Non-Hodgkin's Lymphoma and Type of Tobacco Smoke

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Abstract

Background: In recent decades, the incidence of non-Hodgkin's lymphoma (NHL) has increased in all industrialized countries. Tobacco smoke contains several recognized or putative carcinogenic compounds that differ in concentration depending on which of the two main types, blond or black, is consumed. This investigation sought to evaluate the association between NHL and type of tobacco smoked (blond, black, or mixed), focusing on the Working Formulation (WF) subgroups. Methods: Reanalysis of Italian data from a recent multicenter population-based case-control study. The 1450 cases of NHL and 1779 healthy controls from 11 Italian areas with different demographic and productive characteristics were included in the study, corresponding to ~7 million residents. Odds ratios (ORs) adjusted for age, gender, residence area, educational level, and type of interview were estimated by unconditional logistic regression model. Results: A statistically significant association [OR = 1.4, 95% confidence interval (CI) 1.1–1.7] was found for blond tobacco exposure and NHL risk. A dose-response relationship was limited to men younger than 52 years (χ^2 for trend = 9.95, $P < 0.001$). Subjects starting smoking at an early age showed a higher risk in men younger than 65 years, whereas no clear trend was evident for the other age and gender subgroups. The analysis by WF categories showed the highest risks for follicular lymphoma in blond (OR = 2.1, 95% CI 1.4–3.2) and mixed (OR = 1.8, 95% CI 1.1–3.0) tobacco smokers and for large cell within the other WF group (OR = 1.6, 95% CI 1.1–2.4) only for blond tobacco. Conclusion: Smoking blond tobacco could be a risk factor for NHL, especially follicular lymphoma. (Cancer Epidemiol Biomarkers Prev 2004;13(3):431–437)

Introduction

In the last two decades, the risk of non-Hodgkin's lymphoma (NHL) has rapidly increased in all industrialized countries (1). In Europe, the annual mean percentage change in incidence, estimated by pooling data from eight cancer registries, has risen to +4.8% in males and +3.4% in females (2). Furthermore, the analysis by subcategory showed that such an increase was confined to specific subtypes (*i.e.*, follicle center cell, extranodal B-cell, nodal T-cell, and nodal lymphoma not otherwise specified; 2).

Acquired or congenital immunodeficiency, autoimmune disorders, some viral infections, agriculture chemicals, sunlight radiation, and occupational exposure to solvents, including benzene, are recognized or suspected risk factors for NHL (3). However, the etiology of the disease is still largely unknown, and no single responsible factor has yet been identified to explain the observed increase (3). Furthermore, improvements in diagnostic techniques are thought to have played only a marginal role in influencing the NHL time trend (2).

In the last 10 years, many epidemiological studies have reported cigarette smoking as a possible risk factor for NHL, especially for the follicular subtype, but this evidence has been questioned (4). Some case-control studies performed in areas with prevalence of blond tobacco (*e.g.*, United States) failed to find any association (5–8), even if bias toward null could not be completely excluded (4), while a positive association was found in other investigations (9, 10). In addition, evidence from cohort studies, carried out in the same areas, remains controversial (11–14). Recently, a large case-control study of 11 Italian areas confirmed the association between smoking habit and follicular NHL (15). Moreover, the lack of any association with the risk of diffuse large B-cell lymphoma (the most common subtype of NHL) suggested that such a finding is unlikely to be a spurious observation due to recall bias (15).

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Tobacco smoke contains about 3800 different chemicals, comprising many carcinogens (16), and their presence is sufficient to explain the induction of cancer in man (17). However, exposure from cigarette smoking depends on the type of tobacco, varieties of which may contain chemical carcinogens at different concentrations. In fact, aromatic amines and nitrous compounds (including nitrosamines) are present at higher concentrations in black (air-cured) tobacco smoke, while benzene and polycyclic aromatic hydrocarbons (PAHs) are more concentrated in blond (flue-cured) tobacco smoke (18). These different concentrations of carcinogenic compounds have been indicated as possible responsible factors for different risks for bladder (19–21) and lung (22–24) cancers in subjects smoking different cigarette brands.

This investigation aimed to evaluate the association between type of tobacco smoke and NHL, with particular attention focused on the follicular subtype, by reanalyzing data from the large multicenter case-control study by Stagnaro *et al.* (15).

Materials and Methods

Information about 1450 cases of NHL, diagnosed in 1990–1993, and 1779 healthy controls were extracted from a recent large population-based case-control study carried out to assess the association between hematolymphopoietic malignancies and several putative risk factors, including smoking habit (15). Eleven Italian areas with different demographic and productive characteristics were included in the study (*i.e.*, the Torino municipality and the provinces of Firenze, Forlì, Imperia, Latina, Novara, Ragusa, Siena, Varese, Vercelli, and Verona), corresponding to ~7 million residents (25). Incidence cases of histologically confirmed NHL (*International Classification of Diseases*-9 codes 200 and 202; 26) were identified through a systematic search in all public and private hospital departments (hematology, specialist and general medicine, and surgery) and in specialist hospital outside the areas (National Cancer Institute in Milan and in Genoa, Universities of Pavia and Genoa, and hospitals of Rome, Bologna, and Genoa), where such patients could be admitted. Departments of pathology and cancer registries in the study area were also considered for recruitment. NHL cases were classified by a single experienced pathologist according to the Working Formulation (WF; 27), with the Revised European-American Lymphoma classification (28) still being unavailable. Patients aged 20–74 years were considered eligible for the analyses. Cases with a medical history of HIV were excluded from the study.

The control group, matched by area, calendar period, sex, and age (±5 years), was selected by random sampling of the resident population, carried out each July first in each matched year. In Florence, Forlì, and Ragusa, the computerized and regularly updated municipal electronic demographic files were used. In the other areas, where demographic files were not readily available, control subjects were extracted from the database of the National Health Service, which is updated every 6 months and covers virtually the whole resident population. Both data files are well validated as epidemiological data sources and accepted by the *Journal of the National Cancer Institute* committee that revised the protocol.

Data were collected from person-to-person interviews lasting ~1 h. To participate in the study, each subject gave his/her informed consent at the beginning of the interview. Interviewers were trained through a residential 3-day course (care of Siena University) and provided with a specific manual. When subjects were deceased or too ill to be interviewed, close relatives were interviewed instead. Interviews preferably took place in the subject's home and the same protocol was adopted for both cases and controls. Personnel in charge of case ascertainment was excluded from the collection and treatment of information on exposure to ensure interview blinding. Each interviewer had the same proportion of cases and controls. A standardized questionnaire was used to obtain information about sociodemographic characteristics and the following known or suspected risk factors: occupation, solvents and pesticides, X-rays, specific medications, family medical history, lifelong residential history, previous disease, reproductive history (for women only), beverage consumption, and tobacco smoking. Smoking history included cigarette brand, date of starting and stopping smoking, number of cigarettes smoked, and use of filters. Brands were classified as type of tobacco (blond, black, and unknown) based on information collected from the Italian monopoly of tobacco producers. Eleven cigar and pipe smokers were excluded from the analysis due to the small sample size. People who have smoked also cigars and/or a pipe numbered 45 among cases and 54 among controls. More details about the questionnaire and interview procedures have been published elsewhere (15).

Subjects who stopped smoking within the year prior to inclusion in the study were considered as current smokers due to the tendency of patients in poor health conditions to stop smoking. A three-level intensity-by-duration index (pack-years) was constructed by multiplying the number of packs smoked daily by the number of years of smoking according to the tertile distribution of smoking in controls. Point estimates of odds ratio (OR) and the corresponding 95% confidence intervals (CIs) associated with smoking were computed, adjusting for relevant potential confounders (age, gender, area, education level, and respondent type) by unconditional logistic regression model (29). All analyses were performed using SPSS statistical package for personal computers (30).

Results

The eligible population included 1778 cases and 2391 controls. The interviews were carried out for 1450 cases (82%) and 1779 controls (74%): 10% and 19% of subjects refused the interviews and 8% and 7% were not found, respectively.

The proportion of interviews conducted directly at home and in the same period were 67% for cases and 75% for controls, with 18% and 17% missing, respectively. Next of kin were interviewed for 18% of cases and 4% of controls. A history of smoking (at least 1 cigarette/day for 1 year) was reported for 79% men and 35% women.

Among smokers who began smoking blond tobacco (n = 935), 84.0% have continued to use the same type of

tobacco, while only 32.1% among persons starting with black tobacco (n = 822) have done the same, thus meaning that 39.4% have smoked both types of tobacco during their life. No information about the type of cigarette smoking was available for 51 subjects (21 cases and 30 controls).

Table 1 shows the distribution of cases and controls by gender, age, residence area, education level, and type of interview.

Table 2 shows the results of analysis of NHL risk by type of cigarette tobacco. Blond tobacco showed a stronger association with NHL risk than did black tobacco in ever smokers (OR = 1.4, 95% CI 1.1–1.7 and OR = 0.84, 95% CI 0.61–1.2, respectively), while persons smoking the two types combined (mixed) displayed an intermediate risk (OR = 1.2, 95% CI 0.91–1.5). The same pattern was observed splitting smokers into former and current, but no significant differences were evident between the two groups. No clear dose-response trend was highlighted from the risk estimated for the different levels of pack-years either for blond tobacco smokers or for mixed ones.

Analyzing risk among blond tobacco smokers, a statistically significant association between pack-years and NHL for men in the first tertile of age (younger than 52 years) was observed (χ^2 for trend = 9.95, $P < 0.001$), while no similar trend was evident in females, with a high risk (OR = 2.2, 95% CI 1.2–4.3) seen only for the lowest level of pack-year in middle age (data not shown).

Table 1. Distribution of NHL cases and controls according to gender, age, residence area, educational level, and type of interview

		Cases				Controls			
		Males		Females		Males		Females	
		No.	%	No.	%	No.	%	No.	%
Age (yr)	<35	70	8.6	33	5.2	109	11.9	134	15.6
	35–44	79	9.7	59	9.2	113	12.3	92	10.7
	45–54	132	16.3	119	18.6	170	18.5	139	16.1
	55–64	263	32.4	190	29.7	247	26.9	242	28.1
	65+	267	32.9	238	37.2	279	30.4	254	29.5
Area	Florence	175	21.6	142	22.2	196	21.4	184	21.4
	Forlì	89	11.0	50	7.8	67	7.3	68	7.9
	Imperia	35	4.3	23	3.6	67	7.3	42	4.9
	Novara	44	5.4	48	7.5	60	6.5	53	6.2
	Ragusa	31	3.8	15	2.3	48	5.2	42	4.9
	Siena	34	4.2	28	4.4	27	2.9	33	3.8
	Turin	134	16.5	115	18.0	139	15.1	156	18.1
	Varese	92	11.3	89	13.9	133	14.5	119	13.8
	Vercelli	29	3.6	25	3.9	34	3.7	33	3.8
	Verona	111	13.7	79	12.4	96	10.5	90	10.5
	Latina	37	4.6	25	3.9	51	5.6	41	4.8
Education level	Illiterate	28	3.5	27	4.2	21	2.3	47	5.5
	Primary school	399	49.2	389	60.9	416	45.3	462	53.7
	Middle school	178	21.9	119	18.6	236	25.7	189	22.0
	High school	157	19.4	82	12.8	181	19.7	125	14.5
	University	35	4.3	22	3.4	59	6.4	35	4.1
	Missing	14	1.7			5	0.5	3	0.3
Interview	Direct	635	78.3	536	83.9	873	95.1	833	96.7
	Surrogate	164	20.2	98	15.3	39	4.2	23	2.7
	Missing	12	1.5	5	0.8	6	0.7	5	0.6

Starting smoking in the youngest age class (Table 2) seemed to be weakly or not at all associated with NHL incidence. Nevertheless, in males younger than 65 years (second tertile), elevated risks for blond tobacco (OR > 2.0) were observed for early smokers, although an opposite tendency was seen in females (data not shown).

In former smokers, a higher risk was observed for subjects in both blond and black tobacco groups (Table 2) who had quit smoking for <5 years (OR = 2.0 and 1.8, respectively), with a tendency toward a reduction of risk for those who had quit for a longer time. No clear pattern was evident for the mixed tobacco group.

Table 3 reports the results concerning NHL subtypes according to the WF. A statistically significant association between smoking and follicular lymphoma was observed for blond (OR = 2.1) and mixed (OR = 1.8) but not for black (OR = 0.82) tobacco. The strongest associations were found for C subtype in blond and for B subtype in mixed tobacco groups. A high risk was also observed for H subtype only for blond tobacco. Moreover, the risk for mycosis fungoides (MF) seemed to be elevated in all considered tobacco groups, even if without any statistical significance. ORs < 1 were observed for hairy cell leukemia (HCL) in all groups considered, with statistical significance among blond tobacco smokers.

The association between blond tobacco smoking and follicular lymphoma risk is shown in Table 4. Risks were higher in former (OR = 2.4) than in current smokers (OR = 1.9), although a dose-response relationship was not evident. The risk was more elevated for persons who started smoking after 17 years, even if this observation seems mainly due to a high risk in females (OR = 2.6, 95% CI 1.5–4.7; data not shown). A trend opposite to what was expected emerged for years quitting smoking, with the highest risk being observed for people had quit for >10 years (OR = 2.7).

Discussion

Although most cigarettes smoked throughout the world contain blond tobacco, black tobacco has long been widely consumed in Mediterranean Europe.

In Italy, a rising tobacco consumption was observed in females until the mid-1980s, with a tendency to plateau in the last decade (proportion of smokers about 17–18%), while a strong reduction was observed in males, especially in younger men (31).

The incidence and mortality rates of NHL in Western countries (*e.g.*, England, Wales, and the United States) tend to be very high. In Southern Europe (Italy and France), where NHL rates are lower, the most widely smoked tobacco has been the black variety, with blond tobacco consumption increasing only in recent years (2, 17, 32, 33).

The two types of cigarette tobacco were found to be differently associated with the risks of some cancer sites. In particular, the risk of bladder cancer is reported to be two to three times higher for smoking black rather than blond tobacco, and several studies observed that the higher bladder cancer risk in smokers of black tobacco was correlated with a two to five times higher exposure to carcinogenic aromatic amines present in black tobacco

Table 2. Number of controls and NHL cases, OR, and 95% CI associated with type of tobacco smoking

	Tobacco								
	Blond			Black			Mixed		
	Controls/cases	OR	95% CI	Controls/cases	OR	95% CI	Controls/cases	OR	95% CI
Cigarettes status									
Never smokers	811/581	1.0	–	811/581	1.0	–	811/581	1.0	–
Ever smokers	419/381	1.4	1.1–1.7	148/116	0.84	0.61–1.2	366/327	1.2	0.91–1.5
Former	153/155	1.5	1.1–1.9	107/86	0.89	0.62–1.3	154/136	1.1	0.80–1.5
Current	266/226	1.3	1.1–1.7	41/30	0.72	0.41–1.3	212/191	1.2	0.91–1.6
Pack-years of smoking[a]									
≤12	210/159	1.3	1.0–1.7	42/31	0.80	0.47–1.3	54/37	1.0	0.65–1.7
13–29	142/135	1.5	1.1–2.0	48/37	0.88	0.53–1.5	123/100	1.1	0.79–1.5
≥30	67/87	1.4	0.97–2.0	58/48	0.85	0.53–1.4	189/190	1.2	0.93–1.6
Age (yr) started smoking[a]									
<15	84/66	1.5	1.0–2.2	40/29	0.92	0.53–1.6	117/112	1.3	0.97–1.9
15–17	78/71	1.6	1.1–2.3	18/22	1.5	0.74–2.9	69/62	1.3	0.86–1.9
18–20	149/138	1.4	1.0–1.8	57/49	0.88	0.55–1.4	136/111	0.97	0.70–1.3
21–23	31/20	0.81	0.44–1.5	10/2	0.22	0.04–1.1	19/18	1.2	0.60–2.4
≥24	77/86	1.5	1.1–2.1	23/14	0.52	0.25–1.1	25/24	1.1	0.57–2.0
Years since quitting smoking[a]									
1–4	36/38	2.0	1.2–3.3	6/8	1.8	0.60–5.7	29/23	1.0	0.56–1.9
5–9	41/34	1.3	0.80–2.2	18/17	0.86	0.42–1.8	42/42	1.2	0.75–2.0
≥10	76/83	1.4	0.99–2.0	83/61	0.84	0.56–1.3	83/71	1.0	0.71–1.5

Note: OR and 95% CI were adjusted for age, sex, area of residence, education level, and type of interview.
[a]Those with missing information in these categories (51) were excluded from the analyses.

(17, 19–21). As regards lung cancer, an increased risk was found among smokers of both types of tobacco, but the association was stronger among black tobacco smokers (22–24).

In general, the relation of the type of cigarette smoked to NHL has been analyzed by comparing risks in different countries based on the pattern of geographical distribution of black and blond tobacco consumption all over the world. Most studies, however, have been carried out in countries where the use of blond tobacco is predominant, while only a very few investigations have covered nations where black tobacco smoking is common.

In a recent review, Peach and Barnett (4) reported results from 7 cohort and 14 case-control studies identified from Medline analyzing the association between smoking and NHL. Only four (34–37) studies [all case-control, of which only one was population-based (37)] were

Table 3. Number of controls and NHL subtype cases based on the WF, OR, and 95% CI associated with type of tobacco smoking

		Smokers								
	Nonsmokers	Blond			Black			Blond and black		
	No.	No.	OR	95% CI	No.	OR	95% CI	No.	OR	95% CI
Control	811	419	1.0	–	148	1.0	–	366	1.0	–
A: Small cell	94	34	0.91	0.58–1.4	22	0.96	0.55–1.7	65	1.3	0.88–2.1
Follicular	54	56	2.1	1.4–3.2	6	0.82	0.33–2.0	32	1.8	1.1–3.0
B: Predominantly small cleaved	9	4	0.87	0.26–3.0	2	2.1	0.39–10.8	10	4.6	1.6–13.5
C: Mixed small cleaved and large	40	48	2.6	1.6–4.1	2	0.35	0.08–1.5	20	1.4	0.74–2.5
D: Predominantly large	5	4	1.6	0.39–6.3	2	3.3	0.52–21.1	2	1.7	0.26–10.6
Diffuse	258	148	1.2	0.93–1.6	42	0.65	0.42–0.99	130	1.1	0.79–1.4
E: Small cleaved	69	40	1.2	0.80–1.9	12	0.64	0.32–1.3	35	0.84	0.52–1.4
F: Mixed small cleaved and large	69	30	0.95	0.59–1.5	16	1.2	0.64–2.3	36	1.3	0.78–2.1
G: Large	120	78	1.3	0.91–1.8	14	0.45	0.23–0.87	59	1.1	0.74–1.7
Other	108	97	1.3	0.92–1.8	24	0.77	0.45–1.3	64	0.95	0.65–1.4
H: Large	67	71	1.6	1.1–2.4	16	0.83	0.43–1.6	41	1.0	0.64–1.6
I: Lymphoblastic	4	2	0.70	0.12–3.9	3	4.7	0.83–26.6	3	1.6	0.31–8.7
J: Lymphoblastic small noncleaved	9	11	1.5	0.59–3.9	0	–	–	4	0.66	0.19–2.4
K: angioimmunoblastic lymphadenopathy-like	3	0	–	–	1	1.0	0.09–13.3	3	0.98	0.14–7.0
MF	5	8	3.2	0.97–10.2	2	2.2	0.38–13.2	5	2.4	0.61–9.7
HCL	17	4	0.32	0.10–0.97	2	0.41	0.09–1.9	6	0.46	0.17–1.2
Other	3	1	0.29	0.03–3.3	0	–	–	2	1.0	0.08–13.4

Note: OR and 95% CI were adjusted for age, area of residence, education level, sex, and type of interview. Those with missing information in these categories were excluded from the analyses. Thirty-nine nonsmokers and 74 smokers (NHL cases), unclassifiable for WF, were excluded.

Table 4. Number of controls and follicular lymphoma cases, OR, and 95% CI associated with blond tobacco smoking

	Controls/cases	OR	95% CI
Cigarettes status			
Never smokers	811/54	1.0	–
Ever smokers	419/56	2.1	1.4–3.2
Former	153/22	2.4	1.4–4.1
Current	266/34	1.9	1.2–3.1
Pack-years of smoking[a]			
≤12	210/30	2.3	1.4–3.8
13–29	142/19	2.1	1.2–3.7
≥30	67/7	1.6	0.67–3.7
Age (yr) started smoking[a]			
<18	226/24	1.7	0.99–2.9
≥18	193/32	2.4	1.5–3.9
Years since quitting smoking[a]			
1–4	36/3	1.2	0.36–4.2
5–9	41/6	2.4	0.95–6.0
≥10	56/13	2.7	1.4–5.3

Note: OR and 95% CI were adjusted for age, sex, area of residence, education level, and type of interview.
[a]Those with missing information in these categories were excluded from the analyses.

conducted in areas where the smoking of dark tobacco is or has been rather common: three in Italy and one in Uruguay. This latter (36) is the only study, to our knowledge, examining the relationship between type of smoking and NHL in the same population. The higher risk was found among smokers of black tobacco (OR = 3.5, 95% CI 1.1–10.9), while the corresponding risk for blond tobacco smokers was 1.9 (95% CI 0.7–5.4). However, this finding was based on a quite smaller exposed population (72 smokers among cases and 63 among controls), while our sample size was much larger. Furthermore, results by Freedman *et al.* (10) suggested that heavy cigarette smoking among men who had served in the U.S. military in Vietnam, where blond tobacco was traditionally smoked, may be an important risk factor for NHL. This finding was evident among younger men, and an association emerged with age at which smoking started and the number of years since smoking cessation.

Our results, in agreement with Freedman *et al.*'s (10), support a relationship between the smoking of blond tobacco and an increased risk of NHL among younger men, with an exposure-response gradient ($P < 0.01$) and with a higher risk for persons starting smoking at a younger age. At the same time, there is no evidence in our investigation of a decreased risk with years from quitting smoking and the risk for former smokers was higher than that for current smokers, even if the 95% CI of the corresponding OR were largely overlapped.

Different kind of cigarettes (*i.e.*, hand-rolled or manufactured) may differ in content of tar, nicotine, or other carcinogenic compounds by type of tobacco (18), but such variables cannot be presumed major confounders in our analyses because hand-rolled cigarettes were used only by a small fraction of smokers (1.3% in blond, 8.3% in black, and 5.5% in mixed tobacco smokers). As regards the use of cigarettes without filter only, the small proportion in blond tobacco smokers (2.4%) prevents the yield of reliable results, while a statistically significant effect was observed in neither black nor mixed tobacco smokers (data not shown). Furthermore, a previous study on the same sample data (38) suggested a higher risk for manager and clerks. However, after adjusting for job category, the risk for smokers remains unchanged (data not shown).

Acknowledging the criticism in Peach and Barnett's (4), recent review (*i.e.*, "Many of the studies ignoring the fact that NHL is a heterogeneous group of disorder"), we present the analysis of subtypes of NHL according to the WF classification. We found a stronger relationship between blond tobacco smoking and follicular lymphoma, particularly with mixed small cleaved cell type. Similar results were observed in other studies where the predominant type of tobacco consumed was blond (10–12). Nevertheless, we did not find any dose-response relationship: the risk was higher in persons who quit smoking much earlier (>10 years) and the highest risk was observed in adult age, even if this finding seems to be related to the high risk seen in females.

While no association was evident with black tobacco, use of mixed tobacco showed an intermediate risk. However, no clear trend was evident in this latter subgroup in either the proportion of exposure to blond tobacco or the blond pack-years smoked (data not shown). However, this subgroup could be heterogeneous, comprising people who have smoked two different types of tobacco during their life span, with subjects who decided to switch from one kind of tobacco to another. A recent meta-analysis (22) on lung cancer risk highlighted a decreasing concentration in tar and nicotine exposure but a switch from black to blond tobacco with rise in other carcinogenic compounds (18), probably confounding the relationship between exposure and risk. Moreover, the study design (case-control) did not allow checking for the agreement between reported and actual brand of tobacco smoked, which was found to be poor in a prospective preventive trial in 24 industries by Peach *et al.* (39), and a recall bias could not be excluded. However, there is no reason to assume that such a bias have caused a different distribution of exposures among cases and controls; then, it is likely to have reduced the differences in risk estimated by group of smokers.

A statistically significant association was observed between the exposure to blond tobacco and the risk for large cell lymphoma (H subtype of WF). Our finding is consistent with the observation of a stronger effect of tobacco smoking in high-grade lymphoma cases, including large cell, as reported by Freedman *et al.* (10).

Our study also suggests a relationship between smoking habit and risk for MF in all types of tobacco considered, even if statistical significance was not reached. The epidemiologic data about the possible role of smoking in MF lymphoma are scarce. In one recent case-control study, Morales Suarez-Varela *et al.* (40) reported a dose-dependent increase in the risk of MF with increased smoking habits.

Only two relatively recent studies have examined the relationship of smoking with HCL (41, 42), both finding a negative association that reached statistical significance in the investigation by Clavel *et al.* (41). These studies, carried out in countries with different prevalence of tobacco type consumption, are in good agreement with

our findings that suggest a negative relationship between HCL and cigarette smoking with any type of tobacco.

Tobacco smoke produces many different effects that can be related to cell differentiation, proliferation, or carcinogenesis in the lymphoid tissues. In particular, tobacco smoke has immunomodulatory properties (43) and has been shown to decrease the serum levels of most of the immunoglobulin classes (44, 45). Nicotine may significantly contribute to the immunosuppressive effect (46), together with PAHs, which are also mutagenic (47), even if metals and tobacco glycoproteins present in smoke have immunostimulatory properties (48, 49). It is well known that a unique chromosomal translocation t(14,18) of *bcl*-2 tumor suppressor gene locus occurs in >85% of all follicular NHL (50, 51). This translocation, which is also strongly associated with cigarette smoking in healthy people (52), takes place at an early stage of B-cell development during the rearrangement of the *bcl*-2 gene and is present in 70–95% of cases, resulting in expression of this antiapoptosis gene and possibly contributing to development of the lymphoma (53–55).

Lymphomagenic agents prevalent in blond tobacco smoke include benzene, other PAHs, and formaldehyde (56–58). The association between benzene and NHL risk has been reported on occasion in occupational studies (59). More recently, results from a large cohort exposed to high levels of benzene (60) confirmed the relationship, even if confounding from exposure other than occupational sources could not be completely excluded (61). Nevertheless, benzene has been shown to cause mutagenic alterations in a lymphocyte precursor cell (62, 63). This evidence supports the hypothesis of a higher expected risk for blond tobacco smokers.

This investigation highlights an association between NHL and smoking habits, particularly for blond tobacco exposure, which is limited to follicular and a few other lymphoma subtypes (H-large cell). Our finding is consistent with the nearly 2-fold risk for blond tobacco smokers reported by De Stefani *et al.* (36), even if no association with black tobacco emerged. The increase in blond tobacco consumption in southern European countries could be one of the causes of the rise of NHL rates (4.2%/year) observed in both gender (2, 17, 32, 33), especially for follicular subtype (2).

Even if a clear dose-response relationship is limited to a subgroup of smokers (younger men), the large sample size, the good characterization of NHL, the fairly high participation rate, and the accurate and blind face-to-face interviews at home make it difficult to attribute the findings of the present work merely to biases in study design or conduction. Furthermore, confounding effect from other variables associated to the exposure (*e.g.*, type of cigarettes or job category) should have not influenced our results. Smoking blond tobacco could be an important risk factor for NHL; however, in view of the above-mentioned drawbacks of this investigation and the limited knowledge about the etiology of this cancer, further studies are required for definitive confirmation.

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