Published in final edited form as:
Cancer Res. 2011 July 15; 71(14): 4898–4907. doi:10.1158/0008-5472.CAN-11-0165.

Pre-diagnostic serum levels of cytokines and other immune markers and risk of non-Hodgkin lymphoma

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Abstract

While severe immune dysregulation is an established risk factor for non-Hodgkin lymphoma (NHL), it is unclear whether subclinical immune system function influences lymphomagenesis. To address this question, we conducted a nested case-control study within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial to investigate whether circulating levels of cytokines and other immune markers are associated with future risk of NHL. Selected cytokines [interleukin (IL)-4, IL-6, IL-10, tumor necrosis factor (TNF)-α] and other immune markers [soluble TNF receptor 1 (sTNF-R1), sTNF-R2, C-reactive protein (CRP), sCD27] were measured in prediagnostic serum specimens from 297 incident NHL cases and 297 individually matched controls. Odds ratios (OR) and 95% confidence intervals (CI) relating quartiles of analyte concentration to NHL risk were calculated using conditional logistic regression. Statistically significant associations with increased NHL risk were observed for elevated serum levels of sTNF-R1 (quartile 4 vs. quartile 1: OR 1.7, 95% CI 1.1–2.8; P_{trend}=0.02) and sCD27 (OR 5.3, 95% CI 2.9–9.4; P_{trend}<0.0001). These associations remained in analyses of cases diagnosed 6+ years following blood collection (sTNF-R1: OR 2.1, 95% CI 1.0–4.0, P_{trend}=0.01; sCD27: OR 4.1, 95% CI 1.9–8.5, P_{trend}=0.0001). Elevated levels of IL-10, TNF-α and sTNF-R2 were also significantly associated with increased risk of NHL overall; however, these associations weakened with increasing time from blood collection to case diagnosis, and were null for cases diagnosed 6+ years post-collection. Our findings for sTNF-R1 and sCD27, possible markers for inflammatory and B-cell stimulatory states respectively, support a role for subclinical inflammation and chronic B-cell stimulation in lymphomagenesis.

Keywords

non-Hodgkin lymphoma; cohort studies; serum; cytokines; soluble markers; immune system

INTRODUCTION

Epidemiologic studies of individuals with AIDS and organ transplantation recipients have established severe immune dysregulation as a risk factor for non-Hodgkin lymphoma (NHL) (1–4). The effects of AIDS and drugs for combating transplant rejection include immune

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Potential conflicts of interest: none to declare

suppression, viral reactivation and chronic antigenic stimulation, all of which cause increased B cell division, which increases the potential for unrepaired genetic errors that can lead to lymphomagenesis (5). It is unclear, however, whether subclinical variation in immune system function influences NHL risk in the general population. Insight into this question may come from the study of cytokines -- secreted proteins that modulate various activities of B and T lymphocytes including proliferation, antibody production, chemotaxis, interaction with immunoglobulins, and the production of other cytokines. Cytokines of diverse functions operate in a complex, tightly-regulated network controlling immune system function. Perturbations of this network may increase the potential for chronic B cell division and, ultimately, lymphomagenesis.

Limited evidence from genetic and molecular epidemiologic studies support a role for cytokines in lymphomagenesis. Associations between genetic variants in cytokine genes (e.g., *TNF*, *IL4*, *IL10*) and susceptibility to NHL have been observed (6–10). More recently, findings from prospective studies of circulating levels of cytokines and other secreted immune markers and future NHL risk have been reported. Investigations within a cohort study of AIDS patients have identified associations with increased NHL risk for elevated levels of several immune markers, including interleukin-6 (IL-6), IL-10, and soluble CD27 (sCD27), a proposed marker of immune activation (11–13). A case-control study (92 cases, 184 controls) within the New York University – Women's Health Study (NYU-WHS) cohort identified associations with decreased NHL risk for elevated serum levels of IL-5 and IL-13, and with increased risk for levels of sIL2R, TNF-α and soluble TNF receptor type 2 (sTNF-R2), a possible marker of TNF-α-driven inflammation (14). Another study (86 NHL cases, 86 controls) nested within the EPIC-Italy cohort observed associations with reduced risk for higher levels of IL-2 and TNF-α, and an association with increased risk for intercellular adhesion molecule (ICAM) (15). As well, a small study of prediagnostic plasma C-reactive protein (CRP) and multiple cancers nested within the EPIC-Greece cohort reported a statistically significant positive association with leukemia/lymphoma (27 cases) (16).

The sample sizes of these studies of cytokines and other immune markers were small, limiting their statistical power to detect associations of moderate magnitude and their ability to explore, through analyses stratifying on follow-up time, the possibility that associations are the result of reverse causation. The latter point is particularly relevant given that abnormal levels of immune markers can be caused by lymphomas, both directly (through production of cytokines by cancer cells) and indirectly (through changes in the immune system in response to the presence of disease) (17, 18).

In this report we present findings from a comparatively large case-control study (297 cases, 297 controls) nested within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) designed to investigate whether serum levels of selected cytokines (IL-4, IL-6, IL-10, TNF-α) and other immune markers (sTNF-R1, sTNF-R2, CRP, sCD27) are associated with future risk of NHL. We previously reported from this study an association between prediagnostic circulating soluble CD30 levels and NHL that persisted for the longest period of follow-up, from 6–10 years (19).

MATERIALS AND METHODS

Detailed descriptions of the PLCO Trial have been previously reported (20, 21). In brief, between 1993 and 2001, approximately 155,000 subjects in ten cities (Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St Louis, MO; and Washington, DC) aged 55–74 were recruited from the general population and randomized to the screening or non-screening arm of the study.

All screening-arm subjects provided nonfasting baseline blood samples that were processed and frozen within two hours of collection, and stored at −70°C. Individuals were followed up for all cancer diagnoses by annual mailed questionnaire, in addition to the Trial disease outcomes by annual screening examinations during the first six years of follow-up. All cancer diagnoses were pathologically confirmed through medical record abstraction. The institutional review boards of the US National Cancer Institute and the ten study centers approved the trial, and all participants provided written informed consent.

After follow-up through January 31, 2006, 297 cases of NHL (ICD-O-2-M 9590–9595, 9670–9677, 9680–9688, 9690–9698, 9700–9717, 9823, 9827) were identified from 54,829 eligible screening-arm participants (eligibility criteria: signed informed consent, no history of cancer at enrollment, two or more unthawed serum vials available, did not develop cancer or exit the cohort within the first year of follow-up). Controls were individually matched to cases on a one:one ratio on the basis of age at baseline (five-year categories), sex, race, PLCO center, and date of baseline blood draw (three-month categories) from among subjects who had not been diagnosed with any type of malignancy except non-melanoma skin cancer at the time of the case diagnosis date.

Multiplex assays for use on a Luminex multianalyte profiling (xMAP) system (Luminex Corp., Austin, TX) were used to measure several analytes. The xMAP system is a liquid array system that utilizes bead-bound analyte-specific reporter molecules to permit the simultaneous quantitative analysis of up to 100 different analytes in a single microtiter well. The specific multiplex array system used in these analyses was the Bio-Rad Bio-Plex 200 (Hercules, CA). Analytes measured using the Bio-Plex 200 system included the following: IL-4, IL-6, IL-10 and TNF-α, measured in duplicate (34 subjects per batch) using a 4-plex high-sensitivity kit (Millipore, Billerica, MA); sTNF-R1 and sTNF-R2, measured in duplicate (36 subjects per batch) using a 2-plex kit (Invitrogen/BioSource, Carlsbad, CA); and CRP, measured singly (72 subjects per batch) using a single-plex kit (Invitrogen/ BioSource, Carlsbad, CA). Serum sCD27 was measured in duplicate (36 subjects per batch) by enzyme-linked immunosorbent assay (Bender Medsystems, Burlingame, CA). Values below assay limits of detection (LOD) were imputed using a parametric model-based estimation procedure (22). Using measurements among controls for a target analyte, we used maximum likelihood methods to estimate parameters for the log-normal distribution. For each measurement < LOD, we randomly sampled a value from the appropriate log-normal distribution as the imputed value. Samples from matched case-control sets were assayed consecutively within the same batch.

Masked replicate samples (N=72) were interspersed within and across assay batches to assess quality control. Intraclass correlation coefficients (intra-batch/inter-batch) were 0.98/0.93 for IL-4, 0.97/0.94 for IL-6, 0.96/0.79 for IL-10, 0.96/0.85 for TNF- α, 0.95/0.93 for sTNF-R1, 0.94/0.87 for sTNF-R2, and 0.96/0.93 for sCD27. Coefficients of variation (intra-batch/inter-batch) were 8.0%/26.4% for IL-4, 5.5%/27.5% for IL-6, 14.0%/38.3% for IL-10, 5.6%/31.0% for TNF-α, 3.5%/8.9% for sTNF-R1, 2.8%/11.8% for sTNF-R2, and 6.3%/19.8% for sCD27. For CRP, measurements from blinded quality control replicates suggested possible problems with one of the assay batches. We present results with this batch excluded [resulting sample size: 261 cases, 261 controls; coefficients of variation (intra-batch/inter-batch) 11.9%/40.3%; intraclass correlation coefficients 0.95/0.89], although our findings for the total data set of 297 cases and 297 controls were virtually identical (results not shown).

All statistical tests conducted in the analysis were two-sided. The Wilcoxon signed rank test was used to test for significant differences in levels of each analyte among the matched pairs of cases and controls. For each analyte, odds ratios (OR) and 95% confidence intervals (CI)

relating concentration categories and NHL risk were then computed using conditional logistic regression modeling. For analyses of all NHL, analyte concentrations were categorized using control quartiles as cut-points. An exception is IL-4, measurements of which were below the LOD for 40% of all samples. For this analyte, samples below the LOD formed one category, while samples above the LOD were categorized using control tertiles. Tests for trend were performed by modeling the intra-category medians as a continuous parameter.

We evaluated the impact of follow-up time from blood collection to case diagnosis on our findings using two analytic methods. A "matched approach" involved fitting conditional logistic regression models using data from cases and their matched controls within strata defined by time from blood collection to case diagnosis (1–2, 3–5 and 6–10 years). An "unmatched approach" involved fitting a polytomous regression model comparing case subcategories defined by follow-up time to diagnosis (1–2, 3–5 and 6–10 years from blood collection) to all controls, with model adjustment for baseline age, sex, race, PLCO center, and enrollment year. The two methods have complementary strengths; the matched approach minimizes the impact of measurement error from between-batch assay variability on our findings, while the unmatched approach provides more stable risk estimates through the use of all controls as a single referent group. We tested for differences in analyte associations with NHL across strata of follow-up time in conditional models using the likelihood ratio test.

Analyses within strata defined by sex and age group were performed using conditional logistic regression modeling, with tests of interaction performed using the likelihood ratio test. Polytomous regression models were also fit to compute associations between dichotomized analyte levels (cut-point: control median) and individual NHL histologic subtypes [chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL); diffuse large B-cell lymphoma (DLBCL); follicular lymphoma (FL); other or not otherwise specified histologies (Other/NOS)].

RESULTS

Cases and controls had identical distributions of matching factors (Table 1). The majority of participants were male (63%) and self-identified as white (94%), with a median age at enrollment of 65 years. The most common NHL histologic subtypes were CLL/SLL (N=117, 39% of cases), DLBCL (62, 21%) and FL (44, 15%). The median length of follow-up from blood collection to case diagnosis was 5.0 years (range 1.0–10.8 years). In analyses among controls of immune marker levels, including sCD30 (19), several markers were correlated with one another (Supplemental Table 1), most notably IL-4 and IL-6 [Spearman rank correlation (r) = 0.80], sTNF-R1 and sCD30 (r = 0.39), sTNF-R1 and sTNF-R2 (r = 0.35), sTNF-R2 and TNF-α (r = 0.34), and sCD27 and sCD30 (r = 0.32). Levels of sTNF-R1, unlike sTNF-R2, were only weakly correlated with TNF-α (r = 0.15).

The distributions of analytes among cases and controls are summarized in Table 2. Cases had statistically significant elevated levels of IL-10, TNF-α, sTNF-R1, sTNF-R2 and sCD27 compared to their matched controls, with *P*-values ranging from 0.003 to <0.0001. Serum levels of IL-4, IL-6 and CRP did not differ between cases and controls ($P \geq 0.27$). Similarly, in analyses of analyte categories (Table 3), statistically significant associations with increased NHL risk were observed for the highest categories of IL-10 (fourth quartile vs. first: OR 2.1, 95% CI 1.3–3.4, P_{trend}=0.0001), TNF-α (OR 2.2, 95% CI 1.3–3.9, P_{trend}=0.005), sTNF-R1 (OR 1.7, 95% CI 1.1–2.8, P_{trend}=0.02) and, in particular, sCD27 (OR 5.3, 95% CI 2.9–9.4, P_{trend}<0.0001). Categories of sTNF-R2 concentration were not

associated with NHL at a level of statistical significance (OR 1.4, 95% CI 0.9–2.2, P_{trend}=0.10).

However, several of these associations were found to be affected by the length of time from blood collection to case diagnosis (Table 4). In both unmatched and matched analyses, prediagnostic levels of IL-10, TNF-α, sTNF-R2 were most strongly associated with cases diagnosed 1 or 2 years following blood collection. Associations with cases diagnosed 3–5 years after collection were weaker for IL-10 and TNF-α, and null for sTNF-R2. All three markers were not associated with NHL cases diagnosed 6 years or longer from collection. A test of heterogeneity in analytes associations across strata of follow-up time was statistically significant for sTNF-R2 (P=0.005) but not for TNF-α (P=0.22) or IL-10 (P=0.22). The association with NHL for sTNF-R1 was strongest for cases diagnosed 6 years or longer since blood collection (P_{trend} = 0.07 and 0.01 for matched and unmatched analyses respectively). Serum levels of sCD27 were significantly associated with NHL at each time period. When we fit a polytomous model adjusting simultaneously for sCD27 and sTNF-R1, the association with sCD27 among cases diagnosed 6+ years post-collection remained statistically significant (P_{trend} = 0.0004), while the sTNF-R1 association became of borderline significance (P_{trend} = 0.07). When all other immune markers, including sCD30, were additionally adjusted for in the model, the findings did not materially change (sCD27, P_{trend} = 0.002; sTNF-R1, P_{trend} = 0.08). No evidence of statistical interaction with sex, age at baseline or other immune markers was observed.

We explored further the associations with sTNF-R1 and sCD27 through analyses of common NHL subtypes (Table 5). The strongest subtype-specific association with elevated sTNF-R1 was observed for CLL/SLL, although associations with cases diagnosed 6 years or longer from blood collection were weaker and not statistically significant. Conversely, associations with DLBCL, FL and other/unclassified NHL cases were strongest for cases diagnosed 6–10 years post-collection. For sCD27, associations with CLL/SLL and FL were strongest for cases diagnosed 1–2 years from blood collection, and FL was not associated with elevated sCD27 for cases diagnosed 6–10 years later. DLBCL was significantly associated with elevated sCD27 only for cases diagnosed 6–10 years post-collection. Analyses of the other immune markers did not suggest clear associations with specific NHL subtypes, although elevated levels of IL-10, TNF-α and sTNF-R2 were significantly associated with increased risk of CLL/SLL cases diagnosed 1–2 years following blood collection (Supplemental Table 2).

DISCUSSION

In this investigation of serum levels of immune markers and subsequent risk of NHL, elevated pre-diagnostic levels of sTNF-R1 and sCD27 were significantly associated with increased risk, both overall and for cases diagnosed 6 years or longer from the date of blood collection. Elevated levels of IL-10, TNF-α and sTNF-R2 were also significantly associated with NHL overall; however, these associations weakened with increasing time from blood collection to case diagnosis, and were null for cases diagnosed 6 years or longer post-collection. We did not observe associations with risk for levels of IL-4, IL-6 or CRP.

sTNF-R1 and sTNF-R2 are the respective soluble fragments of tumor necrosis factor receptors I and II, the cell surface receptors through which TNF-α effects are mediated (23). These soluble receptors, shed from the cell surface in response to TNF-α (24) and other inflammatory stimuli (25–27), can bind with TNF-α and may play a regulatory role by limiting its circulating levels in the blood (28). sTNF-R1 and sTNF-R2 have been measured in several studies as possible markers of TNF-mediated inflammation, given the stability of their levels over time (29) and the high sensitivities of assays for these markers (28). In our

study, elevated levels of both sTNF-R1 and sTNF-R2 were associated with future NHL risk. However, whereas the association with sTNF-R1 was consistently observed with increasing follow-up time, the association with sTNF-R2 was present only for cases diagnosed within two years of blood collection – CLL/SLL cases in particular. Given previous evidence that CLL cells release sTNF-R2 (30), our findings for sTNF-R2 may reflect reverse causation. As a possible marker for inflammation, our association with sTNF-R1 supports the hypothesis that pro-inflammatory effects may increase risk of NHL (31). The exact mechanism responsible for elevated sTNF-R1, however, is unclear. We note that among our controls TNF-α was more strongly correlated with sTNF-R2 than sTNF-R1; this is consistent with evidence suggesting that sTNF-R2 secretion is triggered by TNF-α, while sTNF-R1 may be regulated through different inflammatory triggers (32–34). An association with sTNF-R2, but not sTNF-R1, was observed in a smaller study conducted within the NYU-WHS cohort (14), and was found to remain after excluding from the analysis cases diagnosed <2 years from the blood collection date, although the impact of excluding cases with specimens collected longer than 2 years from diagnosis was not explored. Additional evidence from prospective studies is needed to clarify the relationship with NHL risk for these soluble markers.

As with sTNF-R1, levels of sCD27 were associated with future NHL risk, both overall and for cases diagnosed 6–10 years from blood collection. CD27 is a member of the TNF receptor superfamily, and is expressed exclusively on peripheral blood T-cells and a subset of B-cells (35). CD27 appears to be involved in activation of both T-cells and B-cells; the receptor, upon activation by its ligand CD70, stimulates T-cell proliferation (36–38) and enhances immunoglobulin production in B-cells (39, 40). The extracellular portion of CD27 is proteolytically cleaved from CD27-positive cells to produce a soluble form of the receptor (sCD27) detectable in serum. Elevated levels of sCD27 have been observed with many infectious and autoimmune diseases, and has been proposed as a marker of immune activation (35), similar to sCD30 (19). The B-cell stimulatory role of CD27 and the suspected importance of chronic B-cell activation in lymphomagenesis led Widney et al. to investigate the association between sCD27 and subsequent risk of AIDS-NHL in a small study (36 cases, 133 controls) nested within a cohort of HIV+ patients (13). The investigators found elevated sCD27 measured up to 18 months prior to diagnosis to be significantly associated with an increased risk of AIDS-NHL. Our study has now replicated that finding within a general population cohort with follow-up of up to 10 years post-collection. The associations with sCD27 for CLL/SLL and FL weakened with increased time from blood collection to case diagnosis. It is notable that these malignancies are commonly indolent in nature and can remain undetected for a long time. Elevated serum sCD27 has been previously reported in patients with lymphoid malignancies and associated with tumor load, and CD27 is commonly expressed in lymphoma tissue, supporting the idea that the elevated sCD27 levels among some lymphoma patients may be tumor-induced (41). However, we found that the association between sCD27 and DLBCL, an aggressive lymphoma type, was associated with elevated sCD27 only among cases diagnosed 6–10 years from blood collection, consistent with an etiologic effect. Further research is needed to clarify the basis of the association between prediagnostic sCD27 and future risk of NHL.

Serum levels of TNF-α and IL-10 were associated with future NHL risk in our study. However, these associations weakened with longer follow-up time, and were not statistically significant for cases diagnosed 6–10 years after blood collection. Tests of interaction across strata of follow-up time for these analytes were not statistically significant, although our study lacks power to detect such differences. In two smaller prospective studies of circulating immune markers and NHL risk in general population cohorts, inconsistent findings were reported for TNF-α; an association with increased NHL risk was observed in the NYU-WHS study (14), and with decreased risk in the EPIC-Italy investigation (15). In

both studies, prediagnostic IL-10 levels were not associated with NHL risk. Our finding of statistically significant associations with levels of these cytokines only for cases diagnosed within 5 years of blood collection is suggestive of tumor-induced effects, although these results are also compatible with etiologic effects in the late stages of NHL development. Studies of *TNF* and *IL10* knock-out mice (42–44) and of genetic polymorphisms in humans (6–10) strongly implicate both cytokines in B-cell lymphomagenesis. TNF-α is thought to influence lymphoma development through up-regulation of pro-inflammatory and anti-apoptotic signals, possibly via the nuclear transcription factor (NF)-κB pathway (6), while IL-10 may influence NHL risk through its B-cell stimulatory effects (12). Voorzander et al. reported that elevated IL-10 levels observed among NHL patients appeared to be caused by increased expression from lymphoma cells and stimulated lymphoma proliferation, suggesting that IL-10 is an autocrine lymphoma growth factor (45). In contrast, elevated levels of TNF-α among NHL patients appear to be produced by normal immune cells (45), although it is possible that lymphomas may play an indirect role in stimulating such production from local cells.

An important strength of our study is its sample size; the case series (N=297) has more than three times the number of cases included in the three previously published prospective studies of circulating immune markers and NHL risk in community-based cohorts. This comparatively large sample size provided us with good study power to detect associations of moderate magnitude, and to explore associations with individual NHL subtypes. Moreover, it enabled us to more aggressively investigate the effects of time from blood collection to case diagnosis on our findings beyond just the exclusion of cases diagnosed within one or two years from diagnosis, as was done in previous studies. As a result, we were able to discover that the apparent associations with IL-10, TNF-α and sTNF-R2 were not observed for cases diagnosed beyond five years of follow-up, suggestive of reverse causation. Another strength of this study is the standardized blood collection and processing protocol employed in the PLCO Trial, which specified that blood specimens be centrifuged, processed and frozen within two hours of blood collection, thus minimizing blood collection and processing procedures as sources of variation in serum analyte levels. This is particularly important for the measurement of immune markers, given concerns regarding the potential of *ex vivo* expression from leukocytes and enzymatic digestion of cytokines with prolonged time to processing (46, 47). By comparison, serum collected in the NYU-WHS cohort was frozen within approximately 3 hours after collection (48), while the median time from blood collection to storage of samples used in the EPIC-Italy cohort investigation was 4 hours (15).

This study also has limitations. The median follow-up time from blood collection to case diagnosis (5 years) in our study was relatively short for an investigation of NHL etiology, although our analyses restricted to cases diagnosed 6–10 years post-collection were less likely to be affected by reverse causation. As with other recent investigations that have used Luminex-based multiplex assays (14, 15, 49), we observed high between-batch assay variability for some of our analytes. However, our ICCs were high, indicating that the components of analyte variability due to within- and between-batch effects are relatively small compared to the variability between individuals. Measurement error from between-batch variability may have introduced bias, likely towards the null, in our unmatched analyses. Our matched analyses, however, would not have been affected by such bias, given that members of each case-control matched pair were assayed in the same batch.

In conclusion, in this prospective study we observed associations between elevated serum levels of sTNF-R1 and sCD27, possible markers for inflammatory and B-cell stimulatory states respectively, and increased future risk of NHL. These findings support a role for

subclinical inflammation and chronic B-cell stimulation in lymphomagenesis, although more research is needed to better understand the biologic basis underlying these relationships

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial support: This research was supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics and by contracts from the Division of Cancer Prevention, National Cancer Institute, NIH, DHHS.

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. The authors thank Drs. Christine Berg and Philip Prorok, Division of Cancer Prevention, National Cancer Institute, the Screening Center investigators and staff of the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, Mr. Tom Riley and staff, Information Management Services, Inc., Ms. Barbara O'Brien and staff, Westat, Inc., Ms. Jackie King and staff, BioReliance, Inc, and Ms. Georgina Mbisa, the Viral Technology Laboratory at SAIC-Frederick. Most importantly, we acknowledge the study participants for their contributions to making this study possible.

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Table 1

Selected characteristics of non-Hodgkin lymphoma (NHL) cases (N=297) and individually matched controls (N=297) selected from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (1993–2006).

	Cases (N=297)	Controls (N=297)
	N(%)	N(%)
Age at enrollment		
55–59	61 (20.5)	61 (20.5)
60–64	87 (29.3)	87 (29.3)
65–69	89 (30.0)	89 (30.0)
70–74	60 (22.2)	60 (22.2)
Sex		
Female	111 (37.4)	111 (37.4)
Male	186 (62.6)	186 (62.6)
Race		
White	280 (94.3)	280 (94.3)
Black	6 (2.0)	6 (2.0)
Other	11 (3.7)	11 (3.7)
PLCO Center		
Birmingham, AL	9 (3.0)	9 (3.0)
Denver, CO	31 (10.4)	31 (10.4)
Detroit, MI	22 (7.4)	22 (7.4)
Honolulu, HI	7 (2.4)	7 (2.4)
Marshfield, WI	48 (16.2)	48 (16.2)
Minneapolis, MN	55 (18.5)	55 (18.5)
Pittsburgh, PA	46 (16.2)	46 (16.2)
Salt Lake City, UT	35 (11.8)	35 (11.8)
St. Louis, MO	24 (8.1)	24 (8.1)
Washington, DC	20 (6.7)	20 (6.7)
Year of enrollment		
1993–1995	105 (35.4)	105 (35.4)
1996–1997	109 (36.7)	109 (36.7)
1998–2001	83 (28.0)	83 (28.0)
NHL histologic subtype		
CLL/SLL	117 (39.4)	
DLBCL	62 (20.9)	
FL	44 (14.8)	
Other/NOS	74 (24.9)	

Cancer Res. Author manuscript; available in PMC 2012 July 15.

Table 2

Summary of serum levels of selected cytokines and other immune markers in non-Hodgkin lymphoma cases (N=297) and matched controls (N=297) selected from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (1993–2006).

Analyte	Units	Limit of Detection (LOD)	% of samples above LOD	Cases: Median (P5, P95)	Controls: Median (P5, P95)	*P**
IL-4	pg/ml	0.13	60.4	8.3 (<0.1, 493.3)	8.8 (<0.1, 467.6)	0.94
IL-6	pg/ml	0.10	98.8	5.5 (0.9, 68.2)	5.9 (1.3, 79.8)	0.84
IL-10	pg/ml	0.15	95.3	5.5 (0.2, 59.7)	4.0 (0.1–52.4)	0.0002
TNF-α	pg/ml	0.05	99.8	6.5 (2.2, 13.8)	5.8 (2.1–11.9)	0.0003
sTNF-R1	pg/ml	15	99.8	1170 (700, 2170)	1095 (660, 1720)	0.004
sTNF-R2	pg/ml	15	99.8	910 (350, 1930)	855 (320, 1410)	0.003
CRP	pg/ml	2	100.0	620.9 (88.2, 3704.1)	629.8 (83.5, 4806.7)	0.27
sCD27	U/ml	0.20	97.8	75.0 (24.2, 163.9)	58.7 (27.3, 108.0)	<0.0001

Abbreviations: P5, 5th percentile; P95, 95% percentile.

**P*-value from Wilcoxon signed rank test of matched case-control pairs.

Table 3

Associations between serum levels of selected cytokines and immune markers and non-Hodgkin lymphoma: the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (1993–2006).

Analyte	Levels	$N_{Controls}/N_{Cases}$	OR* (95% CI)	P_{Trend}
IL-4 (pg/ml)	≤0.14	116/119	1.0	0.63
	0.15–23.27	61/61	1.0 (0.6–1.5)	
	23.28–102.38	61/52	0.8 (0.5–1.3)	
	>102.38	59/65	1.1 (0.7–1.8)	
IL-6 (pg/ml)	≤2.74	75/77	1.0	0.33
	2.75–5.88	74/75	1.0 (0.6–1.6)	
	5.89–16.11	74/59	0.8 (0.5–1.3)	
	>16.11	74/86	1.2 (0.7–1.9)	
IL-10 (pg/ml)	≤1.93	76/60	1.0	0.0001
	1.94–3.96	74/49	0.8 (0.5–1.3)	
	3.97–7.77	74/73	1.3 (0.8–2.0)	
	>7.77	73/115	**2.1 (1.3–3.4)**	
TNF-α (pg/ml)	≤3.96	76/60	1.0	0.005
	3.97–5.78	74/68	1.3 (0.8–2.1)	
	5.79–7.98	74/72	1.5 (0.9–2.7)	
	>7.98	73/97	**2.2 (1.3–3.9)**	
sTNF-R1 (pg/ml)	≤945	78/60	1.0	0.02
	946–1095	73/59	1.1 (0.7–1.8)	
	1096–1330	74/91	**1.8 (1.1–2.9)**	
	>1330	72/87	**1.7 (1.1–2.8)**	
sTNF-R2 (pg/ml)	≤670	81/84	1.0	0.10
	671–855	69/49	0.7 (0.4–1.2)	
	856–1050	76/65	0.9 (0.5–1.4)	
	>1050	71/99	1.4 (0.9–2.2)	
CRP (pg/ml)	≤242.98	66/57	1.0	0.42
	242.99–629.75	65/74	1.4 (0.8–2.4)	
	629.75–1671.60	65/76	1.5 (0.9–2.5)	
	>1671.60	65/54	1.0 (0.6–1.7)	
sCD27 (U/ml)	≤47.82	75/50	1.0	<0.0001
	47.83–58.71	74/41	0.9 (0.5–1.6)	
	58.72–72.73	74/49	1.3 (0.8–2.3)	
	>72.73	74/157	**5.3 (2.9–9.4)**	

Statistically significant (P < 0.05) results shown in bold-face type.

*Computed using conditional logistic regression modeling.

Table 4

Associations between immune marker levels and non-Hodgkin lymphoma (NHL), by time from blood collection to case diagnosis: the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (1993–2006).

			NHL cases, categorized by time from blood collection to case diagnosis (years)								
			1 – 2			3 – 5			6 – 10		
Analyte	**Level**	**$N_{Controls}$**	**N_{Cases}**	**OR_1* (95% CI)**	**OR_2† (95% CI)**	**N_{Cases}**	**OR_1* (95% CI)**	**OR_2† (95% CI)**	**N_{Cases}**	**OR_1* (95% CI)**	**OR_2† (95% CI)**
IL-10 (pg/ml)	≤1.93	65	14	1.0	1.0	21	1.0	1.0	25	1.0	1.0
	1.94–3.96	74	15	0.6 (0.2–1.5)	1.1 (0.5–2.4)	12	0.7 (0.3–1.7)	0.6 (0.3–1.4)	22	1.1 (0.5–2.5)	0.9 (0.5–1.9)
	3.97–7.77	74	15	1.2 (0.4–3.3)	1.2 (0.5–2.7)	27	1.3 (0.6–2.7)	1.4 (0.7–2.7)	31	1.3 (0.6–2.8)	1.3 (0.7–2.4)
	>7.77	73	35	**3.1 (1.2–8.5)**	**2.8 (1.3–5.9)**	43	**2.4 (1.1–5.3)**	**2.2 (1.2–4.2)**	37	1.6 (0.8–3.2)	1.5 (0.8–2.9)
	P_{Trend}			P = 0.004	P = 0.0006		P = 0.009	P = 0.001		P = 0.20	P = 0.13
TNF-α (pg/ml)	≤3.96	76	15	1.0	1.0	23	1.0	1.0	22	1.0	1.0
	3.97–5.78	74	18	1.2 (0.5–3.0)	1.3 (0.6–2.9)	20	1.1 (0.5–2.6)	0.9 (0.5–1.9)	30	1.6 (0.7–3.6)	1.4 (0.7–2.7)
	5.79–7.98	74	17	1.1 (0.4–3.6)	1.7 (0.7–4.1)	26	1.8 (0.7–4.8)	1.3 (0.6–2.6)	29	1.5 (0.7–3.7)	1.3 (0.6–2.8)
	>7.98	73	29	3.6 (1.0–13.4)	**3.2 (1.4–7.5)**	34	**3.3 (1.2–8.9)**	1.9 (0.9–3.8)	34	1.5 (0.6–3.7)	1.4 (0.7–2.9)
	P_{Trend}			P = 0.07	P = 0.004		P = 0.01	P = 0.05		P = 0.55	P = 0.46
sTNF-R1 (pg/ml)	≤945	78	21	1.0	1.0	18	1.0	1.0	21	1.0	1.0
	946–1095	73	20	1.2 (0.5–2.8)	1.3 (0.6–2.6)	21	1.3 (0.6–2.9)	1.2 (0.6–2.5)	18	0.8 (0.3–2.1)	1.0 (0.5–2.0)
	1096–1330	74	16	1.1 (0.5–2.8)	0.9 (0.4–1.9)	37	2.0 (0.8–4.6)	**2.3 (1.1–4.5)**	38	**2.3 (1.0–5.2)**	**2.1 (1.1–4.2)**
	>1330	72	22	1.8 (0.7–4.9)	1.4 (0.7–2.9)	27	1.6 (0.7–3.6)	1.7 (0.8–3.5)	38	1.8 (0.8–4.2)	**2.1 (1.0–4.0)**
	P_{Trend}			P = 0.23	P = 0.52		P = 0.31	P =0.11		P = 0.07	P = 0.01
sTNF-R2 (pg/ml)	≤670	81	20	1.0	1.0	30	1.0	1.0	34	1.0	1.0
	671–855	69	7	0.5 (0.2–1.7)	0.4 (0.2–1.2)	16	0.6 (0.3–1.4)	0.6 (0.3–1.2)	26	0.9 (0.4–1.9)	0.9 (0.4–1.6)
	856–1050	76	18	2.5 (0.9–7.0)	1.0 (0.5–2.2)	26	1.1 (0.5–2.4)	0.9 (0.5–1.6)	21	**0.4 (0.2–0.9)**	0.6 (0.3–1.2)
	>1050	71	34	**5.6 (1.8–2.2)**	**2.3 (1.2–4.8)**	31	1.1 (0.5–2.4)	1.1 (0.6–2.1)	34	0.8 (0.4–1.8)	1.1 (0.6–2.1)
	P_{Trend}			P = 0.0007	P = 0.004		P = 0.53	P = 0.60		P = 0.41	P = 0.88
sCD27 (U/ml)	≤47.82	75	16	1.0	1.0	21	1.0	1.0	13	1.0	1.0
	47.83–58.71	74	11	0.7 (0.3–1.9)	0.8 (0.3–1.9)	8	0.6 (0.2–1.6)	0.4 (0.2–0.9)	22	1.6 (0.7–4.1)	1.7 (0.8–3.9)
	58.72–72.73	74	12	2.0 (0.6–6.3)	1.1 (0.4–2.5)	13	0.8 (0.3–2.1)	0.6 (0.3–1.4)	24	1.9 (0.8–4.5)	1.9 (0.8–4.1)
	>72.73	74	40	**11.3 (2.7–48.0)**	**3.6 (1.8–7.6)**	61	**4.8 (1.9–12.4)**	**3.3 (1.7–6.2)**	56	**4.3 (1.7–11.1)**	**4.1 (1.9–8.5)**
	P_{Trend}			P = 0.0005	P < 0.0001		P <0.0001	P < 0.0001		P = 0.002	P = 0.0001

Statistically significant ($P < 0.05$) results shown in bold-face type.

*Computed using conditional logistic regression modeling within strata defined by time from blood collection to case diagnosis (1–2, 3–5 and 6–10 years).

†Computed using polytomous regression modeling, comparing case subcategories defined by follow-up time to diagnosis (1–2, 3–5 and 6–10 years from blood collection) to all controls, with model adjustment for baseline age, sex, race, PLCO center, and enrollment year.

Table 5

Associations between immune marker levels and non-Hodgkin lymphoma (NHL) subtypes, by time from blood collection to case diagnosis: the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (1993–2006).

				Cases, categorized by time from blood collection to case diagnosis (years)					
Analyte		**Cases**		**1 – 2**		**3 – 5**		**6 – 10**	
NHL Subtype Level	**N Controls**	**N**	**OR* (95% CI)**	**N**	**OR* (95% CI)**	**N**	**OR† (95% CI)**	**N**	**OR† (95% CI)**
sTNF-R1 (pg/ml)									
Chronic lymphocytic leukemia/small lymphocytic lymphoma									
<1096	151	39	1.0	11	1.0	11	1.0	17	1.0
≥1096	146	78	**2.1 (1.3–3.4)**	20	**2.5 (1.0–5.8)**	32	**2.9 (1.3–6.3)**	26	1.5 (0.7–3.1)
Diffuse large B-cell lymphoma									
<1096	151	30	1.0	11	1.0	8	1.0	11	1.0
≥1096	146	32	1.1 (0.6–2.0)	3	**0.2 (0.1–0.9)**	9	1.6 (0.6–4.4)	20	2.3 (1.0–5.6)
Follicular lymphoma									
<1096	151	21	1.0	9	1.0	6	1.0	6	1.0
≥1096	146	23	1.2 (0.6–2.4)	7	1.0 (0.3–3.0)	8	1.3 (0.4–4.1)	8	1.6 (0.5–5.7)
Other or not otherwise classifiable NHL									
<1096	151	29	1.0	10	1.0	14	1.0	5	1.0
≥1096	146	45	**1.9 (1.1–3.3)**	8	0.6 (0.2–1.8)	15	1.5 (0.6–3.7)	22	**5.7 (1.9–17.0)**
sCD27 (U/ml)									
Chronic lymphocytic leukemia/small lymphocytic lymphoma									
<58.72	149	30	1.0	8	1.0	11	1.0	11	1.0
≥58.72	148	87	**3.2 (2.0–5.3)**	23	**4.2 (1.6–10.6)**	32	**2.9 (1.4–6.3)**	32	**2.8 (1.2–6.3)**
Diffuse large B-cell lymphoma									
<58.72	149	22	1.0	8	1.0	7	1.0	7	1.0
≥58.72	148	40	1.7 (0.9–3.0)	6	0.8 (0.2–2.4)	10	1.9 (0.7–5.3)	24	**2.9 (1.1–7.5)**
Follicular lymphoma									
<58.72	149	15	1.0	5	1.0	3	1.0	7	1.0
≥58.72	148	29	**2.4 (1.2–5.0)**	11	**5.6 (1.5–20.6)**	11	**4.8 (1.1–20.2)**	7	0.8 (0.2–2.7)
Other or not otherwise classifiable NHL									
<58.72	149	24	1.0	6	1.0	8	1.0	10	1.0

Analyte		Cases, categorized by time from blood collection to case diagnosis (years)							
		Cases		1 – 2		3 – 5		6 – 10	
NHL Subtype Level	$N_{Controls}$	N	OR* (95% CI)	N	OR* (95% CI)	N	OR† (95% CI)	N	OR† (95% CI)
≥58.72	148	50	**2.3 (1.3–4.1)**	12	2.5 (0.8–7.6)	21	**3.3 (1.3–8.1)**	17	1.6 (0.7–3.9)

Statistically significant ($P < 0.05$) results shown in bold-face type.

*Computed using polytomous regression modeling, comparing NHL histologic subtypes to all controls, with model adjustment for baseline age, sex, race, PLCO center, and enrollment year.

†Computed using polytomous regression modeling, comparing, for each NHL subtype, subcategories defined by follow-up time to diagnosis (1–2, 3–5 and 6–10 years from blood collection) to all controls, with model adjustment for baseline age, sex, race, PLCO center, and enrollment year.