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Household endotoxin levels and the risk of non-Hodgkin lymphoma

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Abstract

Objective—Endotoxin, a component of the outer membrane of gram-negative bacteria, elicits a strong innate and inflammatory immune response associated with secretion of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF- α). Because TNF- α polymorphisms that increase TNF- α production are associated with an increased risk of non-Hodgkin lymphoma (NHL), we hypothesized that increased levels of household endotoxin would be associated with an increased NHL risk.

Methods—We evaluated this association in the National Cancer Institute/Surveillance, Epidemiology and End Result (NCI/SEER) NHL multi-center population-based case-control study. Used vacuum cleaner bags were collected from participants during a home interview. Dust samples from the bags of 594 cases and 442 controls were analyzed for endotoxin (Endotoxin Unit [EU]/mg of dust) using the kinetic chromogenic *Limulus* amoebocyte lysate assay. Multivariable logistic regression was used to estimate the effect of endotoxin on NHL risk adjusted for age, sex, race, education, study center, and farm exposure.

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Results—Endotoxin was not associated with NHL overall (odds ratio [OR] for highest quartile of endotoxin levels = 0.81, 95% confidence interval [CI]= 0.55,1.20; *P* for trend=0.35), or with diffuse large B-cell lymphoma (OR= 0.63, 95% CI= 0.34, 1.16; *P*= 0.31) or follicular lymphoma (OR= 0.107, 95% CI=0.61, 1.89; *P*=0.73) subtypes. Both working and living on a farm were associated with higher household endotoxin levels compared to never working (*P*=0.009) or living (*P*=0.01) on a farm. Excluding farmers from the analysis did not change the results.

Conclusions—We found no evidence of a role for household endotoxin in NHL etiology.

Keywords

Endotoxin; Non-Hodgkin lymphoma; Epidemiology; Farming; Risk; Case-control

Introduction

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of tumors composed of B or T lymphocytes at various stages of development. The incidence of NHL in the U.S. doubled between 1970 and 1990 but has stabilized since the 1990s [1]. The etiology of NHL is not fully understood but altered immunity, especially acquired or inherited severe immune suppression, is an accepted risk factor [2]. In a large pooled analysis study of over 11,000 NHL patients and almost 12,000 controls, a functional polymorphism in the gene encoding for TNF- α , associated with increased TNF- α secretion, was associated with a 13% increased risk of NHL overall and a 29% increased risk of diffuse large B-cell lymphoma (DLBCL) [3, 4].

Endotoxin is a component of the outer membrane of gram-negative bacteria. It is common in the indoor environment and is principally associated with cigarette smoke, crowding, pets, household cleanliness, and geography in relation to the use of certain types of air conditioners and heating devices [5]. Endotoxin induces B-cell activation through a variety of pathways including stimulation of TNF- α production in monocytes and macrophages [6], although there is individual variation in the response attributable to genetic variation [7, 8]. In a comprehensive literature review, Lundin and Checkoway found evidence of a strong and consistent inverse association between endotoxin exposure and lung cancer risk that was dose dependent, along with suggestive evidence that risks for other cancers may be similarly reduced [9]. We hypothesized that endotoxin might increase the risk of NHL because of its capacity to induce TNF- α secretion, which in turn stimulates B-lymphocyte proliferation [10] (the cell of origin of the majority of lymphomas).

There is also some epidemiologic evidence indirectly linking endotoxin exposure to NHL risk. Endotoxin is associated with atopy [11], which is, in turn, inversely associated with NHL risk [12]. However, the timing of the endotoxin exposure determines whether the atopy association is inverse or positive. Early life exposure may be protective for atopy, while exposure at older ages can increase risk [13]. Persistent allergy is consistently inversely associated with NHL, but the relationship between NHL and childhood atopy that resolves by adulthood has not been carefully examined so the effect of age at onset is not known [12].

Farming is also associated with very high endotoxin exposure and farm homes have higher levels of endotoxin than non-farm homes [14] due to animal and plant exposure. Farming exposure is associated with asthma and atopy, and again, the timing of the exposure determines the direction of the relationship; early exposure is inversely associated, while late exposure is positively associated [15, 16]. Thus, late exposure to farming (e.g. occupational) should increase endotoxin exposure and therefore increase NHL risk. Indeed, in a multi-center population-based case-control study, we found that specific types of farm exposures,

especially those related to animals, were associated with an increased risk of NHL [17], supporting a role for endotoxin, possibly via B-cell activation. We and others found strong evidence that organochlorines measured in plasma, adipose tissue and in household vacuum dust are associated with NHL risk [18-20] and thus the farming association could be due solely to pesticides or to both pesticides and organic exposures like endotoxin.

Thus, because there is immunological and epidemiological evidence supporting a role for endotoxin and NHL risk, we evaluated the association using previously collected samples from a multi-center case-control study of NHL.

Methods

Study Population and Self-Reported Exposures

The study design has been reported in detail elsewhere [21, 22]. In brief, a population-based case-control study of NHL was conducted in four areas covered by Surveillance, Epidemiology and End Results (SEER) cancer registries: Detroit, Iowa, Los Angeles and Seattle to evaluate risk factors for NHL, including pesticides, medical history, sun exposure and diet. Eligible case patients were aged 20-74 years when newly diagnosed with NHL between July 1, 1998 and June 30, 2000. All cases were histologically confirmed and coded at each participating SEER registry according to the International Classification of Diseases – Oncology, 2nd edition and updated to the 3rd edition [23, 24]. Controls were selected from the general population, and random digit dialing was used to identify potential control participants under age 65 years and Center for Medicare and Medicaid Services files were used to identify potential control participants 65 years of age or older. Controls had no NHL diagnosis and were frequency-matched to cases by age (in five-year age groups), sex, race/ethnicity, and study center. Overall response rates were 59% for cases and 44% for controls, resulting in 1,321 cases and 1,057 controls enrolled in the parent study. All participants signed a written informed consent approved by the human subjects review board of each participating institution according to the Declaration of Helsinki in 1964 and modified in 2004.

Trained interviewers conducted a computer-assisted personal interview in each participant's home. A core set of questions administered to all participants ascertained information about demographics, height and weight, pesticide use, occupational history, hair dye use, number of siblings and birth order. Participants were also asked about whether they ever lived or worked on a farm and the number of years they lived on a farm. Smoking-related information was only obtained for approximately half of the participants due to the split questionnaire design and thus was evaluated in only half of the subjects.

Endotoxin Assessment

We took advantage of household dust collection for a sub-study that assessed the association between organochlorine compounds, including organochlorine pesticides and PCBs, and NHL risk [18]. Details of household dust collection, shipment, processing and storage are presented elsewhere [18]. Briefly, during the in-person interview, permission to remove the used bag from the household vacuum cleaner was obtained from participants. Those who possessed more than half of their carpets or rugs for less than 5 years or had not used a vacuum cleaner on carpets or rugs within the past year were excluded. [18]. Out of 1,233 cases and 1,004 controls who gave permission, 695 cases and 521 controls were eligible for dust collection [21]. Some participants with dust samples had insufficient dust remaining for this analysis as a result of previous analyses for organochlorine compounds and a small number of bags were lost during shipping, resulting in 618 cases and 460 controls providing vacuum cleaner bags with dust from which endotoxin levels were successfully measured.

Because response rates were extremely low among Hispanics [25], dust samples from this group were excluded, leaving 594 cases and 443 controls samples for this analysis.

Dust samples were sent to the University of Iowa to be analyzed at the Pulmonary Toxicology Facility (PTF). The details of the endotoxin protocol have been reported elsewhere [5, 26, 27]. Briefly, dust was sieved (425 μm), aliquoted into 100-mg lots, and frozen at -80°C . A 50-mg subsample of each dust sample was extracted with 1.0 ml pyrogen-free water containing 0.05% Tween-20 and analyzed for endotoxin using a modification of the kinetic chromogenic *Limulus* amoebocyte lysate assay with a 12-point standard curve. Five blank wells were included as negative controls for the purpose of quality control. Approximately 10% of the samples were selected randomly for a blinded replication and the Pearson's correlation coefficient between samples analyzed from two different aliquots was 0.89. We also examined the geometric mean and standard deviation of endotoxin levels among controls by assay batch using analysis of variance, and found that endotoxin levels did not significantly differ ($P=0.60$). Endotoxin concentrations were quantified as Endotoxin Units per milligram (EU/mg). Because the distribution of endotoxin concentration was log normal, the geometric mean and geometric standard deviation were used to indicate central tendency and variability.

Statistical Analysis

To evaluate potential selection bias, sociodemographic characteristics were compared between cases with and without endotoxin measurements, and the same comparisons were done between controls with and without endotoxin measurement. Because farm exposures (occupational/residential) are associated with increased levels of endotoxin [14] and are suspected risk factors of NHL [28], we also evaluated whether farming exposures were different between cases with and without endotoxin measurement, and between controls with and without measurement. In our study population, sociodemographic characteristics, farm exposures (occupational/residential) and vacuuming behavior were compared in cases and controls using either independent t-tests (for continuous variables) or Pearson's chi-squared tests (for proportions). One control subject with extremely high endotoxin levels was excluded from the analysis due to likely sampling error (e.g., bacterial growth in the vacuum bag), leaving 594 cases and 442 controls for this analysis. We also examined the effect of the demographic characteristics and farm exposures on endotoxin levels among controls.

In order to examine a possible threshold effect, endotoxin concentrations among controls were categorized into tertiles, with the highest tertile further split into two equal groups and proportions compared in cases and controls. Unconditional multivariable logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI). Median endotoxin concentrations in controls within each endotoxin category were used to conduct tests for linear trend. The effect of endotoxin levels considered as a continuous measure (per unit change in $\text{Ln}(\text{endotoxin})$ EU/mg) and risk of NHL was also evaluated using unconditional multivariable regression. The analyses were adjusted for the frequency-matched variables age (<45, 45-54, 55-64, 65 years), sex, education (12, 13-15, 16 years), race (white, black, Asian or other) and study center (Detroit, Iowa, Seattle, and Los Angeles). Additional potential confounders (e.g., smoking [5], allergy/asthma [29, 30], occupational and residential farm exposure (current, former, never) [14] were considered by assessing whether their inclusion changed the association with endotoxin exposure by at least 10%; only occupational farm exposure and frequency-matched variables were retained in the model. The effect of season of dust collection on endotoxin levels was also examined as there can be seasonal differences in household levels [31, 32]. The association between endotoxin and NHL risk was examined separately by sex, study site and major histological subtype (first for all NHL subtypes combined, and then separately for DLBCL, follicular

lymphoma, and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Endotoxin levels were assessed in cases and controls exposed and unexposed to farming. The associations were examined again excluding participants who had ever lived or worked on a farm to circumvent confounding by this exposure. Statistical significance was defined as having a *P* less than 5% for a two-sided hypothesis. All statistical analyses were conducted using the SAS Version 9.0 (SAS Institute, Inc., Cary, NC) statistical software package.

Results

Cases and controls with endotoxin measurements were older and more likely to be white than cases and controls without measurements (Supplementary Table 1). Seattle and Iowa had the highest proportion of eligible cases and controls, respectively; Detroit had the lowest. There was no evidence of an effect of season of specimen collection on endotoxin levels (data not shown). Controls with endotoxin measurements included in this sub-study were more likely to be current farmers (4.5%) or to have lived on a farm (40.7%) than controls without measurements (1.8% and 34.1%, respectively); there were no such differences for cases.

Cases were, on average, almost 2 years younger than controls (*P*= 0.008). Controls were more likely to live or work on a farm, formerly or currently, and for a longer time (>10 years, *P*=0.03) than cases (Table 1). Endotoxin levels ranged from 1.5 EU/mg to 1011.1 EU/mg in our samples; median levels in cases and controls were 60.7 EU/mg and 62.6 EU/mg, respectively.

Because endotoxin levels may be associated with working and living on a farm [14] and there are geographical differences in household endotoxin levels [5], we tested whether endotoxin levels were affected by these variables among controls. No significant relationship was observed for either working or living on a farm (data not shown). Endotoxin levels were significantly different across study centers, with the highest level in Seattle homes (*P*=0.0003, data not shown).

No association was found between household endotoxin levels and NHL, DLBCL or follicular lymphoma risk (Table 2). Exclusion of participants who had ever lived or worked on a farm did not alter the results (Tables 2). There were no major differences in risk between males and females (data not shown). There was no significant association between household endotoxin levels and CLL/SLL, based on 60 cases (data not shown). No differences were observed when endotoxin levels were modeled as a continuous variable (data not shown). When stratified by study center, a borderline significant inverse association with increasing endotoxin levels was observed in Iowa (*P*_{trend} = 0.05; excluding occupational or residential farm exposures, *P*_{trend}= 0.09) when modeling endotoxin as a categorical variable using tertiles (OR for the highest tertile = 0.57, 95%CI = 0.31-1.04). No association was observed in other centers (data not shown).

As expected, the interval since last vacuum bag change and the time since last vacuuming were significantly correlated ($r^2= 0.31$, *p*<0.0001). Endotoxin levels were lower among participants who replaced their vacuum cleaner bags at least 6 months prior to sample collection compared to those who changed the bags more frequently (data not shown). Among those who changed their bags least frequently, a borderline significant positive association between endotoxin and NHL was observed (OR per Ln(EU/mg) = 1.58, 95%CI = 0.92-2.74, data not shown), while overall no association was observed among those who changed the vacuum cleaner bags more frequently (data not shown).

Discussion

This is the first epidemiologic study to investigate the association between measured levels of endotoxin in households and NHL risk. Endotoxin has been inversely associated with lung and other cancers [9]. Because endotoxin stimulates B-cell activity, via an increase in TNF and other cytokines [6], and B-cell activation is a presumed risk factor for NHL [3, 4, 33], we expected that endotoxin exposure would also increase NHL risk. Instead, we observed no association between household dust endotoxin levels and risk of all subtypes of NHL combined, DLBCL, follicular lymphoma or CLL/SLL. There are several possible reasons for our null finding. Endotoxin levels in house dust do not reflect exposures in the workplace, which are likely important sources of endotoxin exposure [29]. In addition, the response of lymphocytes and macrophages to endotoxin is variable and depends on host factors, such as age, sex and genetic variation in immune response genes, that affect the ability of endotoxin to activate B-cells [34, 35]. Sex and age were accounted for by the adjustment in the multivariable model. Polymorphisms in cytokine genes may also contribute to the inter-individual variation in responsiveness to endotoxin [7, 8], but such genetic influence was not evaluated in our study. Smoking is another factor that may affect household endotoxin levels and was evaluated as a potential confounder in subgroup analysis; effect estimates (odds ratios) remained very similar with adjustment of smoking.

We found the highest levels of endotoxin in homes in Seattle and the lowest in Detroit. The reason for this difference by study center is not known. The comparatively moist weather in Seattle relative to the other centers probably does not account for the higher endotoxin levels since outdoor moisture is not a significant predictor of endotoxin levels [5]. Another possible explanation for the difference is endotoxin exposure from dogs, since dog ownership has been associated with higher home endotoxin levels [36], and Washington state has the highest prevalence of households with dogs compared to the other study center locations (US Pet Ownership & Demographic Sourcebook, 2007, www.avma.org) (information on dog ownership was not collected in our study). Note that dog ownership has been associated with a decreased NHL risk [37, 38]).

The geometric mean (GM) household endotoxin concentration of 61.8 EU/mg in our study is comparable with the value for family room floors in the National Survey of Endotoxin in U.S. Housing (GM=71.1 EU/mg; 95% CI=64.3-78.7 EU/mg) [5]. Samples from both studies were analyzed in the same laboratory according to the same protocol but comparisons are difficult to make because we used dust collected from participants' vacuum cleaner bags in a cross-sectional sampling and the National Survey used room-specific dust collected from a designated area into sampling thimbles by field technicians.

There was a weak inverse association among cases and controls in Iowa, where 94% of the current and 73% of the former farmers were located. Dust endotoxin levels were higher among both cases and controls exposed to farming. However, farmers were over-represented in the study as a whole and among eligible controls compared to cases (Supplementary Table 1). Nevertheless, results were unchanged when lifetime farmers were excluded from the analysis restricted to Iowa, and from the analysis for the combined study centers, so it is unlikely that this potential confounder actually biased the results.

The strengths of our study include gold standard laboratory methods for measuring endotoxin levels in dust by the group that performed the only US nationwide study [5, 14, 39], a population-based ascertainment of participants, and a relatively large sample size. However, our study has several limitations. We used a single measurement of endotoxin using dust collected from vacuum cleaner bags. From contemporaneous sampling in five locations within households, Thorne et al. [39] demonstrated low correlations for paired

samples, with Pearson correlation coefficients ranging from 0.12 to 0.44 (N=2,469); thus reproducibility could be poor and misclassification introduced. However, because we did not specify the locations (e.g. living room, kitchen, or bedroom) where vacuum cleaners were used, endotoxin levels measured from dust in vacuum cleaner bags may represent an integrated measurement representative of the entire home, which could be less subject to measurement variability due to different sampling locations. A single sampling may be used as a reasonable proxy to reflect about 1-year household endotoxin exposure [31, 40] while it may fail to reflect long-term exposure (e.g. up to 6 years) [41]. Thus, a single measurement would be an invalid measure of historical exposure if endotoxin levels changed over time. We also found that persons who changed their vacuum bags infrequently had lower levels of endotoxin than those who changed them frequently but we did not have information on frequency of vacuuming. The association between frequency of vacuuming and endotoxin levels is not clear [42], however in our study, it is possible that persons who changed their bags infrequently did so because they did not have much surface area to vacuum, and therefore had lower endotoxin levels. Dust samples were collected from NHL cases after diagnosis, and it is possible that disease led to changes in lifestyle that, in turn, led to changes in household endotoxin levels. We tried to minimize these potential problems by limiting the dust analysis to people who had owned their carpets or rugs for at least 5 years. Seasonal variation in endotoxin concentrations sampled from dust has also been reported [31, 32], but we found no evidence of an effect of season of specimen collection on endotoxin levels in our study. As above, the lack of information on occupational endotoxin exposure could have led to null results via non-differential misclassification, however the majority of the jobs listed in our questionnaires are not significantly linked to endotoxin exposure, and thus it was not possible to correct for occupational endotoxin exposure.

In summary, we did not find an association between endotoxin levels in carpet dust measured at the time of diagnosis and risk of NHL. Cohort studies with pre-diagnosis dust specimens, multiple longitudinal measurements and detailed residential and occupational histories may help to clarify the relationship.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

LPS	lipopolysaccharide
TNF-	tumor necrosis factor

NCI	National Cancer Institute
SEER	Surveillance, Epidemiology and End-Results program
NHL	non-Hodgkin lymphoma
EU/mg	Endotoxin units per milligram
OR	Odds ratio
CI	confidence interval
DLBCL	diffuse large B-cell lymphoma
PTF	Pulmonary Toxicology Facility
Ln	natural logarithm

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Table 1Characteristics of cases and controls ^a.

	Cases N=594	Controls N=442	P
Mean Age (years)	58.8 ± 11.3	60.6 ± 10.8	0.008
Age (years)			0.001
<45	75 (12.6)	44 (9.9)	
45-54	126 (21.2)	73 (16.5)	
55-64	165 (27.8)	101 (22.9)	
65	228 (38.4)	224 (50.7)	
Sex			0.52
Male	321 (54.0)	230 (52.0)	
Female	273 (46.0)	212 (48.0)	
Race/Ethnicity			0.21
Non-Hispanic White	531 (89.4)	389 (88.0)	
African American	36 (6.1)	38 (8.6)	
Others	27 (4.6)	15 (3.4)	
Study Center			0.07
Detroit	113 (19.0)	57 (12.9)	
Iowa	175 (29.5)	135 (30.5)	
Los Angeles	133 (22.4)	107 (24.2)	
Seattle	173 (29.1)	143 (32.4)	
Education (years)			0.64
12	223 (37.5)	164 (36.9)	
13-15	205 (34.5)	143 (32.6)	
16	166 (28.0)	135 (30.5)	
Occupational farming ^b			0.15
Never	536 (90.5)	393 (88.9)	
Former	42 (7.1)	29 (6.6)	
Current	14 (2.4)	20 (4.5)	
Years lived on a farm ^b			0.03
Never	82 (64.4)	262 (59.3)	
0-5	60 (10.1)	37 (8.4)	
6-10	27 (4.6)	16 (3.6)	
>10	124 (20.9)	127 (28.7)	
Histology			
Follicular	137 (23.1)		
DLBCL	182 (30.6)		
T-cell	40 (6.7)		
Others/Unknown	235 (39.6)		
Last change of vacuum cleaner bag ^b			0.66
< 1 month	220 (37.5)	152 (35.0)	

	Cases N=594	Controls N=442	P
1 month range < 6 months	305 (52.0)	238(54.8)	
6 months	62 (10.6)	44 (10.1)	
Last use of vacuum cleaner			0.92
<1 week	429 (72.2)	322 (72.8)	
1 week range < 1 month	142 (23.9)	105 (23.8)	
1 month	23 (3.9)	15 (3.4)	

Abbreviation: DLBCL, diffuse large B-cell lymphoma.

^aMean (SD) was presented for continuous variable and N (%) for categorical variables.

^bTotal may vary due to missing values.

Table 2

Endotoxin levels in carpet dust and risk of Non-Hodgkin's lymphoma diffuse large B-cell lymphoma (DLBCL), and follicular lymphoma, with endotoxin levels modeled as a categorical variable.

Endotoxin (EU/mg)	Controls		All NHL cases		DLBCL Cases		Follicular Cases	
	N		N	OR (95% CI) ^a	N	OR (95% CI) ^a	N	OR (95% CI) ^a
<47.08	147		220	1	65	1	53	1
47.08 and <90.47	147		194	0.89 (0.66, 1.22)	58	0.85 (0.54, 1.32)	38	0.74 (0.45, 1.22)
90.47 and <140.11	74		95	0.94 (0.64, 1.37)	38	1.24 (0.74, 2.09)	18	0.73 (0.39, 1.36)
140.11	74		85	0.81 (0.55, 1.20)	21	0.63 (0.34, 1.16)	28	1.07 (0.61, 1.89)
<i>P</i> for trend				0.35		0.31		0.73
Excluding Participants Who Ever Lived or Worked on a Farm								
<47.08	93		115	1	49	1	33	1
47.08 and <90.47	90		114	0.77 (0.52, 1.14)	36	0.73 (0.42, 1.27)	22	0.68 (0.36, 1.30)
90.47 and <140.11	41		60	0.98 (0.60, 1.61)	22	1.15 (0.59, 1.26)	12	0.90 (0.40, 2.01)
140.11	37		48	0.83 (0.49, 1.40)	14	0.80 (0.37, 1.71)	15	1.19 (0.55, 2.56)
<i>P</i> for trend				0.65		0.83		0.53

Abbreviations: NHL, non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; OR, Odds Ratio; 95% CI, Confidence Intervals.

^a Adjusted for age (<45, 45-54, 55-64, 65), sex, race/ethnicity (non-Hispanic white, African American, others), education (12, 13-15, 16), study center (Seattle/Detroit/Iowa/Los Angeles), and (for first set of results only) farming occupation status (never/former/current).