

CEBP Focus: Update on Lymphoma

Exposure to Solvents and Risk of Non-Hodgkin Lymphoma: Clues on Putative Mechanisms

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Abstract

Malignant lymphomas are a group of diseases of uncertain etiology. Both environmental factors and genetic susceptibility have been reported as risk factors. We have conducted a population-based case-control study in Italy: all newly diagnosed cases of malignant lymphoma, in males and females ages 20 to 74 years in the 1991 to 1993 period, were identified; the control group was comprised of a random sample of the general population resident in each of the areas under study, stratified by sex and 5-year age groups. Overall, 1,428 non-Hodgkin lymphoma (NHL) cases and 1,530 controls were interviewed. Experts from each geographic area examined questionnaire data and assigned a level of probability and intensity of exposure to a range of chemical groups and individual chemicals. For those in the medium/high level of exposure, there was an increased risk of NHL for exposure to benzene, xylene,

and toluene. We have examined the hypothesis that the effect of solvents is related to their immunotoxicity by analyzing the interaction with a previous history of autoimmune disease. We have found an apparent, though not statistically significant, increased risk of NHL in those with both exposure to benzene and a history of autoimmune disease (odds ratio, 16.3; 95% confidence interval, 0.8-321). In addition, an odds ratio of 29.8 (95% confidence interval, 1.4-650.2, based on nine exposed cases) was associated with high-level exposure to benzene in those with a positive family history of malignant hematologic neoplasms. Both hypotheses (i.e., the interaction with autoimmune diseases and with familial predisposition) indirectly suggest that an immunologic mechanism could be involved in lymphomagenesis from solvents. (Cancer Epidemiol Biomarkers Prev 2007;16(3):381-4)

Introduction

Malignant lymphomas are a group of diseases of uncertain etiology. They are malignancies of the cells of the immune system; thus, it seems suggestive that immunologic conditions may be related to the occurrence of this group of cancers. A more than 100-fold increased risk of non-Hodgkin lymphoma (NHL) has been described among renal and cardiac transplant recipients receiving immunosuppressive therapy even after only 1 year of latency (1). An increased NHL incidence is also found among subjects with primary immunodeficiency (e.g., ataxia telangiectasia), acquired immunodeficiency (HIV), and autoimmune diseases (e.g., lupus erythematosus, coeliac disease, and Sjögren syndrome; refs. 2, 3). Other autoimmune diseases (such as, e.g., diabetes type 1, multiple sclerosis, or rheumatoid arthritis) have been associated with the risk of NHL in some studies but not in others (2, 3). In relation to environmental exposures, recent studies have suggested an association between NHL and exposure to organic solvents (4-7). Organic solvents represent a class of chemicals that have been used in many industrial sectors, and exposure is also widespread among the general population. We have published in detail, elsewhere, the associa-

tion between organic solvents and NHL, based on a large population-based multicenter case-control study in Italy (8). Solvents are suspected of exerting an immunotoxic effect (9).

Familial/genetic susceptibility has also been suggested. In one recent study, the risk of NHL was elevated in relatives of NHL cases [hazard ratio, 2.9; 95% confidence interval (95% CI), 0.95-8.53]; the aggregation seemed to be stronger for siblings (hazard ratio, 7.6; 95% CI, 0.98-58.8) and for male relatives (hazard ratio, 6.2; 95% CI, 0.77-50.0; ref. 10). These results are consistent with previous findings of familial aggregation of NHL. Evidence on genetic susceptibility for NHL is still scanty, except for some specific genetic syndromes. These fall into five main categories: DNA repair defects (e.g., Bloom, Fanconi, and Ataxia telangiectasia), defects leading to secondary mutations, tumor suppressor defects (e.g., Down syndrome and Li-Fraumeni), immunodeficiency (e.g., X-linked lymphoproliferative disorder), and defects in apoptosis (11). Both familial aggregation and the mechanisms involved in familial syndromes shed light on potential pathways involved in lymphomagenesis and can contribute to the identification of candidate genes. Currently, the search for candidate genes points mainly to factors regulating immune function. Several genes have been proposed, most coding for inflammatory and regulatory cytokines (*TNF*, *IL- α 1*, *IL-1 β* , *IL-1RN*, *IL-6*, and *LTA*), *Th1/Th2* cytokines (*IL-10*, *IL-4*, and *IL-4RA*), or chemokines (*IL-8*, *CCR2*, *CCR5*, and *SDF-1*) and others involved in innate immunity (myeloperoxidase, *MPO*).

The present study examines hypotheses regarding the putative mechanisms of action of solvents in lymphomagenesis, based on a large multicenter case-control study conducted in Italy. Because the main hypothesis concerning the mechanism of action of solvents in lymphomagenesis

Received 12/19/06; accepted 12/21/06.

Grant support: National Cancer Institute grant CA51086, European Community (Europe against Cancer Programme), and Italian Alliance against Cancer (Lega Italiana per la Lotta contro i Tumori).

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doi:10.1158/1055-9965.EPI-07-0124

has been immunotoxicity, we focused on the interaction between autoimmune disease and solvents. We also examined effect modification by family history of hematolymphopoietic malignancy.

Materials and Methods

We have conducted a population-based case-control study in 11 areas in Italy. We included all newly diagnosed cases of NHL that occurred during the period 1991 to 1993 among residents aged 20 to 74 years. Eight of the 11 areas were chosen because of the presence of manufacturing industries where solvents have been largely used. In each center, NHL cases were identified through periodic surveys of the hospital and pathology departments where such cases are diagnosed and treated and in some hematologic centers in Milan, Pavia, Bologna, and Rome, outside the areas under study. In Varese, all cases were identified through the Varese Cancer Registry. As a result of this systematic case ascertainment, it is unlikely that many cases were lost. NHL cases were classified following the Working Formulation.⁴ If the original diagnosis was outside the Working Formulation definitions, the cases were reclassified within the Working Formulation by a pathologist experienced in the field. Following the Working Formulation, chronic lymphocytic leukemia cases were grouped in the "small lymphocytic consistent with chronic lymphocytic leukemia" category. All doubtful NHL diagnoses (i.e., diagnoses for which the local pathologist had expressed uncertainties about the allocation to a specific NHL category) and, additionally at random, one case of five were submitted to a panel of three pathologists to validate diagnoses. In total, 334 cases were reviewed, and the NHL diagnosis was confirmed for all cases.

The control group was comprised of a random sample of the general population, resident in each of the areas under study, stratified by sex and 5-year age groups. The size of the control group was somewhat greater than the largest diagnostic group (NHL and chronic lymphocytic leukemia cases combined). Details on case ascertainment and recruitment and control sampling procedures have been described previously (8).

We conducted in-person interviews primarily at the interviewee's home to obtain information on education; relevant lifestyle factors, such as tobacco smoking and beverage consumption; a detailed occupational history (including detailed data on specific jobs through the use of a job-specific questionnaire); extra-occupational exposure to solvents and pesticides; hair-dye use; lifelong residential history; previous diseases; use of diagnostic or therapeutic X-rays; specific medications; family medical history; and reproductive history. The medical history contained detailed questions on several autoimmune diseases, including date of diagnosis. We focus here on a history of lupus, rheumatoid arthritis, and scleroderma.

The approach used in this study to assess exposure used job- or industry-specific questionnaires with subsequent expert ratings to assign exposure to a defined list of agents. Industrial hygiene experts from each geographic area examined questionnaire data and assessed the level of probability, and intensity, of exposure to groups or classes of solvents as well as certain individual substances. The assessors were blind to case-control status. The experts made exposure judgments for

the following categories: a general category "solvents" covering all substances used as solvents and specific chemical classes (aromatic hydrocarbons, chlorinated hydrocarbons, aliphatic hydrocarbons, and oxygenated derivative hydrocarbons). Furthermore, when the information was sufficient to assess individual chemicals, they made exposure judgments for aromatic hydrocarbons (benzene, styrene, xylene, and toluene), among chlorinated hydrocarbons (dichloromethane, tetrachloroethylene, trichloroethylene, 1,1,1-trichloroethane, and chloroform), and among oxygenated derivative hydrocarbons (1,4-dioxane).

Exposures were rated on two scales: "probability" represented the certainty/uncertainty of exposure based on knowledge of the materials used and technologies applied in the particular activity/production process reported in a given calendar period. It was classified into three levels: low, medium, and high. The second scale was "intensity," which represented the estimated concentration of the agent in the work environment and was measured on a four-point scale: very low, low, medium, and high. The first class of intensity ("very low") was used for subjects judged to have occupational exposure intensities comparable to the upper end of the reference range of exposures for the general population. "Low level" intensity was assigned when workplace exposure was judged to be low because of control measures but nevertheless higher than in the general population. Medium exposure was assigned to subjects who worked where only moderate or poor control measures were judged to exist. The highest category was used for subjects who worked in workplaces with no control measures. To ensure a standardized approach, the assessors were centrally trained prior and periodically during their independent evaluations of questionnaires. Furthermore, to reduce the exposure assessment variability of the different experts, a job-exposure matrix was developed, collecting the minimum overall consensus for the most frequent job titles/sectors in the study areas, and this was used as baseline for the individual exposure assessment. Participants were asked to provide a family history of illness (including cancer) among first-degree relatives. Illness type was reported in an open-ended response. We asked also age at diagnosis for each illness and the cause of, and age at, death of respondent's mother and father.

Data Analysis. Data analyses were done with SAS (SAS Institute, Cary, NC). Odds ratios (OR) and the corresponding 95% CI were calculated. All analyses were done using multiple logistic regression models, taking into account relevant potential confounders (sex, age, area, and education level). Subjects who were never exposed to any of the listed chemicals were used as the referent population.

Table 1. Exposure to solvents with probability level and exposure level >1 (see text)

	Exposed cases	Exposed controls	OR* (95% CI)
Solvents			
AI yes	10	5	3.0 (0.7-13.1)
AI no	268	254	1.1 (0.9-1.4)
Aromatic			
AI yes	5	3	1.7 (0.2-13.5)
AI no	164	154	1.1 (0.8-1.4)
Benzene			
AI yes	4	1	16.3 (0.8-321.6)
AI no	78	74	1.0 (0.7-1.4)

Abbreviations: AI, autoimmune diseases (lupus, rheumatoid arthritis, and scleroderma).

*OR adjusted by sex, age, center, and educational level.

⁴ For details, see http://training.seer.cancer.gov/ss_module08_lymph_leuk/lymph.

Results

We were able to interview personally 84.6% of NHL cases and 96.5% of controls in the eight areas where exposure assessment for solvents was completed and interviewed the remainder by proxy (for details, see ref. 8). For proxy respondents, we have evaluated the distribution of some confounding factors between direct responders and proxies. We observed that the distribution by sex, smoking, and educational level was very similar in the two groups (8).

Analyses for specific chemical substances revealed elevated NHL risks associated with medium/high exposures to toluene (OR, 1.8; 95% CI, 1.1-2.8), xylene (OR, 1.7; 95% CI, 1.0-2.6), and benzene (OR, 1.6; 95% CI, 1.0-2.4). Table 1 shows the distribution of exposure to selected solvents and a history of previous autoimmune diseases. We excluded one center (Imperia) because the prevalence of rheumatoid arthritis was not credible and was much higher than in population surveys. Table 1 shows an apparent, though not statistically significant, increased risk of NHL in those with both exposure to benzene and a history of autoimmune disease. Table 2 shows the interaction between solvent exposure and a family history for a malignancy of the hematolymphopoietic system. A strong and statistically significant association is shown, in particular with benzene (OR, 29.8; 95% CI, 1.4-650.2), for exposure intensity greater than "low." Interactive terms between benzene exposure and autoimmune disease or family history were not statistically significant ($P > 0.05$).

Mechanistic Hypothesis. In the present study, the data suggest that solvent-related lymphomagenesis can be modulated by a history of autoimmune diseases and by a family history of hematolymphopoietic malignancies, the first observation of such possible modulatory effects.

The IARC has classified benzene as carcinogenic to humans (group 1) based on an increased risk of hematopoietic malignancies, notably acute myeloid leukemia, but not NHL. Among the same group of aromatic hydrocarbons, styrene was classified as possibly carcinogenic to humans (group 2B); xylene and toluene were not classifiable as to their carcinogenicity for humans (group 3). Among chlorinated hydrocarbons, trichloroethylene and tetrachloroethylene were both classified by IARC as probably human carcinogens (group 2A); dichloromethane was recently evaluated as possibly carcinogenic to humans (group 2B); 1,1,1-trichloroethane was considered not classifiable as to its carcinogenicity to humans.⁵

The main hypothesis concerning the mechanism of action of solvents in lymphomagenesis has been immunotoxicity. Immunotoxicity studies on organic solvents suggested that professional and/or environmental exposure may determine effects on the immune system of humans. Among aromatic solvents, most of the studies referred to benzene. This chemical may affect both humoral and cellular acquired immunity in animals. The Agency for Toxic Substances and Disease Registry reported in a review on benzene (9) that an association between benzene exposure and adverse effects on functional immunity is supported by the literature; in particular, benzene determines an immunosuppressive response. Benzene has a greater depressive effect on T lymphocytes than B cells (12). Biro et al. found that solvents, such as benzene and styrene, activate peripheral lymphocytes and cause changes in the prevalence of CD25⁺/CD4⁺ T lymphocytes (13). In a study among workers exposed to benzene concentrations lower than 15 ppm, depression of the

Table 2. Cases and controls exposed to solvents with probability level >1 and exposure level >1, by family history (at least one hematolymphopoietic malignancy in the first-degree relatives)

Family history	Exposed cases	Exposed controls	OR* (95% CI)
Solvents			
No	284	284	1.1 (0.9-1.4)
Yes	18	9	1.4 (0.3-5.2)
Benzene			
No	85	86	1.0 (0.7-1.4)
Yes	10	3	4.2 (0.6-31.8) [†]
Xylene			
No	94	102	1.0 (0.7-1.4)
Yes	6	5	2.1 (0.3-16.2)
Toluene			
No	100	105	1.1 (0.8-1.5)
Yes	7	5	2.5 (0.3-19.0)
BTX			
No	61	61	1.0 (0.7-1.5)
Yes	5	3	2.5 (0.3-22.7)

Abbreviation: BTX, benzene, toluene, and xylene.

*OR adjusted by sex, age, center, and educational level.

[†]For exposure level > 2 ("low"): family history, yes; exposed cases, 9; exposed control, 1; OR, 29.8; 95% CI, 1.4-650.2.

circulating B-lymphocyte level was induced (14). Other disparate and immunologic effects in workers exposed to solvents have been described (15-18). In a study in mice, immunotoxicity induced by benzene treatment alone was characterized by involution of thymic mass and suppression of both B- and T-cell mitogenesis (19). This study also suggested that toluene, in sufficient amounts, may have an antagonistic effect on benzene immunotoxicity in mice (19).

We have tested in our data set a new hypothesis (i.e., an interaction between a history of autoimmune disease and solvent exposure). We found a possible interaction between a history of autoimmune diseases and solvent exposure, which suggests that already existing immunologic impairment can increase the risk of NHL in subjects exposed to solvents and, in particular, to benzene. However, the association needs to be confirmed; biological plausibility is also currently lacking, as the immunosuppressive effect of solvents would be expected to attenuate the manifestations of autoimmune disease. We found also a stronger association between exposure to benzene and NHL in the presence of a family history of hematolymphopoietic malignancies. Most hypotheses concerning genetic predisposition to NHL, in fact, refer to genes involved in immune function. Thus, genetic predisposition to benzene-related lymphomagenesis deserves further investigation.

Appendix A

The following are the members of the Working Group: Alessandra Benvenuti, David Kriebel, Vanessa Bolejack, Rosario Tumino, Valerio Ramazzotti, Stefania Rodella, Emanuele Stagnaro, Paolo Crosignani, Dino Amadori, Dario Mirabelli, Letizia Sommani, Isabella Belletti, Loredana Trotschel, Luciano Romeo, Giuseppe Miceli, Giulio Andrea Tozzi, and Igino Mendico.

Acknowledgments

We thank S. Alberghini Maltoni, S. Barcellini, G. Barni, L. Bellesini, V. Cacciarini, R. Carlini, M. Casale, G. Castellino, G. Cremaschi, L. Davico, A. Fiorio, R. Gibilisco, L. Guzzo, R. Hirvas, S. Legrotti, L.

⁵ <http://www-cie.iarc.fr/>

Migliaretti, R. Monteleone, G. Osella, T. Palma, G. Panizza, C. Picoco, G. Piergiovanni, G. Righetti, R. Sguanci, M. Tedeschi, D. Tiberti, G. Tonini, P. Trada, T. Vescio, and M. Zanetta for their cooperation; Giuseppe Gorini and Angela Veraldi for their contribution and suggestions; and the members of the panel of pathologists (S. di Lollo, L. Fiore Donati, U. Magrini, F. Menestrina, D. Novero, G. Palestro, A. Paolucci and M. Paulli) and the clinical department staffs for their involvement in patient recruitment.

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