Research Article

Cancer Epidemiology, Biomarkers & Prevention

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

Fatemeh Saberi Hosnijeh[1,3], Esmeralda J.M. Krop[1], Chiara Scoccianti[4,10], Vittorio Krogh[5], Domenico Palli[6], Salvatore Panico[7], Rosario Tumino[8], Carlotta Sacredote[9], Niga Nawroly[10], Lützen Portengen[1], Jakob Linseisen[11], Paolo Vineis[10], and Roel Vermeulen[1,2]

Abstract

Background: Recently, biological markers related to the immune system such as cytokines have been studied to further understand the etiology of non-Hodgkin Lymphoma (NHL). However, to date, there are no studies that have studied cytokine levels prospectively in relation to NHL risk in the general population.

Methods: Using bead-based immunoassays, plasma levels of 11 cytokines, 4 chemokines, and 1 adhesion molecules were measured in prediagnostic blood samples of 86 NHL cases and 86 matched controls (average time between blood collection and diagnosis, 4.5 y). Conditional logistic regression adjusted for body mass index and alcohol consumption was used to analyze the association between individual plasma cytokine levels and the risk of developing NHL.

Results: In multivariate models, excluding cases diagnosed within 2 years after inclusion, we observed a significant association for interleukin 2 (IL2; *P* trend = 0.004), interferon (IFN)-γ (*P* trend = 0.05), and intercellular adhesion molecule (ICAM) (*P* trend = 0.04). Subanalyses of B-cell NHL patients showed a significant association with IL2 (*P* trend = 0.003), tumor necrosis factor-α (TNF-α; *P* trend = 0.03), and ICAM (*P* trend = 0.04) and a borderline association with IL5 (*P* trend = 0.07) and IFN-γ (*P* trend = 0.08).

Conclusions: The results of this study suggest, in a prospective setting, a possible association between plasma levels of IL2, ICAM, IFN-γ, and TNF-α with NHL risk and provide some evidence that risk of NHL might be related to a downregulation of T helper 1 cytokines.

Impact: Identification of subtle changes in immune response regulation quantified by plasma cytokine levels possibly provides new insights in the etiology of NHL. *Cancer Epidemiol Biomarkers Prev; 19(6); 1577–84. ©2010 AACR.*

Introduction

Non–Hodgkin lymphomas (NHL) are a heterogeneous disease group belonging to malignancies of the lymphoid tissue, which vary in histologic characteristics, clinical manifestations, and etiologic factors (1-5). Incidence rates of NHL have shown a significant rise especially in highly aggressive subtypes during the past several decades worldwide (6). In European countries, an overall increase in NHL incidence of 4.2% per annum was observed from 1985 to 1992 (7) and there is still a small (3%) but continuing increase in incidence (8). Despite extensive research in recent years, reasons for this increase are still largely unknown (9).

The most established risk factors of NHL are those related to severe immunocompromised individuals such as genetically determined or acquired immune deficiencies, including HIV infection or iatrogenically induced immune suppression after transplantation (3-5). As an extension of this observation, it has been hypothesized that many of the known and unknown NHL risk factors might influence NHL risk through (subtle) modulations of the immune system (10). A possible role of cytokines in the development of NHL is supported by recent reports on NHL risk related to genetic variation in genes encoding proinflammatory and anti-inflammatory cytokines [tumor necrosis factor (TNF), LTA, IL10, and IL4; refs. 11-13]. These reports offer important evidence linking immune function to NHL

Authors' Affiliations: [1]Institute for Risk Assessment Sciences, Division of Environmental Epidemiology, Utrecht University; [2]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, Netherlands; [3]Zanjan University of Medical Science, Zanjan, Iran; [4]IARC, Lyon, France; [5]Nutritional Epidemiology Unit, National Cancer Institute, Milan, Italy; [6]Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Centre, Scientific Institute of Tuscany, Florence, Italy; [7]Department of Clinical and Experimental Medicine, Federico II University of Naples, Napoli, Italy; [8]Ragusa Cancer Registry, Ragusa, Italy; [9]Institute for Scientific Interchange Foundation, Torino, Italy; [10]Imperial College, London, United Kingdom; and [11]Institute of Epidemiology, Helmholtz Zentrum München, München, Germany

Note: P. Vineis and R. Vermeulen are co-senior authors.

Corresponding Author: Roel Vermeulen, Institute for Risk Assessment Sciences, Division of Environmental Epidemiology, Utrecht University, Jenalaan 18D, 3584 CK Utrecht, the Netherlands. Phone: 31-30-253-9448; Fax: 31-30-253-9499. E-mail: R.C.H.Vermeulen@uu.nl

doi: 10.1158/1055-9965.EPI-09-1237

among nonimmunocompromised individuals. However, functional relevance of the found single-nucleotide polymorphisms (SNP) is still unclear. Therefore, studies incorporating direct measurements of the immune environment, in addition to investigation of genetic polymorphisms, are needed to further elucidate the role of immunomodulatory factors in lymphomagenesis.

Biological markers related to the immune system such as cytokines have been studied to explain the etiology of NHL (14-16). However, there are only few studies that have studied cytokine levels prospectively in relation to NHL risk and none have been in the general population. In a study nested within the Multicenter AIDS Cohort Study (17) in which serum samples were obtained preceding a diagnosis of AIDS lymphoma, Breen et al. reported an increased serum level of IL6 in Burkitt's lymphoma patients compared with CD4-matched AIDS controls who did not have lymphoma. In another study (18), a significant increase in detectable serum levels of IL10 in AIDS lymphoma patients was found compared with HIV^+/HIV^- subjects. However, in this study, >81% of the cases developed NHL within 1 year of blood collection, and as such, it can be questioned if the observed increases in IL10 were a result of the disease itself or the cause of the disease. Furthermore, as this study was conducted among HIV^+ subjects, it is questionable if these results can be extrapolated to the general population.

We hypothesize that given the pivotal role of cytokines in immune function, blood levels of multiple cytokines collectively might reflect the subtle status of a deregulated immune response or a failure to modulate the immune response appropriately. To illustrate innate and acquired variations in immune responses that affect the risk of developing NHL, a prospective study is the only appropriate epidemiologic design. Only in this way factors related to disease can be studied years before the onset of the disease, excluding the possibility that the measured response is actually caused by the disease and not the cause of the disease itself. We therefore did a case-control study nested within the Italian contribution to the European Prospective Investigation into Cancer and Nutrition (EPIC) on plasma cytokine and chemokine levels potentially related to NHL risk.

Materials and Methods

Study population

The EPIC project is a European network of prospective cohorts that was set up to examine relationships of cancer risk with nutrition and metabolic risk factors (19). In the period 1993 to 1998, EPIC Italy completed the recruitment of 47,749 volunteers (15,171 men and 32,578 women, ages 35-65 y) in four different areas covered by cancer registries: Varese (12,083 volunteers) and Turin (10,604) in the northern part of the country and Florence (13,597) and Ragusa (6,403) in central and southern Italy, respectively. An associated center in Naples enrolled 5,062 women. The present study included incident cases of NHL until the end of 2004 according to the International Classification of Diseases for Oncology, third edition.

For each case subject, one random control was selected among all cohort members alive and free of cancer at the time of diagnosis of the index case matched by center, gender, date of recruitment, age at diagnosis, and age at recruitment (±3 y).

A total of 91 patients with NHL were eligible to be included in the study. Excluded were cases without suitable control samples (n = 2) and cases for whom plasma specimen was missing (n = 3), so a total of 86 pairs entered the study. NHL diagnosis was the second malignancy for five cases. Analyses excluding these cases did not change the results of the study.

Laboratory assay

Blood samples collected from EPIC participants were stored at −196°C in liquid nitrogen until they were pulled for laboratory analysis. None of the plasma specimens were thawed before the analyses. We measured 11 cytokines [interleukin (IL) 1α, IL1β, IL2, IL4, IL5, IL6, IL10, IL12, IL13, IFN-γ, and TNF-α], 4 chemokines (IL8, RANTES, eotaxin, and IP10), and 1 adhesion molecules (ICAM) in stored citrate plasma samples (50 μL) of all cases and controls using the Luminex multianalyte profiling technology (Lab-MAP) according to the protocol described by de Jager et al. (20), except that, instead of a 1-hour incubation, an overnight incubation at 4°C was used (21). The Luminex multianalyte assay has been validated previously against standard ELISA tests (20). In a recent study, we showed that this assay provides reproducible results in plasma and serum samples and that the rank correlations between measured and analyte levels in serum and plasma were relatively high (Spearman rank correlation, 0.74-0.98) for most of the analytes (21). All laboratory personnel were blinded with regard to case-control status. Median time interval between sample collection and freezing was 4 hours for both cases and controls (Table 1). All samples were run in duplicate with matched case-control sets assayed in the same batch. Quality control sets (low- and high-concentration cytokine quality control samples) were run in duplicate with the case-control sets in each batch. The median intrabatch coefficient of variation for all of cytokines based on these quality control duplicate sets was 6.7% (4.3-30), and the median interbatch coefficient of variation was 30.7% (9.6-110). The lower limits of detection (LOD) based on the standard curve were 0.24 pg/mL for IL4; 0.61 pg/mL for IL12; 1.22 pg/mL for IL1β, IL2, IL5, IL6, IL8, IL10, IL13, IFN-γ, and TNF-α; 2.44 pg/mL for IL1α, RANTES, and eotaxin; 4.88 pg/mL for IP10; and 73.24 pg/mL for ICAM.

Statistical analysis

Values of cytokine levels below the LOD or above the maximum range of detection were imputed based on a

Table 1. Baseline characteristics of NHL cases and control subjects

	Case (n = 86)	Control (n = 86)	P_{diff}
Matching variables			
Age at recruitment, y*	55 (36-74)	54 (35-73)	0.845
Age at diagnosis, y*			
Male (%), y*	39.5%; 60 (41-69)	—	
Female (%), y*	60.5%; 59.5 (41-77)	—	
Center (%)			
Florence	20.9%	20.9%	
Naples	7%	7%	
Ragusa	5.8%	5.8%	
Turin	20.9%	20.9%	
Varese	45.3%	45.3%	
Other variables			
BMI*	24.8 (17.8-36.6)	25.3 (18.8-35.3)	0.896
Physical activity (sex-specific quartiles), %			0.665
Qrt1	14%	15.1%	
Qrt2	27.9%	20.9%	
Qrt3	45.3%	52.3%	
Qrt4	9.3%	10.5%	
Missing	3.5%	1.2%	
Education			0.241
None	4.8%	0	
Primary school completed	53%	55.3%	
Technical/professional school	10.8%	16.5%	
Secondary school	22.9%	18.8%	
Longer education (including University degree)	8.4%	9.4%	
Ethanol intake at recruitment (g/d)	12.3 (0-104.4)	11.7 (0-93.9)	0.685
Storage time of samples (h)*	4 (1:30-30:07)	4 (1:30-74:23)	0.216
Histologic subtype of NHL (%)			
B-NHL	92%	—	
Follicle center cell	29.1% (31.6%)†	—	
Diffuse large B cell	16.3% (17.7%)†	—	
Marginal zone B-cell lymphoma	11.6% (12.7%)†	—	
Chronic lymphocytic leukemia	10.5% (11.4%)†	—	
Mantle cell lymphoma	4.7% (5.1%)†	—	
Miscellaneous nodal lymphomas	19.8% (21.5%)†	—	
T-cell NHL	8%	—	
Extranodal T cell	4.7%	—	
Nodal peripheral T-cell lymphoma	3.5%	—	

*Median (range); for individuals without self-report and measured anthropometric data, center, age, and gender-specific average values of anthropometric variables have been imputed.
†Percentage of B-NHL subjects.

maximum likelihood estimation method (22). In all analyses, levels of cytokines were log transformed to normalize their distributions. For individuals without self-reported and measured anthropometric data, center/age/gender-specific average values of anthropometric variables have been imputed (n = 7). Differences between cases and controls in mean plasma concentrations of cytokines and baseline covariates were assessed using paired t test. For categorical variables, the statistical significances of case-control differences were tested by the χ^2 test.

Odds ratios (OR) and 95% confidence intervals (95% CI) for NHL in relation to plasma cytokine concentrations (as continuous variables) were calculated by conditional logistic regression (CLR) using the PHREG procedure (SAS statistical software, version 9.1; SAS Institute). Risk estimates were computed both as crude and with additional adjustments for potential confounders, including body mass index (in kg/m^2; continuous) and alcohol intake (g/d; continuous). The effect of physical activity (sex-specific quartiles of combined recreational,

household, and occupational physical activity) and educational level (indicator of socioeconomic status, categorical) as potential confounding variable was examined, but they did not appreciably change the risk estimates and therefore were not included in the models. Tertiles of plasma cytokine concentrations were calculated based on the distribution in control subjects, and CLR models were used to estimate the association between tertiles of cytokine levels and risk of NHL. We also investigated the possible associations by histologic subtype of B-cell NHL (B-NHL) and by excluding samples that were not stored within 12 hours of collection and cases diagnosed with <2 years of follow-up. The latter exclusion was made to remove the possibility that cytokine levels may have been changed in cases compared with control subjects due to preclinical disease status ($n = 18$ cases). In addition, potential modification of the effect of cytokines by gender and centers was tested.

Statistical analyses were done using SPSS software (version 11.5; SPSS, Inc.) and SAS (version 9.1; SAS institute). All P values are two-sided, with $P < 0.05$ considered as statistically significant.

Results

Description of the study population

This study included 86 cases and an equal number of controls (68 men and 104 women). The median age at cancer diagnosis was 60 (range, 41-69) and 59.5 (range, 41-77) years for men and women, respectively (Table 1). Median time between recruitment (i.e., blood collection) in the study and diagnosis of NHL was 4.5 years (range, 0.12-10.4). Of all NHL cases, 92% were diagnosed with B-NHL, with the most common diagnosed subtypes being follicular lymphoma (31.6%), miscellaneous nodal lymphomas (21.5%), and diffuse large B-cell lymphoma (17.7%). Cases and control subjects did not differ considerably with regard to most risk factors and covariates (Table 1).

Plasma cytokine concentration

Geometric mean and SD for plasma levels of individual cytokines are shown in Table 2. Cytokine levels for cases seemed to be lower than for controls. For chemokines, this seemed to be reversed. Cases and controls showed small differences in mean level of IFN-γ ($P = 0.05$), IP10 ($P = 0.07$), TNF-α ($P = 0.07$), and ICAM ($P = 0.07$) in the paired t test. By excluding case patients ($n = 18$) diagnosed within <2 years of follow-up, significant differences between levels of TNF-α ($P = 0.04$) and ICAM ($P = 0.02$) and borderline significant differences for IFN-γ ($P = 0.06$) and IL5 ($P = 0.06$) between cases and controls were found (data not shown).

Risk estimation

CLR analyses based on tertiles of cytokine levels showed a significant inverse association for IL2 and a borderline significant association for IFN-γ and ICAM with the occurrence of NHL in both the crude and fully adjusted regression model (Table 3). When we restricted the analyses to the case patients diagnosed after the first 2 years of follow-up ($n = 136$), these associations became slightly stronger: IL2 (P trend = 0.004), IFN-γ (P trend = 0.05), and ICAM (P trend = 0.04). A further restriction to B-NHL patients ($n = 130$) showed a significant association for IL2 (P trend = 0.003), TNF-α (P trend = 0.03), and ICAM (P trend = 0.04) and a borderline association for IL5 (P trend = 0.07) and IFN-γ (P trend = 0.08; data not shown).

Discussion

To date, little is known about blood immune marker changes that may be related to the development of NHL, except for a few small studies among HIV patients (17, 18). In several studies, it has been documented that an immunosuppressed state plays a key role in development of lymphomas (3-6). Of the many mechanisms contributing to immune suppression, much attention was recently given to inflammatory cells and to inflammatory mediators in general. Adhesion molecules and their ligand(s) such as ICAM-1 play an important regulatory role in the inflammatory process. It is shown that ICAM can be upregulated on many cell types during an inflammatory or immune response, particularly under the influence of various cytokines (23, 24). Interaction of all activated leukocytes with ICAM-1 may be a crucial step in the induction and protraction of an inflammatory response (23). In the multivariate CLR model, we found that increased levels of ICAM were associated with higher NHL risk. Several clinical studies have shown that blood level of ICAM is increased in NHL patients; however, to our knowledge, there are no reports until now relating this adhesion molecule prospectively to the risk of NHL.

As immune dysfunction is thought to be the underlying basis of lymphomagenesis, an imbalance in the regulation and expression of T helper 1 (Th1) and Th2 cytokines could play an important role in the etiology of NHL and its major subtypes (9, 25). Th1 cytokines generate and activate CTLs and natural killer cells, which play crucial roles in antitumor immune responses. Mori et al. (25) studied $CD4^+$ cells of diffuse large B-cell lymphoma patients and concluded that the Th1/Th2 balance was polarized to Th2 in untreated patients and to Th1 in patients in complete remission. We found similar results in that a lower risk of NHL with increasing IL2, IFN-γ, and TNF-α (Th1) plasma levels was observed. It should be noted, however, that reevaluation of the Th1 and Th2 paradigm and discovery of other Th cells, such as Th3 and Th17, have led to the realization that a number of inflammatory conditions in which Th1 was previously considered central may actually be related to other types of Th cells. So, although the Th1/Th2 model still provides a valuable framework to describe the immune changes found in this study, the

actual regulatory mechanisms involved are likely more complicated.

Recent genetic studies showed that SNPs from candidate genes, including TNF (10, 12-14); LTA (10); IL4, IL5, and IL6 (11); and IL10 (10-12, 14), may be risk factors for NHL overall or for certain NHL subtypes. Our study showed evidence of a possible role of TNF-α in lymphomagenesis, particularly B-NHL. Based on laboratory evidence, both TNF G308A and IL10 T357A polymorphisms result in overall elevated expression of TNF-α and thus contribute to a shift in the Th1/proinflamatory immune response (13), whereas our results showed low expression of Th1 cytokines (IL2, IFN-γ, and TNF-α) in prospective NHL cases compared with controls. Lan et al. (12), in a study of cytokine polymorphisms in the Th1/Th2 pathway, documented that SNPs in Th2 cytokine genes may be associated with risk of NHL. These findings raise the possibility that a shift in the balance of the Th1/Th2 response could have crucial consequences for lymphomagenesis. However, it should be considered that malignant diseases develop and progress as a result of multifactorial processes, in which genomic alterations and modifications in gene expression in premalignant cells are joined by a tumor-supporting microenvironmental setting (23). Further, functional significance of cytokine SNPs is not clear, as genotype-phenotype correlation studies have shown conflicting results possibly due to the extensive networks of gene products regulating the production, modulation, and decay of cytokines. It is therefore likely that any influence of individual cytokine genes on respective phenotypes will be relatively minor.

Our study is one of the first studies that used prospectively collected samples, thus avoiding inverse causation bias that may occur when variation in blood level of cytokines is induced by the disease or by cancer treatments or lifestyle changes after cancer diagnosis. There were, however, several limitations in our study. First, we only had one blood sample for each individual to characterize their immune profile. There are little data on the interindividual and intraindividual variation of cytokines. However, in a previous study (21), we looked at the interindividual and intraindividual variability of cytokines measured in this study. Intraclass correlation coefficients for most cytokines, based on two repeated blood samples collected 1 to 2 weeks apart and tested on two different days, were generally above 0.8, indicating that cytokine levels did not vary much in time within a

Table 2. Descriptive statistics of plasma cytokine levels for cases and control subjects

	Control (n = 86)					Case (n = 86)					P*
	Geometric mean	Geometric SD	Min-max	<LOD	>Range	Geometric mean	Geometric SD	Min-max	<LOD	>Range	
IL1β	1.65	3.39	0.14-71.95	28		1.51	3.422	0.07-51.2	23		0.75
IL1α	67.56	8.97	0.46-32,976.9	3		48.62	9.89	0.7-29,806.4	4	2	0.24
IL2	70.61	8.76	0.16-3,854.4	5	2	41.13	12.63	0.0004-18,044.2	4		0.11
IL4	0.71	4.17	0.02-25.8	7		0.59	4.44	0.02-45.45	5		0.39
IL5	27.84	6.78	0.2-3,640.1	9		18.23	6.38	0.19-5,509.4	13		0.09
IL6	58.38	6.04	0.61-7,122.8	5		61.73	6.28	0.25-2,785.1	3		0.56
IL8	6.97	6.16	0.37-951.8	14		5.31	7.29	0.16-1,474.9	16		0.35
IL10	46.44	6.8	0.77-7,117.2	1		42.39	7.58	0.38-8,754.2		1	0.76
IL12	14.91	7.05	0.17-1,931.2	3	1	11.77	8.04	0.23-3,395.9	1	1	0.40
IL13	1.88	4.69	0.06-199.3	24		1.45	5.23	0.09-103.01	24		0.21
IFN-γ	6.81	7.97	0.16-702.7	21		3.47	13.9	0.0002-2,539.6	29		0.05
TNF-α	1.03	4.42	0.04-208.1	32		0.65	6.47	0.001-76.21	30		0.07
RANTES	9,459.68	2.51	1,237-16,581,337	1	30	9,981.54	2.09	1,320-74,929		31	0.38
ICAM	117,761.8	1.66	28,986-179,640			128,143.4	1.4	38,274.8-210,085			0.07
Eotaxin	74.34	2.16	8.9-837.5	1		83.82	2.15	22.24-985.7			0.28
IP10	40.54	1.68	10.9-180.1	1		46.95	1.97	13.01-397.9			0.07

NOTE: Number of samples with cytokine value lower than LOD (<LOD) and higher than maximum value (>Range) were imputed.
Abbreviations: Min, minimum values of cytokine concentration; Max, maximum values of cytokine concentration; RANTES, regulated on activation, normal T cell expressed and secreted; IP10, IFN-inducible protein 10.
*P value of paired t test (two-sided) based on log-transformed values of cytokine concentrations.

Table 3. Crude and multivariable-adjusted ORs and 95% CIs of NHL cases by tertiles of plasma cytokine concentrations in EPIC Italy

Cytokine	Univariate model					Multivariate model					
	n = 172					*n* = 172			More than 2 y of follow-up (*n* = 136)		
	Tertile limits	No. cases/ no. controls	OR (95% CI)	*P**	*P* trend†	OR (95% CI)	*P**	*P* trend†	OR (95% CI)	*P**	*P* trend†
IL1β				0.91	0.7		0.98	0.98		0.8	0.5
	<–0.242	31/29	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	–0.242 to 0.848	29/29	0.94 (0.46-1.92)			0.93 (0.44-1.96)			0.85 (0.39-1.86)		
	>0.848	26/28	0.83 (0.36-1.93)			0.97 (0.39-2.45)			0.73 (0.25-2.07)		
IL1α				0.98	0.8		0.86	0.9		0.5	0.4
	<3.078	29/28	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	3.078-4.896	29/29	0.95 (0.44-2.04)			0.8 (0.36-1.82)			0.5 (0.21-1.42)		
	>4.896	28/29	0.92 (0.41-2.07)			0.8 (0.33-1.98)			0.6 (0.23-1.66)		
IL2				0.05	0.01		0.05	0.04		0.02	0.004
	<3.524	43/28	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	3.524-5.166	21/29	0.39 (0.16-0.91)			0.34 (0.14-0.86)			0.17 (0.04-0.6)		
	>5.166	22/29	0.41 (0.18-0.96)			0.38 (0.14-1)			0.23 (0.07-0.78)		
IL4				0.59	0.5		0.9	0.9		0.9	0.7
	<–1.129	32/29	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	–1.129 to –0.115	22/28	0.68 (0.29-1.59)			0.83 (0.34-2.06)			0.8 (0.28-2.32)		
	>–0.115	32/29	0.95 (0.19-2.24)			1.04 (0.41-2.54)			0.8 (0.29-2.23)		
IL5				0.25	0.2		0.15	0.1		0.1	0.08
	<2.639	40/29	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	2.639-3.742	21/29	0.57 (0.28-1.15)			0.46 (0.2-1.07)			0.38 (0.15-0.98)		
	>3.742	25/28	0.67 (0.30-1.45)			0.61 (0.27-1.38)			0.52 (0.2-1.3)		
IL6				0.85	0.8		0.77	0.5		0.9	0.8
	<3.906	28/29	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	3.906-4.620	27/29	0.99 (0.44-2.23)			1.2 (0.51-2.79)			1.17 (0.45-3.05)		
	>4.620	31/28	1.24 (0.50-3.1)			1.42 (0.54-3.72)			1.00 (0.33-3.04)		
IL8				0.32	0.2		0.15	0.3		0.1	0.06
	<1.021	37/29	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	1.021-2.546	20/28	0.55 (0.25-1.19)			0.43 (0.18-1)			0.35 (0.13-0.95)		
	>2.546	29/29	0.79 (0.36-1.73)			0.73 (0.3-1.77)			0.58 (0.21-1.58)		
IL10				0.74	0.7		0.5	0.4		0.3	0.3
	<2.912	33/29	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	2.912-4.512	25/29	0.73 (0.34-1.59)			0.61 (0.26-1.42)			0.48 (0.18-1.27)		
	>4.512	28/28	0.87 (0.38-1.98)			0.71 (0.28-1.8)			0.62 (0.22-1.7)		
IL12				0.54	0.3		0.42	0.3		0.3	0.2
	<1.845	35/29	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	1.845-3.146	23/28	0.63 (0.28-1.43)			0.58 (0.25-1.35)			0.47 (0.17-1.26		
	>3.146	28/29	0.76 (0.35-1.67)			0.64 (0.27-1.55)			0.61 (0.23-1.61)		
IL13				0.52	0.3		0.6	0.4		0.4	0.2
	<–0.049	36/29	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	–0.49 to 1.125	25/29	0.67 (0.31-1.43)			0.7 (0.32-1.56)			0.82 (0.33-2.03)		
	>1.125	25/28	0.70 (0.33-1.48)			0.7 (0.32-1.55)			0.53 (0.22-1.3)		
IFN-γ				0.17	0.06		0.13	0.04		0.1	0.05
	<1.019	39/29	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	1.019-2.910	28/29	0.69 (0.33-1.44)			0.64 (0.28-1.44)			0.61 (0.24-1.56)		
	>2.910	19/28	0.44 (0.19-1.03)			0.38 (0.15-0.97)			0.34 (0.12-0.96)		

(*Continued on the following page*)

Table 3. Crude and multivariable-adjusted ORs and 95% CIs of NHL cases by tertiles of plasma cytokine concentrations in EPIC Italy (Cont'd)

Cytokine	Univariate model					Multivariate model					
	n = 172					*n* = 172			More than 2 y of follow-up (*n* = 136)		
	Tertile limits	No. cases/ no. controls	OR (95% CI)	*P**	*P* trend†	OR (95% CI)	*P**	*P* trend†	OR (95% CI)	*P**	*P* trend†
TNF-α				0.38	0.2		0.41	0.18		0.3	0.1
	<–0.928	36/28	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	–0.928 to 0.3	27/29	0.71 (0.34-1.47)			0.73 (0.33-1.62)			0.66 (0.27-1.59)		
	>0.3	23/29	0.58 (0.26-1.28)			0.56 (0.24-1.31)			0.46 (0.18-1.17)		
RANTES				0.34	0.2		0.28	0.16		0.09	0.1
	<8.828	24/29	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	8.828-9.145	25/29	1.06 (0.46-2.46)			0.98 (0.39-2.47)			0.57 (0.2-1.71)		
	>9.145	37/28	1.69 (0.76-3.74)			1.81 (0.78-4.2)			2.1 (0.8-5.46)		
ICAM				0.15	0.06		0.14	0.05		0.1	0.04
	<11.675	19/28	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	11.675-11.887	27/29	1.40 (0.64-3.09)			1.36 (0.6-3.15)			1.68 (0.64-4.41)		
	>11.887	40/29	1.25 (0.99-5.12)			2.44 (0.98-6.08)			3.1 (1.08-8.92)		
Eotaxin				0.61	0.6		0.5	0.8		0.7	0.9
	<4	28/28	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	4-4.435	24/29	0.78 (0.35-1.73)			0.64 (0.28-1.48)			0.67 (0.26-1.73)		
	>4.435	34/29	1.16 (0.58-2.31)			0.91 (0.43-1.94)			0.91 (0.42-2)		
IP10				0.23	0.4		0.18	0.3		0.4	0.8
	<3.537	29/28	1.00 (reference)			1.00 (reference)			1.00 (reference)		
	3.537-3.907	20/29	0.61 (0.24-1.55)			0.56 (0.19-1.66)			0.52 (0.16-1.65)		
	>3.907	37/29	1.27 (0.53-3.05)			1.32 (0.51-3.44)			0.97 (0.35-2.73)		

NOTE: ORs estimated by CLR models for tertiles of plasma cytokine concentrations (tertile cut points based on the distribution of cytokine levels for control subjects). *P* trend = *P* value (two-sided) were calculated by including the median of each tertile of cytokine concentrations as continuous variables in addition to all covariates to the multivariate models.

*Type III *P* value.

†*P* value of Wald χ^2 test.

subject compared with the variance between subjects at least in a 2-week period. It was concluded that, therefore, a single plasma or serum cytokine measurement possibly could be used to characterize an individual's immune profile.

We had a relatively high interbatch coefficient of variation (~30%) in the current study. However, as the case-control pair samples were analyzed next to each other (randomized order) in the same batch, the interbatch variability will not have affected the case-control comparisons. In contrast, the intrabatch coefficient of variation was on average small (~7%). Another potential limitation is that after blood collection cytokines are being excreted from cells as an emergency response. Therefore, the time between blood collection and storage can be important. In our study, the time between blood collection and storage was relatively short (median, 4 h) and did not differ between cases and controls. We explored this issue further by excluding samples that were not stored within 12 hours of collection. These analyses resulted in observations similar to the main analyses. Although we could not control for some of the known risk factors for NHL (i.e., occupation exposures, viral infections, or some previous immune diseases), they are unlikely to have confounded our results, as the prevalence of these risk factors is low in the general population. Previous studies on polymorphisms in cytokine genes have shown that the effects of these genes were related to specific NHL subtypes. Unfortunately, our study was too small to investigate the association of cytokines with specific subtypes of NHL.

Blood cytokines are produced not only by those cell types considered to play pivotal roles in the immune system as well as in inflammatory responses, including lymphocytes, monocytes, and mast cells, but also by macrophages and, for some cytokines, also fibroblasts, neutrophils, and endothelial cells (26). So, it should be

noted that plasma level of cytokines may not necessarily reflect activity in the target tissue (lymph nodes). Lastly, although we found some evidence of cytokine levels being associated with NHL risk, it is important to notice that analyses of a large number of cytokines may produce statistically significant associations simply by chance. Moreover, due to small sample size of the study, it is possible that some associations between these immune system molecules and NHL were missed. It is therefore important that our findings are replicated in larger studies.

In conclusion, our findings suggest a possible association between plasma levels of IL2, ICAM, IFN-γ, and TNF-α with NHL risk and provided some evidence that risk of NHL might be related to a chronic inflammatory environment as well as a shift in the balance of Th1/Th2 cytokines.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

The EPIC study was funded by "Europe Against Cancer" Programme of the European Commission (SANCO), Italian Association for Research on Cancer, Italian National Research Council, and Compagnia di San Paolo. This work was supported by the Environmental Cancer Risk, Nutrition and Individual Susceptibility Network of Excellence, operating within the European Union 6th Framework Program, Priority 5: Food Quality and Safety (FOOD-CT-2005-513943). F. Saberi Hosnijeh acknowledges the Iranian Ministry of Health and Medical Education for support of a Ph.D. program at Utrecht University.

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Received 12/09/2009; revised 03/09/2010; accepted 03/22/2010; published OnlineFirst 05/25/2010.