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Circulating cytokines and risk of B-cell non-Hodgkin lymphoma: a prospective study

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Abstract

Cytokines play important roles in B-cell activation, proliferation, and apoptosis, thus may be etiologically related to risk of B-cell non-Hodgkin lymphoma (B-NHL). However, the association

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between circulating levels of cytokines and B-NHL risk has not been prospectively studied in non-HIV populations. The objective of this study was to assess this association by conducting a case–control study nested within a prospective cohort of non-HIV-infected, healthy women. Fifteen cytokines were measured in samples collected a median of 8.2 years prior to diagnosis in 92 cases and two matched controls per case. Only cytokines that showed adequate temporal reproducibility over a two-year period were included. The odds ratio (OR) for the highest tertile relative to the lowest was elevated for soluble IL-2 receptor (sIL-2R) (OR = 2.5, 95% CI = 1.4–4.7, $p_{trend} < 0.01$) and decreased for IL-13 (OR = 0.5, 95% CI = 0.2–1.0, $p_{trend} = 0.05$). Three other cytokines were marginally associated with risk of B-NHL: TNF-α (OR = 1.7, 95% CI = 0.9–3.3, $p_{trend} = 0.11$), sTNF-R2 (OR = 1.9, 95% CI = 0.9–3.5, $p_{trend} = 0.06$), and IL-5 (OR = 0.5, 95% CI = 0.3–1.0, $p_{trend} = 0.06$). No association was observed between B-NHL risk and levels of the other cytokines measured (IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-10, IL-12, IL-12p70, CRP and sTNF-R1). This study suggests that dysregulated cytokines may be involved in B-NHL development.

Keywords

B-cell non-Hodgkin lymphoma; Nested case-control study; Cytokine; Interleukin; C-reactive protein

Introduction

Mature B-cell non-Hodgkin lymphoma (B-NHL), representing around 83% of all NHLs, is a heterogeneous group of malignancies involving uncontrolled clonal expansion of transformed B cells in the periphery [1]. The etiology of B-NHL remains elusive, but the strongest and most consistent risk factors are altered immunity conditions. Immunodeficiency, including both congenital immunodeficiency disorders and acquired conditions such as those observed in HIV-positive/AIDS patients and in transplant patients receiving immunosuppressive drugs, is a well-described and strong risk factor for NHL [2]. Chronic antigenic stimulation by Epstein–Barr virus (EBV), *H. pylori* or hepatitis C virus, especially in a setting of impaired immune surveillance, is also associated with increased B-NHL risk [3]. Finally, autoimmune conditions, such as rheumatoid arthritis, systemic lupus erythematosus, and Sjögren's disease, are consistently associated with an increased risk of B-NHL [4], while atopic conditions, such as asthma, hay fever, and eczema, may be associated with a decreased risk of NHL [2,5]. A common characteristic of these conditions is the dysregulation of cytokines, which are known to play essential roles in immune cell development and immune functions [6–8]. In addition, cytokines can directly stimulate B-cell proliferation, prevent B-cell apoptosis, and promote B-cell V(D)J recombination and isotype switching, which collectively enhance the likelihood of the chromosome translocations that are a hallmark of B-NHL [9]. Translocations can activate proto-oncogenes such as *c-myc* and/or inactivate tumor suppressor genes which may ultimately lead to malignant transformation of B cells [10]. Hence, it can be hypothesized that cytokines might play a key role in B-cell lymphomagenesis.

Indeed, a role for cytokines in B-NHL development is supported by epidemiologic studies which examined polymorphisms in genes coding for cytokines in relation to the risk of B-NHL [11]. In particular, fairly consistent results have been observed for variants in the interleukin-10 (*IL-10*) and tumor necrosis factor (*TNF*) genes. The *IL-10* –1082G [12–15] and *IL-10* –3575A variants [13–16] have been associated with an increased risk of NHL (overall and for certain subtypes), and the *TNF* –308A variant with an increased risk of diffuse large B-cell lymphoma (DLBCL) [14,17–19], whereas the *TNF* –857T variant was linked to a decreased risk of follicular lymphoma (FL) [15,20]. However, the functional effects of single-nucleotide polymorphisms are often unknown, and other factors besides

genetic variation affect cytokine levels. To obtain a more direct assessment of the role of endogenous cytokines in B-NHL development, it is of value to investigate the associations between circulating levels of cytokines and B-NHL risk. To date, only one study has prospectively investigated these associations based on a HIV-infected population [21–27]. Results showed strikingly higher pre-diagnostic circulating levels of IL-10, IL-6, and other markers of B-cell activation and differentiation in AIDS-lymphoma subjects compared to non-lymphoma groups, suggesting a strong role of these cytokines in the pathogenesis of B-cell lymphoma in individuals with HIV infection. In addition, the circulating concentration of soluble CD30, which, although not a cytokine, is a marker for chronic B-cell stimulation, was recently reported by Purdue et al. [28] to be associated with increased risk of NHL in a prospective cohort of immunocompetent healthy subjects. These results support the notion that subclinical immune dysregulation, associated with variation in cytokine levels, may be implicated in the development of B-NHL [2].

This study aimed to assess whether circulating levels of cytokines, cytokine receptors, and C-reactive protein (CRP) (which we will refer to generically as “cytokines”) are associated with risk of B-NHL by conducting a case–control study nested within a prospective cohort of non-HIV-infected, healthy women. More specifically, we hypothesized that pro-inflammatory cytokines, including IL-1β, IL-2, IL-6, soluble IL-6 receptor (sIL-6R), IL-12, IL-12p70, TNF-α, soluble TNF receptor 1 (sTNF-R1), sTNF-R2, interferon-γ (IFN-γ), and CRP, and B-cell stimulatory cytokines (IL-5) are associated with an increased risk of B-NHL, whereas anti-inflammatory cytokines, including IL-1 receptor antagonist (IL-1RA), IL-4, IL-10, IL-13, and sIL-2R are associated with a decreased risk of B-NHL. We selected these 17 cytokines based on: (1) their putative biological relevance to B-NHL pathogenesis; and (2) results of a pilot study done in the same population of women which showed that these cytokines were detectable in the majority of samples, and that levels over 2 years showed good temporal reliability, i.e., a single measurement was representative of the average long-term levels in an individual, relative to other individuals [29]. In the present study, though, levels of two of our initially selected cytokines, sIL-6R and IFN-γ, were not detectable in the majority of our case–control samples and no further results on these two cytokines are presented in this report.

Materials and methods

The New York University Women’s Health Study

Between March 1985 and June 1991, 14,274 women 35–65 years old were enrolled as volunteers in the New York University Women’s Health Study (NYUWHS) at the Guttman Institute, a mammography screening center in New York City [30]. At the time of enrollment and at annual screening visits thereafter, subjects were asked to complete questionnaires on demographic, medical, anthropometric, reproductive, and dietary factors and to provide 30 ml of non-fasting peripheral venous blood, drawn using collection tubes without anticoagulant. Serum samples were stored at −80°C for future analyses. Ascertainment of vital status and disease incidence is obtained through cohort follow-up, which involves a combination of active follow-up using questionnaires mailed every 2–4 years and telephone calls for non-respondents, and passive methods, by linkage with the US National Death Index and with the statewide tumor registries of New York, New Jersey and Florida. When a new cancer is reported, written permission from the patient (or next of kin, if deceased) to request medical and pathological reports from hospitals or physicians is solicited. The Institutional Review Board of New York University School of Medicine annually reviewed and approved the study.

Study design and subjects

Case subjects were all incident cases of mature B-NHL (ICD-9 codes 200.*, 202.0, 202.4, 202.8, 202.9, 203.0, 204.1, 238.6, and 273.3), as defined by the WHO classification [31]. Major subtypes of B-NHL include DLBCL, FL, plasma-cell neoplasms (mainly multiple myeloma (MM)), and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) [1]. Because the risk factors for MM may differ from those for other types of B-NHL, and because epidemiologic studies have traditionally examined MM separately, it is advisable to conduct the analysis of B-NHL epidemiologic studies both including and excluding MM. Hence, we decided a priori to include MM cases in the study but to perform a sensitivity analysis by excluding MM cases later in the analysis.

Because of a lag in the reporting of cancers to tumor registries and to prevent selection bias, only cases diagnosed before July 2003, the starting date of the last complete round of follow-up, were included. A total of 147 subjects reported a diagnosis of lymphoma or leukemia. Among these, 67 B-NHL and 17 other hematological tumors were confirmed through medical/pathological records review performed by a pathologist. All the 63 self-reported cases of lymphoma/leukemia not confirmed by medical records were further checked with linkage to tumor registries, which allowed us to identify an additional 26 cases of mature B-NHL. Finally, 6 cases that were not self-reported were identified by linkage to tumor registries. In total, 99 cases were identified. Seven cases were excluded because of a previous primary cancer other than non-melanoma skin cancer. Therefore, 92 primary B-NHL cases were included in the current study, with 61 (66%) confirmed by medical records review and the remaining by a tumor registry.

For each case, two controls were selected at random from the appropriate risk set, i.e., women alive and free of cancer (except non-melanoma skin cancer) at the date of diagnosis of the case and matching the case on age at enrollment (±6 months), date at blood donation (±3 months), and race/ethnicity (European Descent, African-American, or Latina).

Laboratory assay

Frozen 1-ml aliquots of serum which had never been thawed were packed in dry ice and sent to the laboratory for cytokine analyses. Laboratory personnel were blinded to the case/control status of the serum samples. Samples from a case and her two matched controls were always assayed on the same plate. Ten percent blinded duplicate aliquots were included to assess intra-batch and inter-batch variability. Cytokines were analyzed using Luminex xMap™ technology which combines the principle of a sandwich immunoassay with fluorescent-bead-based technology allowing multiplex analysis of up to 100 different analytes in a single microtiter well [32]. CRP was measured using a kit from Linco/Millipore Research (Billerica, MA); IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, TNF-α, and IFN-γ were measured using a high-sensitivity (hs-) kit from Linco/Millipore Research; IL-1RA, sIL-2R, sIL-6R, IL-12, sTNF-R1, and sTNF-R2 were measured using kits from Biosource International (Camarillo, CA). The assays were performed as previously described [29], in accordance with the manufacturers' protocols. The intra-batch coefficients of variation (CVs) were below 11% except for sIL-2R (13.9%), IL-4 (19.8%), and IL-1β (22.3%). The inter-batch CVs were below 15% except for IL-1β (31.2%), IL-1RA (19.7%), and IL-2 (29.3%).

Statistical methods

Subject characteristics were compared between cases and controls using mixed-effects regression models for continuous variables and conditional logistic regression for categorical variables, to take into account the matched design. To compare cytokine levels in cases and controls, the difference between the log-transformed cytokine level of a case and

the average of the log-transformed levels of her two controls was calculated for each matched set, and the median of these differences was compared to zero using the Wilcoxon signed-rank test. Spearman rank-order correlations (*r*) were calculated for continuous variables. The Kruskal–Wallis non-parametric ANOVA test was used to examine the differences in cytokine levels according to categorical variables (e.g., smoking).

Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (95% CIs). Subjects with undetectable levels of cytokines were assigned the midpoint between zero and the limit of detection (LD) of the cytokine assay. Analyses were conducted using tertiles, with cutpoints based on the frequency distribution of both cases and controls, treating the lowest tertile as the reference group (an analysis using cutpoints based on the distributions of controls showed similar results). The likelihood ratio test (LRT) was used to test for linear trend by entering the cytokine tertiles as an ordinal variable (0, 1, and 2) into the conditional logistic regression models. In case more than 33% of the samples were below the LD, all undetectable samples were included in the reference group, and the median of the remaining samples defined the second cutpoint, to ensure balanced numbers in the 2nd and 3rd categories. Analyses were also conducted on the continuous scale. To reduce the right skewness of the distributions of the cytokine concentrations, the base 2 logarithmic transformation was used, which leads to a convenient interpretation: the OR associated with a change of one unit on the $\log_2$ scale corresponds to the OR associated with a doubling in cytokine level on the original scale.

In addition to crude models, models adjusted for BMI, alcohol consumption, and smoking are presented because these variables were associated with the levels of some of the cytokines. Adjusting for other factors, i.e., education, use of non-steroidal anti-inflammatory drugs (NSAIDs) in the 4 weeks prior to the baseline visit, regular use of aspirin prior to the baseline visit did not materially affect the results and are therefore not presented. Only 6 of the subjects (3 cases and 3 controls) had a family history of hematological neoplasms, so adjustment for family history was not conducted.

To reduce the potential for reverse causation (i.e., subclinical tumor affecting cytokine levels), we conducted an analysis limited to the matched sets with a lag-time between the date of blood donation and date of case diagnosis of more than 2 years (83 cases and 166 controls). We also performed an analysis excluding MM cases ($n = 12$) and their controls ($n = 24$), since previous studies of NHLs have often excluded these cases. We additionally investigated cytokine-risk associations the largest histologic subtypes, i.e., SLL/CLL and DLBCL. Because of the smaller sample sizes in these analyses, cytokine levels were classified as high or low using the medians of controls as cutpoints.

All analyses were conducted using SAS release 9.1 (SAS Institute, Cary, NC). All *p*-values were based on two-sided tests. The significance level was set at 0.05 for all tests.

Results

Characteristics of the study subjects are shown in Table 1. The median age at blood donation for both cases and controls was 57 years (range, 36–66 years). The median age at diagnosis among cases was 65 years (range, 38–79 years), and the median lag-time between blood donation and diagnosis was 8.2 years. The majority of the subjects (87%) were of European descent. Cases tended to drink alcohol less frequently than controls, were less likely to use NSAIDs within 4 weeks prior to baseline, and were less likely to regularly use aspirin prior to the baseline visit, but these differences were not statistically significant. Cases were similar to controls for the other variables. CLL/SLL was the most common subtype (30% of all cases), followed by DLBCL (26%), FL (14%), and MM (13%).

We examined the associations of cytokine levels with subject characteristics among controls. Age was significantly positively correlated with CRP ($r = 0.20$), TNF-α ($r = 0.27$), sTNF-R1($r = 0.35$), sTNF-R2 ($r = 0.30$), and marginally correlated with IL-6 ($r = 0.14$) and IL-13 ($r = -0.14$) (Table 2). BMI was significantly positively correlated with CRP ($r = 0.49$) (Table 2). Lower levels of CRP, sTNF-R1, and sTNF-R2 were observed among alcohol drinkers. The median level of CRP in alcohol drinkers was 3.7 vs. 5.8 mg/l in non-drinkers ($p = 0.04$), the median level of sTNF-R1 was 1,159 vs. 1,403 pg/ml ($p = 0.001$), and the median sTNF-R2 was 925 vs. 1,090 pg/ml ($p = 0.01$). Levels of IL-4, IL-5, and IL-6 varied according to smoking categories. The median levels were 1.97, 0.07, 16.0 pg/ml for IL-4 ($p = 0.01$), 0.13, 0.02, and 0.23 pg/ml for IL-5 ($p = 0.01$), and 5.3, 2.7, and 8.0 pg/ml for IL-6 ($p = 0.01$) for current, past, and never smokers, respectively. Other variables (education, NSAIDs use or aspirin use) were not associated with cytokine levels.

Spearman correlations were estimated for all cytokines among controls (Table 2). High correlations were observed between IL-1β and IL-2 ($r = 0.78$); among IL-4, IL-5, and IL-6 ($r > 0.70$); as well as among IL-10, IL-12p70, and IL-13 ($r \approx 0.60$). Partial Spearman correlations adjusted for age and BMI were similar to the unadjusted values (data not shown).

Table 3 displays the distributions of cytokines by case–control status. Compared with controls, cases had slightly higher pre-diagnostic concentrations of sIL-2R ($p = 0.05$). Levels of sTNF-R2 were also marginally higher in cases than in controls ($p = 0.08$), but there were no significant differences in levels of the other cytokines or biomarkers.

Table 4 presents the results of the conditional logistic regression models with cytokines classified in tertiles. In unadjusted logistic regression models, the odds ratio for the highest tertile relative to the lowest was elevated for sIL-2R (OR = 2.5, 95% CI = 1.4–4.7, $p < 0.01$) and decreased for IL-13 (OR = 0.5, 95% CI = 0.2–1.1, $p = 0.05$). sTNF-R2 and IL-5 were marginally associated with risk of B-NHL, with the OR of the highest tertile compared to the lowest being 1.9 (95% CI = 1.0–3.5, $p = 0.06$) for sTNF-R2 and 0.6 (95% CI = 0.3–1.0, $p = 0.06$) for IL-5. The odds ratio for TNF-α also was elevated (OR = 1.7, 95% CI = 0.9–3.3), although the test for trend was not statistically significant ($p = 0.11$). After adjusted for BMI, alcohol drinking, and smoking status, variables that were associated with the levels of some of the cytokines, the ORs did not change substantially, although the p-values showed the associations tended to become less significant. Similar results were observed in analyses of cytokines on the continuous, $\log_2$-transformed, scale. The OR associated with a doubling in sIL-2R level was 1.4 (95% CI = 1.1–1.7, $p < 0.01$) in the unadjusted model, and 1.3 (95% CI = 1.1–1.7, $p = 0.01$) in the multivariate-adjusted model. The OR associated with a doubling in IL-13 level was 0.9 (95% CI = 0.8–1.0, $p = 0.08$) in both unadjusted and adjusted models. Other cytokines were not associated with B-NHL risk (Table 4).

The ORs were slightly attenuated, but in the same direction, in analyses limited to the 83 matched sets with lag-time greater than 2 years (Table s1). The odds ratio for B-NHL associated with the highest tertile relative to the lowest was elevated for sIL-2R (OR = 2.0, 95% CI = 1.1–3.8, $p < 0.01$) and decreased for IL-5 (OR = 0.5, 95% CI = 0.2–1.0, $p = 0.03$). It was marginally decreased for IL-13 (OR = 0.6, 95% CI = 0.3–1.2, $p = 0.10$) and elevated for sTNF-R2 (OR = 1.8, 95% CI = 0.9–3.5, $p = 0.09$). Finally, excluding risk sets with MM cases did not affect the results substantially for sIL-2R or IL-13: compared to the lowest tertile, the OR for the highest tertile was 2.8 (95% CI = 1.4–5.5, $p < 0.01$) for sIL-2R, and 0.5 (95% CI 0.2–1.1, $p = 0.08$) for IL-13 (Table s1).

We did some exploratory analyses for the two largest B-NHL subtypes. CLL/SLL cases had a higher median (inter-quartile) level of sIL-2R [309 (422–595) pg/ml] than matched

controls [248 (135–407) pg/ml], a difference that was marginally significant ($p = 0.05$). None of the other 14 cytokines were significantly different between cases and controls. There were no statistically significant differences in the 15 cytokines between DLBCL cases and their matched controls (data not shown). The risk for CLL/SLL associated with levels above the median relative to levels below the median was elevated only for sIL-2R (OR = 4.0, 95% CI = 1.3–12.5, $p = 0.02$) in the unadjusted model, and OR = 6.0 (95% CI = 1.0–34.7, $p = 0.05$) in the adjusted model. The large confidence limits observed are due to the small number of CLL/SLL cases ($n = 28$). None of the cytokines was associated with DLBCL subtype (data not shown).

Discussion

To our knowledge, this is the first study to prospectively examine circulating levels of cytokines and CRP in relation to subsequent development of mature B-NHL in a population without HIV infection. We found that sTNF-R2 was associated with an increased risk of mature B-NHL, and the anti-inflammatory cytokine IL-13 was associated with a decreased risk of mature B-NHL. These results can be largely explained by the pivotal roles TNF-α and IL-13 play in the inflammatory response, as well as their direct roles in B-cell activation and stimulation. TNF-α has strong pro-inflammatory effects and can induce the production of other pro-inflammatory cytokines such as IL-1, IL-6, and IL-8 [33]. In our study, it was found to be moderately positively correlated with IL-1β ($r = 0.11$) and IL-6 ($r = 0.18$). TNF-α also plays important roles in mediating activation, growth, differentiation, apoptosis, and migration of B cells [34]. Many studies have examined the association of polymorphisms of cytokine-encoding or related genes with susceptibility to NHL. Among these polymorphisms, the high-producing *TNF* –308A variant allele has been the most widely studied, and a positive association between this allele and risk of DLBCL has been reported [14,17–19]. Although sTNF-R2 inhibits the biological activity of TNF-α at high concentrations, it binds with TNF-α and protects it from breakdown, thus acting as a slow release reservoir [35] and has been increasingly used as a reliable surrogate for TNF-α since it has a longer half-life and can be measured easily and reliably in frozen blood samples [36,37]. Thus, the observed positive association between sTNF-R2 and B-NHL is in the expected direction. IL-13 is a potent anti-inflammatory cytokine that down-regulates pro-inflammatory cytokine production by monocytes [38] and has been shown to inhibit the proliferation and clonal growth of normal and malignant B-cell precursors [39].

The positive association of sIL-2R and negative association of IL-5 with risk of B-NHL may be explained by their immunoregulatory functions. IL-2 is considered a pro-inflammatory cytokine, and activated B cells proliferate in response to the binding of IL-2 to cellular receptors [40,41]. The major function of IL-2, though, is to generate a cytotoxic immune response through the promotion of natural killer (NK) and lymphokine-activated killer (LAK) cells, both of which are important in the immune surveillance of potentially malignant cells [40]. Soluble IL-2R, released from the cell membrane by cleavage of IL-2R, binds free IL-2 and inhibits its tumor surveillance function [41], which may explain its positive association with B-NHL. IL-5 induces both proliferation and differentiation of antigen-activated B cells [42] and also promotes immunoglobulin class switching in activated B cells [43], and we had hypothesized that high IL-5 levels would be associated with increased risk of B-NHL due to its B-cell stimulatory effects. However, IL-5 also has been shown to substitute for IL-2 as a growth factor for cytotoxic T cell induction [44]; thus, the inverse association of IL-5 with B-NHL may be due to its ability to stimulate tumor surveillance.

Malignant B-NHL cells may produce some of the studied cytokines [45], and previous evidence has shown that circulating IL-10 levels can be elevated prior to diagnosis in AIDS-

lymphoma up to at least 3 years before diagnosis [21], suggesting the possibility of reverse causation in this study. However, analyses limited to blood samples collected more than 2 years before diagnosis did not change the results materially; moreover, for half of the cases, more than 8 years elapsed between blood sample and diagnosis.

We selected a multiplex assay to measure cytokines because it is fast and convenient and can measure a large number of analytes in a small volume of biological sample, an important consideration in prospective epidemiologic studies with a limited amount of biological material from each participant. Furthermore, multiplex is based on the ELISA principle, and multiplexing itself does not affect the sensitivity of assays [46]. Some studies have shown a good correlation between the two methods (mutiplex and ELI-SAs), at least for some cytokines [46–48], but others have not [49]. Just as discrepancies existing with ELISA kits from different vendors likely represent differences in antibody pairs [46], a low correlation between multiplex and ELISA assays may be due to the different antibodies used. Although it is possible that use of ELISA assays would have identified additional associations with B-NHL risk (in particular for cytokines for which a substantial proportion (≥30%) of subjects had levels below the limit of detection of the assay, i.e., IL-1β, IL-2, IL-4, IL-12p70, and IL-13), it should be noted that high-sensitivity assays were used for these cytokines, and it is not clear that current ELISA assays would have been better able to measure the very low levels of these cytokines than the multiplex assay used.

Our study has some limitations. First, information on autoimmune and infectious conditions at time of blood donation, which might have affected cytokine levels, was not collected. However, the cohort participants were healthy middle-aged women presenting for mammographic screening in 1985–1991, and the probability of HIV or other serious viral or bacterial infections in this population is expected to be extremely low. Second, serum cytokine levels were measured at only one time point, which might not capture an individual's long-time average levels. However, we limited our study to cytokines that showed reasonably high temporal reliability in a pilot study that compared serum levels collected at multiple annual visits in women from the same population [29]. Specifically, cytokines measured in the current study had moderate [the intraclass correlation coefficients (ICC) = 0.44 for IL-13 and 0.49 for sIL-2R] to high [ICC = 0.57–0.92 for others] temporal reproducibility [29]. Third, it is also possible that circulating cytokines do not reflect target cell levels due to autocrine production of cytokines. An additional limitation of the present study was its relatively small sample size. We did not adjust for multiple comparisons because we measured cytokines selected a priori based on their biological functions, but we cannot exclude that some of the observed associations may be due to chance. Larger studies are needed to confirm our results and to evaluate associations for subtypes of B-NHL. Finally, the majority of study participants were non-Hispanic white, middle-aged women, so the results may not be generalizable to other populations.

The major strength of this study is that circulating cytokine levels were measured prior to the diagnosis of B-NHL, which is essential for evaluation of their role in the etiology of B-NHL, because blood samples obtained after disease diagnosis (as in traditional case–control studies) may largely reflect the effects of the disease on cytokine levels. The prospective design of the study, with a high cancer ascertainment rate [50] and 100% serum-sample availability also minimized the potential for selection bias and covariate-recall bias.

In conclusion, this study is the first to evaluate prospectively the association between cytokine levels and B-NHL in a non-HIV-positive population. It suggests that serum levels of certain cytokines, including sIL-2R, IL-5, IL-13, TNF-α, and sTNF-R2, are associated with risk of B-NHL, and that multiple mechanisms, such as B-cell stimulation, pro-

inflammatory activity, and impaired tumor surveillance, might be involved. Larger studies are needed to confirm these preliminary findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AIDS	Acquired immune deficiency syndrome
B-NHL	B-cell non-Hodgkin lymphoma
BMI	Body mass index
CRP	C-reactive protein
CLL	Chronic lymphocytic leukemia
CI	Confidence interval
DLBCL	Diffuse large B-cell lymphoma
FL	Follicular lymphoma
IL	Interleukin
IL-1RA	IL-1 receptor antagonist
LD	Limit of detection
MM	Multiple myeloma
OR	Odds ratio
SLL	Small lymphocytic lymphoma
sIL-2R	Soluble IL-2 receptor
TNF-*α*	Tumor necrosis factor-*α*

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Table 1

Characteristics of the case and control subjects

Characteristic	Case (n^a = 92)	Control (n^a = 184)	p^b
Age at blood donation, mean (SD), years	55.0 (8.0)	55.0 (8.0)	Matched
Median (range)	57.3 (36.0–65.8)	57.3 (36.5–65.8)	
Race[c], *n* (%)			Matched
European descent	75 (87.2)	150 (87.2)	
African-American	7 (8.1)	14 (8.1)	
Latina	4 (4.7)	8 (4.7)	
Height, mean (SD)[c], cm	162.9 (6.5)	162.5 (6.1)	0.60
Weight, mean (SD)[c], kg	67.4 (11.0)	67.2 (13.3)	0.86
Body mass index, mean (SD)[c], kg/m^2	25.5 (4.2)	25.5 (4.9)	0.94
Education[c], *n* (%)			
Some college or less	40 (54.8)	90 (53.6)	
Completed college or above	33 (45.2)	78 (46.4)	0.85
Smoking status at baseline[c], *n* (%)			
Never-smoker	45 (51.1)	89 (50.9)	
Current smoker	12 (13.6)	32 (18.3)	
Past smoker	31 (35.2)	54 (30.9)	0.71
Alcohol intake at baseline, drinks per week[c], *n* (%)			
0	58 (69.0)	102 (62.6)	
1–5	19 (22.6)	36 (22.1)	
≥6	7 (8.4)	25 (15.3)	0.16
NSAIDs use within 4 weeks prior to baseline, *n* (%)			
No	65 (70.6)	112 (60.9)	
Yes	27 (29.4)	72 (39.1)	0.11
Regular use of aspirin prior to baseline[c], *n* (%)			
No	76 (90.5)	138 (84.1)	
Yes	8 (9.5)	26 (15.9)	0.16
Lag-time between blood donation and diagnosis, *n* (%)			
< 5 years	26 (28.2)		
6–10 years	32 (34.8)		
11–15 years	24 (26.1)		
> 15 years	10 (10.9)		
Age at diagnosis, mean (SD), years	63.3 (9.2)		
Median (range)	65.1 (38.3–79.0)		
Histologic subtype, *n* (%)			
Chronic lymphocytic leukemia (CLL)	28 (30.4)		
Diffuse large B-cell lymphoma (DLBCL)	24 (26.1)		
Follicular lymphoma (FL)	13 (14.1)		
Multiple myeloma (MM)	12 (13.0)		

Characteristic	Case (n^a = 92)	Control (n^a = 184)	p^b
Others or malignant lymphoma not otherwise specified (ML, NOS)	15 (16.3)		

[a] *n* = Number of subjects

[b] *p*-Values from mixed-effects regression models for continuous variables and conditional logistic regression models for categorical variables

[c] Numbers of subjects with missing values for the covariates are as followings: race [18], height [1], weight [3], body mass index [4], education [35], smoking status at baseline [13], alcohol intake at baseline [29], and regular use of aspirin prior to baseline [28]

Table 2

Spearman correlations between age, body mass index (BMI), and cytokines, among 184 controls

	Age	BMI	IL-1β^a	IL-1RA	IL-2^a	sIL-2R	IL-4^a	IL-5^a	IL-6^a	IL-10^a	IL-12	IL-12p70^a	IL-13^a	CRP	TNF-α^a	sTNF-R1
BMI	0.06	1														
IL-1β^a	0.01	0.01	1													
IL-1RA	0.00	0.08	**0.40**^{b}	1												
IL-2^a	0.01	0.05	**0.78**	**0.34**	1											
sIL-2R	0.07	−0.09	0.13	0.11	**0.16**	1										
IL-4^a	0.00	−0.05	0.10	0.00	**0.21**	0.08	1									
IL-5^a	0.01	−0.05	**0.18**	0.01	**0.23**	**0.16**	**0.73**	**1**								
IL-6^a	0.14	0.06	**0.21**	−0.02	**0.32**	0.13	**0.81**	**0.72**	1							
IL-10^a	0.02	−0.03	**0.40**	0.08	**0.35**	**0.21**	0.10	**0.25**	**0.20**	1						
IL-12	−0.02	−0.01	**0.33**	**0.59**	**0.23**	**0.29**	0.02	0.05	0.00	**0.22**	1					
IL-12p70^a	−0.04	−0.01	**0.48**	0.05	**0.51**	**0.17**	0.14	**0.28**	**0.32**	**0.60**	0.11	1				
IL-13^a	−0.14	−0.05	**0.36**	−0.07	**0.29**	**0.18**	0.07	**0.19**	**0.20**	**0.60**	0.14	**0.62**	1			
CRP	**0.20**	**0.49**	0.03	**0.23**	0.07	0.02	0.004	−0.02	**0.15**	0.04	0.08	0.02	−0.13	1		
TNF-α^a	**0.27**	0.12	0.11	−0.09	0.03	**0.31**	0.04	0.10	**0.18**	**0.15**	0.12	0.08	0.09	**0.15**	1	
sTNF-R1	**0.35**	0.14	**0.17**	**0.42**	**0.15**	**0.40**	−0.08	−0.02	0.06	0.13	**0.47**	0.04	−0.02	**0.38**	**0.26**	1
sTNF-R2	**0.30**	0.05	−0.09	0.12	−0.11	**0.20**	−0.09	−0.05	−0.01	0.03	**0.22**	−0.04	−0.04	**0.18**	**0.27**	**0.44**

[a] Measured using a high-sensitivity (hs-) kit

[b] Statistically significant ($p < 0.05$) Spearman correlation coefficients are in bold

Table 3

Numbers of serum samples above the assay limit of detection, medians, and inter-quartile ranges of cytokines among cases and controls

Cytokine	LD[a] (pg/ml)[b]	Cases (total number = 92)		Controls (total number = 184)		p^c
		Number (%) of cases above LD	Median (25th–75th percentiles)	Number (%) of controls above LD	Median (25th–75th percentiles)	
IL-1β[d]	0.06	33 (36)	< LD (< LD–0.2)	69 (38)	< LD (< LD–0.33)	0.48
IL-1RA	30	61 (66)	121 (< LD–427)	131 (71)	149 (< LD–427)	0.43
IL-2[d]	0.16	53 (58)	0.4 (< LD–2.1)	111 (60)	0.4 (< LD–2.4)	0.65
sIL-2R	30	91 (99)	342 (229–586)	170 (92)	286 (169–415)	0.05
IL-4[d]	0.13	56 (61)	3.6 (< LD–25)	106 (58)	4.1 (< LD–77)	0.65
IL-5[d]	0.01	58 (63)	0.06 (< LD–0.28)	129 (70)	0.1 (< LD–0.8)	0.11
IL-6[d]	0.1	90 (98)	3.4 (1.8–10)	178 (97)	4.7 (1.6–16)	0.18
IL-10[d]	0.15	88 (96)	3.7 (1.8–6.9)	177 (96)	3.1 (1.9–6.8)	0.58
IL-12	15	91 (99)	221 (145–353)	183 (99)	209 (139–309)	0.82
IL-12p70[d]	0.11	52 (57)	0.2 (< LD–0.7)	111 (60)	0.4 (< LD–1.5)	0.23
IL-13[d]	0.48	54 (59)	0.6 (< LD–1.9)	114 (62)	1.1 (< LD–2.9)	0.26
CRP	6×10^{-6}	92 (100)	5.7 (2.5–15)	184 (100)	4.4 (1.9–12)	0.11
TNF-α[d]	0.05	93 (100)	3.7 (2.8–5.5)	184 (100)	3.6 (2.6–4.7)	0.16
sTNF-R1	15	94 (100)	1,301 (1,152–1,582)	184 (100)	1,332 (1,099–1,655)	0.66
sTNF-R2	15	95 (100)	1,060 (892–1,319)	184 (100)	1,041 (807–1,238)	0.08

[a] LD, limit of detection

[b] Units for the cytokines were pg/ml except for CRP, which was mg/l

[c] *p*-Values from Wilcoxon signed-rank test

[d] Measured using a high-sensitivity (hs-) kit

Table 4

Odds ratios and 95% CIs for B-cell non-Hodgkin lymphoma associated with baseline serum levels of cytokines

Cytokine	Cutpoints of tertiles (pg/ml)[a]	Number of cases/controls	OR (95% CI)[b]	p^c for trend	OR (95% CI)[b,d]	$p^{c,d}$ for trend
IL-1β[e]	< LD	59/115	1		1.00	
	0.06–0.68	14/37	0.7 (0.4–1.5)		0.8 (0.4–1.6)	
	0.71–64.4	19/32	1.1 (0.6–2.3)	0.86	1.1 (0.6–2.2)	0.86
IL-1RA	< LD–80	31/64	1		1.00	
	100–275	28/59	1.0 (0.5–1.9)		1.1 (0.5–2.1)	
	291–35,846	33/61	1.1 (0.6–2.2)	0.40	1.1 (0.6–2.2)	0.77
IL-2[e]	< LD	39/73	1		1.00	
	0.18–1.60	28/54	1.0 (0.5–1.9)		1.1 (0.5–2.2)	
	1.77–351.3	25/57	0.8 (0.4–1.5)	0.51	0.9 (0.4–1.7)	0.63
sIL-2R	15–220	22/71	1		1.00	
	221–398	29/63	1.5 (0.8–2.9)		1.6 (0.8–3.0)	
	403–5,372	41/50	2.5 (1.4–4.7)	< 0.01	2.5 (1.3–4.8)	< 0.01
IL-4[e]	< LD	36/78			1.00	
	0.13–37.4	35/46	1.7 (0.9–3.3)		1.8 (0.9–3.4)	
	37.8–883	21/60	0.8 (0.4–1.5)	0.54	0.8 (0.4–1.6)	0.62
IL-5[e]	< LD	34/55	1		1.00	
	0.01–0.25	35/60	0.9 (0.5–1.7)		1.0 (0.5–1.9)	
	0.26–14	23/69	0.5 (0.3–1.0)	0.06	0.6 (0.3–1.1)	0.09
IL-6[e]	< LD–2.33	32/60	1		1.00	
	2.34–9.53	35/57	1.2 (0.6–2.2)		1.2 (0.6–2.2)	
	9.56–125	25/67	0.7 (0.4–1.3)	0.28	0.7 (0.4–1.4)	0.35
IL-10[e]	< LD–2.28	28/63	1		1.00	
	2.29–5.06	29/64	1.0 (0.5–1.9)		1.0 (0.5–1.9)	
	5.09–1,090	35/57	1.4 (0.7–2.5)	0.30	1.4 (0.7–2.5)	0.32
IL-12	< LD–161	29/63	1		1.00	
	162–272	29/63	1.0 (0.6–1.9)		1.0 (0.6–1.9)	
	274–23,780	34/58	1.3 (0.7–2.4)	0.42	1.3 (0.7–2.4)	0.49

Cytokine	Cutpoints of tertiles (pg/ml)[a]	Number of cases/controls	OR (95% CI)[b]	p^c for trend	OR (95% CI)[b,d]	$p^{c,d}$ for trend
IL-12p70[e]	< LD	40/73	1		1.00	
	0.11–0.82	30/51	1.1 (0.6–2.0)		1.1 (0.6–2.1)	
	0.86–427	22/60	0.6 (0.3–1.2)	0.22	0.7 (0.4–1.3)	0.28
IL-13[e]	< LD	38/70	1		1.00	
	0.49–2.13	35/49	1.2 (0.6–2.5)		1.3 (0.6–2.7)	
	2.14–266.4	19/65	0.5 (0.2–1.0)	0.05	0.5 (0.2–1.1)	0.06
CRP	0.2–2.748	29/63	1		1.00	
	2.75–9.37	31/61	1.1 (0.6–2.1)		1.1 (0.6–2.1)	
	9.38–146	32/60	1.2 (0.6–2.2)	0.63	1.2 (0.6–2.4)	0.64
TNF-α[e]	0.3–2.9	26/65	1			
	2.9–4.4	30/63	1.2 (0.6–2.3)		1.2 (0.6–2.2)	
	4.5–27	36/56	1.7 (0.9–3.3)	0.11	1.7 (0.9–3.2)	0.14
sTNF-R1	269–1,167	29/63	1			
	1,167–1,476	36/56	1.4 (0.7–2.5)		1.4 (0.7–2.7)	
	1,478–14,180	27/65	0.9 (0.5–1.8)	0.73	0.9 (0.4–1.9)	0.74
sTNF-R2	204–913	26/66	1			
	918–1,167	28/64	1.1 (0.6–2.1)		1.1 (0.6–2.2)	
	1,174–2,817	38/54	1.9 (0.9–3.5)	0.06	1.9 (1.0–3.7)	0.07

[a] Units for CRP, mg/l

[b] Odds ratios (ORs) from conditional logistic regression models, controlling only for matching factors

[c] P for trend calculated by likelihood ratio test, by entering the cytokine tertiles as an ordinal variable (0, 1, 2) into the conditional logistic regression models

[d] Adjusted for BMI, alcohol drinking and smoking status

[e] Measured using a high-sensitivity (hs-) kit