y, Shore RE, Arslan AA, Koenig KL, Liu M, Ibrahim S, et al. : Circulating cytokines and risk of B-cell non-Hodgkin lymphoma: a prospective study. Cancer Causes Control 2010, 21(8):1323–1333. [PubMed: 20373009]
14. Purdue MP, Lan Q, Martinez-Maza O, Oken MM, Hocking W, Huang WY, et al. : A prospective study of serum soluble CD30 concentration and risk of non-Hodgkin lymphoma. Blood 2009, 114(13):2730–2732. [PubMed: 19638620]
15. Vermeulen R, Hosnijeh FS, Portengen L, Krogh V, Palli D, Panico S, et al. : Circulating soluble CD30 and future risk of lymphoma; evidence from two prospective studies in the general population. Cancer Epidemiol Biomarkers Prev 2011, 20(9):1925–1927. [PubMed: 21784955]
16. Purdue MP, Lan Q, Bagni R, Hocking WG, Baris D, Reding DJ, et al. : Prediagnostic serum levels of cytokines and other immune markers and risk of non-hodgkin lymphoma. Cancer Res 2011, 71(14):4898–4907. [PubMed: 21632552]
17. Purdue MP, Hofmann JN, Kemp TJ, Chaturvedi AK, Lan Q, Park JH, et al. : A prospective study of 67 serum immune and inflammation markers and risk of non-Hodgkin lymphoma. Blood 2013, 122(6):951–957. [PubMed: 23814017]

18. Purdue MP, Lan Q, Kemp TJ, Hildesheim A, Weinstein SJ, Hofmann JN, et al. : Elevated serum sCD23 and sCD30 up to two decades prior to diagnosis associated with increased risk of non-Hodgkin lymphoma. Leukemia 2015, 29(6):1429–1431. [PubMed: 25567136]
19. De Roos AJ, Mirick DK, Edlefsen KL, LaCroix AZ, Kopecky KJ, Madeleine MM, et al. : Markers of B-cell activation in relation to risk of non-Hodgkin lymphoma. Cancer Res 2012, 72(18):4733–4743. [PubMed: 22846913]
20. Edlefsen KL, Martinez-Maza O, Madeleine MM, Magpantay L, Mirick DK, Kopecky KJ, et al. : Cytokines in serum in relation to future non-Hodgkin lymphoma risk: evidence for associations by histologic subtype. Int J Cancer 2014, 135(4):913–922. [PubMed: 24488825]
21. Conroy SM, Maskarinec G, Morimoto Y, Franke AA, Cooney RV, Wilkens LR, et al. : Non-hodgkin lymphoma and circulating markers of inflammation and adiposity in a nested case-control study: The Multiethnic Cohort. Cancer Epidemiol Biomarkers Prev 2013, 22(3):337–347. [PubMed: 23300021]
22. Bassig BA, Shu XO, Koh WP, Gao YT, Purdue MP, Butler LM, et al. : Soluble levels of CD27 and CD30 are associated with risk of non-Hodgkin lymphoma in three Chinese prospective cohorts. Int J Cancer 2015, 137(11):2688–2695. [PubMed: 26095604]
23. Epstein MM, Rosner B, Breen EC, Batista JL, Giovannucci EL, Magpantay L, et al. : Pre-diagnosis plasma immune markers and risk of non-Hodgkin lymphoma in two prospective cohort studies. Haematologica 2018, 103(10):1679–1687. [PubMed: 29930163]
24. Vermeulen R, Saberi Hosnijeh F, Bodinier B, Portengen L, Liquet B, Garrido-Manriquez J, et al. : Pre-diagnostic blood immune markers, incidence and progression of B-cell lymphoma and multiple myeloma: Univariate and functionally informed multivariate analyses. Int J Cancer 2018, 143(6):1335–1347. [PubMed: 29667176]
25. Spath F, Wibom C, Krop EJ, Johansson AS, Bergdahl IA, Vermeulen R, et al. : Biomarker Dynamics in B-cell Lymphoma: A Longitudinal Prospective Study of Plasma Samples Up to 25 Years before Diagnosis. Cancer Res 2017, 77(6):1408–1415. [PubMed: 28108506]
26. Purdue MP, Lan Q, Hoffman-Bolton J, Hildesheim A, Callahan CL, Strickland P, et al. : Circulating sCD27 and sCD30 in pre-diagnostic samples collected fifteen years apart and future non-Hodgkin lymphoma risk. Int J Cancer 2019, 144(8):1780–1785. [PubMed: 30230539]
27. Purdue MP, Lan Q, Langseth H, Grimsrud TK, Hildesheim A, Rothman N: Prediagnostic serum sCD27 and sCD30 in serial samples and risks of non-Hodgkin lymphoma subtypes. Int J Cancer 2020, 146(12):3312–3319. [PubMed: 31523805]
28. Conn D, Ngun T, Li G, Ramirez CM: Fuzzy Forests: Extending Random Forest Feature Selection for Correlated, High-Dimensional Data. Journal of Statistical Software 2019, 91(9):1–25.
29. Turner JJ, Morton LM, Linet MS, Clarke CA, Kadin ME, Vajdic CM, et al. : InterLymph hierarchical classification of lymphoid neoplasms for epidemiologic research based on the WHO classification (2008): update and future directions. Blood 2010, 116(20):e90–98. [PubMed: 20699439]
30. Perdue CL, Cost AA, Rubertone MV, Lindler LE, Ludwig SL: Description and utilization of the United States Department of Defense Serum Repository: a review of published studies, 1985-2012. PLoS One 2015, 10(2):e0114857. [PubMed: 25723497]
31. Breen EC, Hussain SK, Magpantay L, Jacobson LP, Detels R, Rabkin CS, et al. : B-cell stimulatory cytokines and markers of immune activation are elevated several years prior to the diagnosis of systemic AIDS-associated non-Hodgkin B-cell lymphoma. Cancer Epidemiol Biomarkers Prev 2011, 20(7):1303–1314. [PubMed: 21527584]
32. Levin LI, Breen EC, Birmann BM, Batista JL, Magpantay LI, Li Y, et al. : Elevated Serum Levels of sCD30 and IL6 and Detectable IL10 Precede Classical Hodgkin Lymphoma Diagnosis. Cancer Epidemiol Biomarkers Prev 2017, 26(7):1114–1123. [PubMed: 28341757]
33. Breen EC, Boscardin WJ, Detels R, Jacobson LP, Smith MW, O'Brien SJ, et al. : Non-Hodgkin's B cell lymphoma in persons with acquired immunodeficiency syndrome is associated with increased serum levels of IL10, or the IL10 promoter −592 C/C genotype. Clin Immunol 2003, 109(2):119–129. [PubMed: 14597210]

34. Breen EC, Fatahi S, Epeldegui M, Boscardin WJ, Detels R, Martinez-Maza O: Elevated serum soluble CD30 precedes the development of AIDS-associated non-Hodgkin's B cell lymphoma. Tumour Biol 2006, 27(4):187–194. [PubMed: 16651853]
35. Widney DP, Gui D, Popoviciu LM, Said JW, Breen EC, Huang X, et al. : Expression and function of the chemokine, CXCL13, and its receptor, CXCR5, in AIDS-associated non-Hodgkin's lymphoma. AIDS Res Treat 2010, 2010:164586. [PubMed: 21490903]
36. Skibola CF, Berndt SI, Vijai J, Conde L, Wang Z, Yeager M, et al. : Genome-wide association study identifies five susceptibility loci for follicular lymphoma outside the HLA region. Am J Hum Genet 2014, 95(4):462–471. [PubMed: 25279986]
37. Zhang Y, Xia ZG, Zhu JH, Chen MB, Wang TM, Shen WX, et al. : Association of Interleukin-10 −3575T>A and −1082A>G polymorphisms with non-Hodgkin lymphoma susceptibility: a comprehensive review and meta-analysis. Mol Genet Genomics 2015, 290(6):2063–2073. [PubMed: 25977148]
38. Nielsen KR, Steffensen R, Haunstrup TM, Bodker JS, Dybkaer K, Baech J, et al. : Inherited variation in immune response genes in follicular lymphoma and diffuse large B-cell lymphoma. Leuk Lymphoma 2015, 56(12):3257–3266. [PubMed: 26044172]
39. van der Weyden CA, Pileri SA, Feldman AL, Whisstock J, Prince HM: Understanding CD30 biology and therapeutic targeting: a historical perspective providing insight into future directions. Blood Cancer J 2017, 7(9):e603. [PubMed: 28885612]
40. Schwab U, Stein H, Gerdes J, Lemke H, Kirchner H, Schaadt M, et al. : Production of a monoclonal antibody specific for Hodgkin and Sternberg-Reed cells of Hodgkin's disease and a subset of normal lymphoid cells. Nature 1982, 299(5878):65–67. [PubMed: 7110326]
41. Falini B, Pileri S, Pizzolo G, Durkop H, Flenghi L, Stirpe F, et al. : CD30 (Ki-1) molecule: a new cytokine receptor of the tumor necrosis factor receptor superfamily as a tool for diagnosis and immunotherapy. Blood 1995, 85(1):1–14. [PubMed: 7803786]
42. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A: Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001, 19:683–765. [PubMed: 11244051]
43. Levy Y, Brouet JC: Interleukin-10 prevents spontaneous death of germinal center B cells by induction of the bcl-2 protein. J Clin Invest 1994, 93(1):424–428. [PubMed: 8282815]
44. Vignali DA, Collison LW, Workman CJ: How regulatory T cells work. Nat Rev Immunol 2008, 8(7):523–532. [PubMed: 18566595]
45. Mauri C, Bosma A: Immune regulatory function of B cells. Annu Rev Immunol 2012, 30:221–241. [PubMed: 22224776]
46. Stirm K, Leary P, Bertram K, Nunez NG, Wust D, Boudesco C, et al. : Tumor cell-derived IL-10 promotes cell-autonomous growth and immune escape in diffuse large B-cell lymphoma. Oncoimmunology 2021, 10(1):2003533. [PubMed: 34858727]
47. Epeldegui M, Conti DV, Guo Y, Cozen W, Penichet ML, Martinez-Maza O: Elevated numbers of PD-L1 expressing B cells are associated with the development of AIDS-NHL. Sci Rep 2019, 9(1):9371. [PubMed: 31253857]
48. Takagi R, Higashi T, Hashimoto K, Nakano K, Mizuno Y, Okazaki Y, et al. : B cell chemoattractant CXCL13 is preferentially expressed by human Th17 cell clones. J Immunol 2008, 181(1):186–189. [PubMed: 18566383]
49. Ansel KM, Ngo VN, Hyman PL, Luther SA, Forster R, Sedgwick JD, et al. : A chemokine-driven positive feedback loop organizes lymphoid follicles. Nature 2000, 406(6793):309–314. [PubMed: 10917533]
50. Legler DF, Loetscher M, Roos RS, Clark-Lewis I, Baggiolini M, Moser B: B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. J Exp Med 1998, 187(4):655–660. [PubMed: 9463416]
51. Reif K, Ekland EH, Ohl L, Nakano H, Lipp M, Forster R, et al. : Balanced responsiveness to chemoattractants from adjacent zones determines B-cell position. Nature 2002, 416(6876):94–99. [PubMed: 11882900]
52. Smith JR, Braziel RM, Paoletti S, Lipp M, Uguccioni M, Rosenbaum JT: Expression of B-cell-attracting chemokine 1 (CXCL13) by malignant lymphocytes and vascular endothelium in primary central nervous system lymphoma. Blood 2003, 101(3):815–821. [PubMed: 12393412]

53. Husson H, Freedman AS, Cardoso AA, Schultze J, Munoz O, Strola G, et al. : CXCL13 (BCA-1) is produced by follicular lymphoma cells: role in the accumulation of malignant B cells. Br J Haematol 2002, 119(2):492–495. [PubMed: 12406091]

54. Trentin L, Cabrelle A, Facco M, Carollo D, Miorin M, Tosoni A, et al. : Homeostatic chemokines drive migration of malignant B cells in patients with non-Hodgkin lymphomas. Blood 2004, 104(2):502–508. [PubMed: 15001469]

55. Kazanietz MG, Durando M, Cooke M: CXCL13 and Its Receptor CXCR5 in Cancer: Inflammation, Immune Response, and Beyond. Front Endocrinol (Lausanne) 2019, 10:471. [PubMed: 31354634]

56. Widney DP, Olafsen T, Wu AM, Kitchen CM, Said JW, Smith JB, et al. : Levels of murine, but not human, CXCL13 are greatly elevated in NOD-SCID mice bearing the AIDS-associated Burkitt lymphoma cell line, 2F7. PLoS One 2013, 8(8):e72414. [PubMed: 23936541]

57. Hussain SK, Zhu W, Chang SC, Breen EC, Vendrame E, Magpantay L, et al. : Serum levels of the chemokine CXCL13, genetic variation in CXCL13 and its receptor CXCR5, and HIV-associated non-hodgkin B-cell lymphoma risk. Cancer Epidemiol Biomarkers Prev 2013, 22(2):295–307. [PubMed: 23250934]

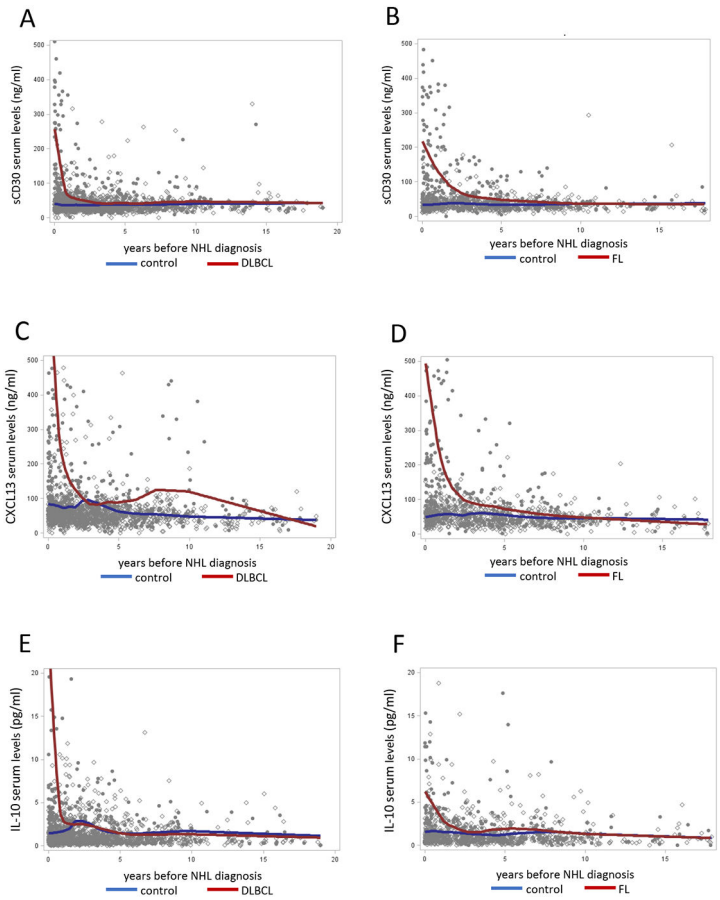

Figure 1. Serum levels of sCD30, CXCL13 and IL-10 among DLBCL and FL cases and controls in specimens collected in the years prior to diagnosis.

Displays the results of the lowess analysis for DLBCL (**A** - sCD30, **C** - CXCL13, and **E** - IL-10) and for FL (**B** - sCD30, **D** - CXCL13, and **F** - IL-10), with individual biomarker levels plotted on the y-axis vs. years to diagnosis on the x axis. sCD30 and CXCL13 levels represent ng/ml; IL-10 levels pg/ml. Cases are indicated by solid circles (●) and controls by open diamonds (◇).

Table 1.

Demographic and service-related characteristics of study population

Characteristic	Cases (N =500)	Controls (N = 985)
Age group, n (%)		
20 and under	21 (4.2)	38 (3.9)
21-30 years	143 (28.6)	288 (29.2)
31-40 years	168 (33.6)	310 (31.5)
41-50 years	143 (28.6)	310 (31.5)
> 50 years	25 (5.0)	39 (4.0)
Median age	36	36
Gender, n (%)		
Male	455 (91.0)	898 (91.2)
Female	45 (9.0)	87 (8.8)
Race/ethnicity, n (%)		
White	333 (66.7)	657 (66.7)
Black	86 (17.2)	169 (17.2)
Hispanic	46 (9.2)	91 (9.2)
Other/Unknown	35 (7.0)	68 (6.9)
Branch of service, n (%)		
Army	176 (35.2)	348 (35.3)
Air Force	98 (19.6)	194 (19.7)
Navy	168 (33.6)	329 (33.4)
Marines	53 (10.6)	104 (10.6)
Coast Guard	5 (1.0)	10 (1.0)
Military Rank, n (%)		
Enlisted	385 (77.0)	805 (81.7)
Officer/Warrant Officer	115 (23.0)	185 (13.3)
Year of Diagnosis, n (%)		
1992-1996	94 (18.8)	188 (19.1)
1997-2000	121 (24.2)	235 (23.9)
2001-2004	143 (28.6)	281 (28.5)
2005-2008	142 (28.4)	281 (28.5)
Year of earliest serum sample, n (%)		
1989 and before	68 (13.6)	135 (13.7)
1990-1994	153 (30.6)	295 (29.9)
1995-1999	212 (42.3)	413 (41.9)
2000 and after	67 (13.4)	142 (14.6)
Median time of follow up earliest serum sample to Dx (year)	5.5	5.4
Number of Available Serum Samples		
1	69 (13.8)	138 (14.0)
2	76 (15.2)	151 (15.3)
3	355 (71.0)	696 (70.7)

Table 2.

WHO codes for B-cell NHL histologic subtypes

	WHO Codes	N	%
Diffuse large B-cell lymphoma	9679, 9680, 9681, 9682, 9683, 9684, 9685, 9686	211	34.53
Follicular lymphoma	9690, 9691, 9692, 9593, 9694, 9695, 9696, 9697, 9698	142	23.24
Chronic lymphocytic leukemia/small lymphocytic lymphoma	9823	39	6.38
Burkitt's lymphoma	9687, 9688, 9826	32	5.24
Marginal zone lymphoma	9689, 9699, 9710, 9711, 9715, 9760, 9764	29	4.75
B-cell not otherwise specified	9591, 9592, 9593, 9595	20	3.27
Mantle cell lymphoma	9673, 9674, 9677	14	2.29
Hairy cell leukemia	9940, 9941	13	2.13

Table 3.

Association of DLBCL and FL with B-cell activation molecules

	DLBCL			FL		
	Case/Ctrl	**OR**	**95% CI***	**Case/Ctrl**	**OR**	**95% CI***
Continuous						
sCD30	537/1065	2.69	(2.22-3.29)**	358/694	4.42	(3.41-5.84)**
CXCL13		2.31	(1.97-2.73)**		2.90	(2.34-3.63)**
IL-10		1.83	(1.55-2.17)**		1.85	(1.47-2.33)
IL-5		1.46	(0.99-2.14)		1.34	(0.82-2.18)
IFN-γ		1.60	(1.25-2.07)		0.92	(0.64-1.30)
TNF-α		1.23	(1.09-1.39)		1.08	(0.93-1.25)
VEGF		1.33	(1.17-1.52)**		1.02	(0.87-1.18)
IL-6		1.13	(1.06-1.21)		1.01	(0.92-1.10)
GM-CSF		1.23	(1.04-1.45)		0.96	(0.78-1.18)
IgE		1.07	(0.98-1.17)		0.96	(0.86-1.07)
IL-8		1.04	(0.98-1.10)		0.99	(0.92-1.06)
IL-1β		1.03	(0.96-1.10)		0.93	(0.85-1.02)
Detectable/Undetectable						
IL-2	257/492	1.07	(0.87-1.32)	176/318	1.16	(0.90-1.50)
IL-4	252/483	1.07	(0.87-1.32)	150/290	1.01	(0.78-1.31)
IL-12	172/291	1.25	(1.00-1.57)	79/158	0.97	(0.71-1.31)

*Adjusted for age, race, and sex

**Remained significant with mutual adjustment for other biomarkers

Table 4.

Changes in mean levels of sCD30, CXCL13, IL-10, TNF-α and IFN-γ from earliest to most recent measurement before diagnosis in DLBCL cases compared with controls

	Case			Control			P-Value	
		N	Mean		N	Mean		
sCD30	Earliest	186	3.70 (0.56)	Earliest	369	3.55 (0.50)	Earliest	0.0008
	Middle	147	3.65 (0.55)	Middle	292	3.53 (0.48)	Middle	0.02
	Newest	204	4.21 (0.97)	Newest	405	3.51 (0.51)	Newest	<0.0001
	Delta	179	0.53 (0.99)	Delta	355	−0.04 (0.56)	Delta	<0.0001
	P-Value for Delta		<0.0001	P-Value for Delta		NS		
CXC13	Earliest	186	4.13 (0.85)	Earliest	369	3.78 (0.76)	Earliest	<0.0001
	Middle	147	4.24 (0.80)	Middle	292	3.99 (0.69)	Middle	0.001
	Newest	204	4.82 (1.23)	Newest	405	4.00 (0.70)	Newest	<0.0001
	Delta	179	0.75 (1.13)	Delta	355	0.21 (0.83)	Delta	<0.0001
	P-Value for Delta		<0.0001	P-Value for Delta		<0.0001		
IL-10	Earliest	186	0.73 (0.52)	Earliest	369	0.66 (0.56)	Earliest	NS
	Middle	147	0.88 (0.68)	Middle	292	0.73 (0.56)	Middle	0.03
	Newest	204	1.20 (1.05)	Newest	405	0.68 (0.52)	Newest	<0.0001
	Delta	179	0.49 (1.14)	Delta	355	0.02 (0.64)	Delta	<0.0001
	P-Value for Delta		<0.0001	P-Value for Delta		NS		
TNF-α	Earliest	186	1.81 (0.83)	Earliest	369	1.78 (0.79)	Earliest	NS
	Middle	147	2.14 (0.97)	Middle	292	2.04 (0.85)	Middle	NS
	Newest	204	2.33 (0.92)	Newest	405	2.02 (0.82)	Newest	0.0001
	Delta	179	0.51 (0.99)	Delta	355	0.23 (0.80)	Delta	0.001
	P-Value for Delta		<0.0001	P-Value for Delta		<0.0001		
IFN-γ	Earliest	186	0.40 (0.31)	Earliest	369	0.39 (0.36)	Earliest	NS
	Middle	147	0.46 (0.37)	Middle	292	0.40 (0.36)	Middle	NS
	Newest	204	0.55 (0.62)	Newest	405	0.39 (0.40)	Newest	0.0008
	Delta	179	0.15 (0.70)	Delta	355	0.01 (0.45)	Delta	0.02
	P-Value for Delta		0.005	P-Value for Delta		NS		

Table 5.

Changes in mean levels of sCD30, CXCL13, and IL-10 from earliest to most recent measurement before diagnosis in FL cases compared with controls

		Case			Control		P-Value	
		N	Mean		N	Mean		
sCD30	Earliest	125	3.76 (0.71)	Earliest	243	3.49 (0.44)	Earliest	0.0001
	Middle	94	3.78 (0.54)	Middle	181	3.51 (0.40)	Middle	<0.0001
	Newest	139	4.46 (1.05)	Newest	271	3.48 (0.40)	Newest	<0.0001
	Delta	122	0.70 (0.99)	Delta	237	0.00 (0.43)	Delta	<0.0001
	P-Value for Delta		<0.0001	P-Value for Delta		NS		
CXCL13	Earliest	125	4.03 (0.91)	Earliest	243	3.72 (0.65)	Earliest	0.0009
	Middle	94	4.10 (0.71)	Middle	181	3.83 (0.64)	Middle	0.002
	Newest	139	4.90 (1.11)	Newest	271	3.91 (0.43)	Newest	<0.0001
	Delta	122	0.91 (1.11)	Delta	237	0.18 (0.61)	Delta	<0.0001
	P-Value for Delta		<0.0001	P-Value for Delta		<0.0001		
IL-10	Earliest	125	0.74 (0.56)	Earliest	243	0.67 (0.53)	Earliest	NS
	Middle	94	0.79 (0.56)	Middle	181	0.64 (0.43)	Middle	0.02
	Newest	139	1.06 (0.78)	Newest	271	0.71 (0.55)	Newest	<0.0001
	Delta	122	0.36 (0.79)	Delta	237	0.06 (0.64)	Delta	0.0003
	P-Value for Delta		<0.0001	P-Value for Delta		NS		