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Cigarette Smoking and Bladder Cancer

Barbara D'Avanzo, Eva Negri, Carlo La Vecchia, Annagiulia Gramenzi, Cosetta Bianchi, Silvia Franceschi and Peter Boyle

The relation between cigarette smoking and risk of bladder cancer was analysed in a case-control study in Northern Italy of 337 cases of histologically confirmed invasive bladder cancer and 392 controls admitted to the same network of hospitals with acute, non-neoplastic, non-urological conditions. Compared with never-smokers, the multivariate relative risk (RR) was 1.9 (95% confidence interval, CI 1.2–3.1) for ex-smokers and 3.3 (95% CI 2.2–5.0) for current smokers. The risk was directly and significantly related to duration of smoking (RR 3.5 for 30 years or more) and dose (RR 3.9 for 20 cigarettes per day or more), and consistent among strata of sex and age (though the RRs were systematically higher at older ages). Smokers of black tobacco only had a RR of 3.7, compared with 2.6 for smokers of blond cigarettes or mixed types . The interaction between tobacco and several occupations associated with bladder cancer risk fitted an additive rather than a multiplicative model: compared with non-exposed never-smokers, RR was 2.5 for exposed non-smokers, 2.8 for non-exposed smokers and 3.7 for occupationally exposed smokers.
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INTRODUCTION

BLADDER CANCER is a known tobacco-related neoplasm, but the strength of the association is uncertain: the relative risks (RR) for smokers compared with non-smokers ranged between 1.4 and 2.9 in eight cohort studies, and the range of variation was even larger (1.2–7.3) in twenty case-control studies [1–12]. An Italian case-control study found a strong association between cigarette smoking and bladder cancer risk, with RR of 5.1 for smokers and of over 10 for heavy smokers (30 or more cigarettes per day) [3]. The proposed explanation for these elevated risks in an Italian population was the high frequency of dark tobacco smoked in the past in Italy [3, 4]. We present data from another

Table 1. Sociodemographic data of 331 cases of bladder cancer and 392 controls

	Males				Females			
	Cases		Controls		Cases		Controls	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Age (yr)								
<50	21	(7.5)	50	(17.1)	4	(6.9)	7	(7.1)
50–59	71	(25.4)	94	(32.1)	16	(27.6)	27	(27.3)
60–69	130	(46.6)	123	(42.0)	25	(43.1)	47	(47.5)
≥70	57	(20.4)	26	(8.9)	13	(22.4)	18	(18.2)
Education (yr)*								
<7	162	(58.1)	152	(51.9)	33	(56.9)	72	(72.7)
7–11	71	(25.4)	79	(27.0)	22	(37.9)	16	(16.2)
≥12	46	(16.5)	60	(20.5)	3	(5.2)	11	(11.1)
Social class†								
I or II	23	(8.2)	29	(9.9)	4	(6.9)	6	(6.1)
III	94	(33.7)	101	(34.5)	20	(34.5)	29	(29.3)
IV or V	131	(47.0)	123	(42.0)	24	(41.4)	51	(51.5)
Other	31	(11.1)	40	(13.7)	10	(17.2)	13	(13.1)

*Sum of strata does not add up to the total because of missing values.
†Based on head-of-household's occupation.

case-control study of smoking and bladder cancer in Northern Italy.

SUBJECTS AND METHODS

The data were obtained from a case-control study, which included cases of urinary tract neoplasms, starting in January 1985, in the Greater Milan area [13, 14]. Trained interviewers identified and questioned patients admitted to a network of teaching and general hospitals in the area under surveillance for histologically confirmed invasive bladder cancer (cases) and for a wide spectrum of acute, non-neoplastic, non-urinary-tract conditions (controls). Participation was almost complete, since less than 3% of cases and controls refused to be interviewed. The present analysis is based on data collected before October 1989.

The standard questionnaire included information on sociodemographic factors, personal characteristics and lifestyle; consumption of alcohol and coffee and other methylxanthine-containing beverages; a few indicator foods; a problem-oriented medical history; drug use; and history of occupation or occupational exposure, including age at starting and stopping for nineteen industries or occupations, the job in terms of involvement in production, and on exposure to fourteen agents or groups of agents [14].

Questions on tobacco included smoking status (ever-smokers being defined as subjects who had smoked at least 1 cigarette, or pipe or cigar, per day for at least a year; ex-smokers those who had stopped at least a year previously). The smokers and ex-smokers were asked the total duration (in years) of the habit, the average quantity smoked per day, and the names of up to three cigarette brands they had smoked for the longest time.

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Table 2. Relative risk (95% CI) of bladder cancer according to smoking habits

	Cases		Controls		Relative risks	
	No.	(%)	No.	(%)	M–H*	MLR†
Never-smokers	75	(22.3)	168	(42.9)	1‡	1‡
Ex-smokers	89	(26.4)	95	(24.2)	1.7 (1.1–2.9)	1.9 (1.2–3.1)
Current smokers	173	(51.3)	129	(32.9)	3.3 (2.2–4.9)	3.3 (2.2–5.0)
Cigarettes per day						
<10	24	(7.1)	20	(5.1)	2.6 (1.3–5.6)	2.9 (1.4–3.9)
10–19	49	(14.5)	40	(10.2)	2.8 (1.6–4.9)	2.9 (1.7–5.1)
≥20	100	(29.7)	69	(17.6)	3.6 (2.2–5.9)	3.9 (2.4–6.4)
χ^2_1 trend					31.6§	33.7§
Duration of smoking (yr)						
<30	56	(16.6)	84	(21.4)	1.6 (1.0–2.8)	1.8 (1.1–3.0)
≥30	187	(55.5)	119	(30.4)	3.3 (2.1–4.9)	3.5 (2.3–5.2)
Undefined	19	(5.6)	21	(5.4)	—	—
χ^2_1 trend					34.2§	38.1§

*Mantel–Haenszel estimates adjusted for sex and age.
†Estimates from multiple logistic regression equations, including terms for age, sex, area of residence, education and occupation.
‡Reference category.
§$P < 0.001$.

All the brands of cigarettes were classified according to their tar yield (laboratory of the British Government Chemist, and/or the Italian State Monopoly [15]) and colour of tobacco (black/air-cured or blond/flue-cured). The two variables (tar yield/colour) were strongly correlated, as well as related to calendar year, since most Italian cigarettes were high-tar/black-tobacco up to the early 1970s, later being replaced by blond, lower tar cigarettes [15]. Since for bladder carcinogenesis a hypothesis has been put forward about black tobacco and its nitrosamine yield [3, 4, 11], this variable has been used throughout the analyses.

The cases were 337 patients aged below 75 (279 males, 58 females) with histologically confirmed invasive bladder cancer diagnosed within the year preceding interview, who had been admitted to the National Cancer Institute, to several university clinics (mainly surgical), or to the Ospedale Maggiore, which includes the four largest teaching and general hospitals in Milan. The age range was 34–74, and the median age was 63.

The comparison group comprised 392 subjects (293 males, 99 females), admitted for acute conditions to the same network of hospitals where cases had been identified. Of these, 30% were admitted for trauma, 17% for non-traumatic orthopaedic conditions, 13% for acute medical diseases, 6% for surgical diseases, and 34% for miscellaneous conditions, including skin, ear, nose and throat, and dental disorders. The median age was 61. The distribution of cases and controls according to sex, age group, social class and education is given in Table 1. The

catchment area of cases and controls was similar. Overall, 78% of the cases and 83% of the controls lived in Lombardy; 88% of the cases and 90% of the controls came from Northern Italy.

Data analysis and control of confounding

RR of bladder cancer, with their approximate 95% confidence intervals [16], according to various tobacco-related variables, were first obtained from data classified by sex and quinquennia of age by the Mantel–Haenszel procedure [17]. Significance was assessed by the linear trend test [18]. To account simultaneously for the potential confounding effect of several identified factors, unconditional multiple logistic regression was used [19]. Five occupations or occupational exposures were associated with bladder cancer risk (chemical industry, dyestuff, painting, pharmaceutical and coal/gas) [14]. Thus the regression equations included terms for age, sex, area of residence, education and exposure to any of these occupations.

RESULTS

Cases of bladder cancer were slightly less educated than controls (14.5% vs. 18.1% reported 12 years of education or more) and male cases tended to be of lower social class (Table 1).

Compared with never-smokers, the age-adjusted RR was 1.8 for ex-smokers, and 3.3 for current smokers (Table 2). Among current smokers, the risk rose with increasing quantity, from 2.6 for light smokers (less than 10 cigarettes per day) to 2.8 for moderate (10–19 cigarettes per day) to 3.6 for heavy smokers. This trend in risk was statistically significant. Likewise, a relation was evident for duration of smoking, with RR of 1.7 for less than 20 years and 3.3 for long duration. These results were not materially modified by allowance for major identified potential confounding factors in multivariate analysis.

*Table 3. Relative risk of bladder cancer according to smoking habits in strata of sex and age**

	Sex		Age	
	Males	Females	<60	≥60
Never-smokers	1	1	1	1
Ex-smokers	1.9 (1.2–3.3)	0.5 (0.1–3.2)	1.9 (0.8–4.9)	1.7 (0.9–3.1)
Current smokers	3.3 (2.0–5.2)	3.2 (1.3–7.7)	2.9 (1.5–5.6)	3.6 (2.1–6.1)
Cigarettes per day				
<10	2.5 (1.1–6.0)	3.1 (0.4–26.0)	2.1 (0.5–9.2)	3.0 (1.1–8.1)
10–19	2.3 (1.2–4.4)		2.7 (1.0–6.9)	2.9 (1.4–5.9)
≥20	4.0 (2.3–6.8)	3.2 (1.3–7.7)	3.0 (1.4–6.6)	3.9 (2.0–7.6)
χ^2_1 trend	26.4†	9.1†	10.0†	22.2†
Duration of smoking (yr)				
<30	1.7 (0.9–3.1)	1.5 (0.3–6.8)	2.1 (1.0–4.7)	1.2 (0.5–3.3)
≥30	3.1 (1.9–4.9)	4.6 (1.6–13.8)	3.2 (1.5–6.8)	3.3 (2.0–5.5)
χ^2_1 trend	25.8†	8.8†	12.2†	22.1†

*Mantel–Haenszel estimates adjusted for sex and age.
†$P < 0.01$.

*Table 4. Relative risk of bladder cancer according to age at starting and time since quitting**

	Cases		Controls		Relative risks	
	No.	(%)	No.	(%)	M–H	MLR
Age at starting						
>20	156	(46.3)	132	(33.7)	2.5 (1.6–3.8)	1.8 (0.9–3.8)
≤20	89	(26.4)	70	(17.9)	3.2 (2.0–5.3)	2.7 (1.8–4.1)
Undefined	17	(5.0)	22	(5.6)	—	—
χ^2_1 trend					22.8‡	25.0‡
Time since quitting†						
>15	16	(4.7)	27	(6.9)	1.0 (0.9–1.1)	1.2 (0.6–2.5)
5–14	39	(11.5)	42	(10.7)	1.9 (1.0–3.5)	1.8 (1.0–3.2)
2–4	32	(9.5)	22	(5.6)	2.8 (1.4–5.7)	3.1 (1.6–6.2)
χ^2_1 trend					10.6‡	11.8‡

Percentage of total series.
*Reference category = never-smokers
†Sum of strata does not add up to the total because of missing values.
‡$P < 0.01$.

The same smoking-related variables are considered in Table 3 in strata of sex and age. The results were broadly consistent in males and females (although some of the estimates for females were rather unstable because of the limited number of subjects), and in the two age strata considered. Nonetheless the risk estimates for current smokers (and for number of cigarettes per day) were higher at older ages.

The RR was higher for subjects who had started before age 20 than for those who had started later (Table 4). For ex-smokers, the risk declined linearly with time since stopping.

Most smokers (78% of cases and 83% of controls who had ever smoked) smoked both black and blond tobacco, most frequently switching to blond tobacco in the past one or two decades. Their RR was about 40% lower than that of smokers who had smoked black tobacco only throughout their life (Table 5).

*Table 5. Relative risks of bladder cancer according to type of tobacco**

	Cases		Controls		Relative risks	
Type of tobacco		(%)		(%)	M–H	MLR
Blond or mixed	205	(60.8)	187	(47.7)	2.6 (1.7–3.8)	2.7 (1.8–4.0)
Black	38	(11.3)	19	(4.8)	3.7 (1.9–7.3)	3.8 (2.0–7.4)
Undefined	19	(5.6)	18	(4.6)	—	—
χ^2_1 trend					25.8†	28.9†

Percentage of total series.
*Reference category: never smokers.
†$P < 0.001$.

Table 6. Interaction between cigarette smoking and high-risk occupation on risk of bladder cancer

	RR for ever-employed in high-risk occupation*	
	No	Yes
Never-smokers	1†	2.5 (1.0–6.8)
Ever-smokers	2.8 (1.9–4.2)	3.7 (1.9–7.3)

*Mantel–Haenszel estimates adjusted for sex and age.
†Reference category.

The interaction between tobacco and occupational exposures associated with bladder cancer risk is shown in Table 6. Compared with never-smokers, never occupationally exposed, the RR was 2.8 for ever-smokers non-exposed, 2.5 for never-smokers exposed to high-risk occupations, and 3.7 for smokers exposed to such occupations. Thus, the interaction between smoking and occupational exposure is additive rather than multiplicative on the relative risk. In the logistic model, interaction was significantly negative, which confirms a less than multiplicative effect of these two variables.

DISCUSSION

We have further confirmed the association between smoking and bladder cancer [1–12]. The risk was significantly above unity, with RRs of 1.9 for ex-smokers and of 3.3 for current smokers, and there were significant trends in risk with dose and duration. Further, the risk was higher in smokers who had started at younger ages and declined after cessation of exposure, reaching a level compatible with the risk of never-smokers 15 years after stopping. This pattern of risk, together with the consistency of the results across strata of age and sex, and the absence of substantial apparent confounding, strongly suggests a causal association. In terms of population attributable risk [20], approximately 50% of bladder cancer cases in this population could be attributable to smoking.

Even in quantitative terms, our findings are in broad agreement with most prospective and case-control studies, showing a two-fold to three-fold increased risk of bladder cancer in smokers. However, the dose–response relation, although in line with most patterns described in the United States or Northern Europe [1, 2, 5, 10, 12] was weaker than that reported from another hospital-based Italian study [4, 5], where the RR for 30 cigarettes per day or more was of the order of 10, and less strong than the RR of 5 for current smokers and 6.9 for heavy smokers from a French study [11]. In addition, smokers of black tobacco had a RR 'only' about 40% higher, and not approximately double compared with smokers of blond tobacco.

The upper confidence limits of our study are compatible with a five-fold increased risk in smokers, and more than seven-fold RR for black tobacco smokers. Further, over the past one or two decades, the market share of cigarettes made from black tobacco, and the number of smokers of black cigarettes only, has decreased, at least in Greater Milan [15]. Our study, on average, was done about 7 years later than the previous Italian bladder cancer case-control study from the Turin area [3, 4], and part of the difference in results could be attributed to the change in Italian cigarette smoking habits. This would be consistent with the higher estimates observed in older subjects, whose exposure to black tobacco was greater, as well as with a late stage effect of tobacco on bladder carcinogenesis, observed in this as well as in previous studies [1, 4–12], with the steady decline in risk after stopping. An early stage effect, however, is also suggested by the inverse relation with age at starting [4].

Chance or bias might explain some of the apparent discrepancies. For bias, we tried to exclude all tobacco-related diagnoses from the comparison group, and checked the comparability of smoking habits across various diagnostic categories of the controls. Participation, moreover, was practically complete, cases and controls came from similar catchment areas, and allowance for major identified potential confounding factors did not materially modify any of the results.

The interaction between smoking and occupational exposures fits an additive rather than a multiplicative model. This is consistent with some [3, 21], but not all [4] previous observations, and can be related to the carcinogen composition of occupational exposure and tobacco smoking—since at least part of the major bladder carcinogens (benzidine or β-naphthylamine) are shared in both exposures. A clearer definition of the tobacco/occupation interaction would have important implications for prevention and public health for occupationally exposed workers: under extreme conditions of exposure to aromatic amines, for instance, the cumulative probabilities of bladder cancer death for a heavy smoker have been estimated to range between 14% assuming an additive model and 60% assuming a multiplicative one [22].

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Anti-oestrogenic and Anti-tumour Properties of Prolonged Tamoxifen Therapy in C3H/OUJ Mice

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The anti-oestrogenic and anti-tumour properties of a sustained-release preparation of tamoxifen were evaluated in female C3H/OUJ mice. Tamoxifen decreased uterine weight compared with controls in intact mice but caused an initial uterotrophic response for 2 months in ovariectomized mice. Prolonged tamoxifen therapy in ovariectomized mice resulted in a uterine weight no different from controls, but these uteri were eventually (at 6 months) refractory to oestradiol. Spontaneous mammary tumours were detected in female mice between 6 months and 1 year of age during continuous cycles of pregnancy and weaning. A similar tumour frequency occurred after one cycle of pregnancy and weaning initiated at 3½ months. Prolonged tamoxifen, started at 3½ or 4½ months of age (following pregnancy/weaning), reduced the appearance of tumours. Similarly ovariectomy at 3½ months prevented mammary tumorigenesis and prolonged tamoxifen could not increase tumour incidence consistently in ovariectomized mice. Although tamoxifen is oestrogenic in short-term tests the compound has the properties of an anti-oestrogen during prolonged administration.
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INTRODUCTION

SPONTANEOUS mammary tumours often occur in inbred mice. The cause was identified by Bittner [1, 2] as the mouse mammary tumour virus, which is transferred to offspring via mother's milk. The tumour frequency in female mice can be decreased by early ovariectomy [3]. Oestrogen is linked to tumorigenesis because male mice do not generally acquire mammary tumours but tumours can be induced by oestrogen injections [4]. This observation led Lacassagne [5] to predict that an agent could be developed that would antagonize the actions of oestrogen in the mammary tissue and prevent tumours. Hence, after some fifty years, tamoxifen, which is used to treat breast cancer [6], has been advocated as a prophylactic in women at high risk [7–9]. The concept has some merit.

Our goal was to study whether tamoxifen could prevent spontaneous mouse mammary tumorigenesis. However, tamoxifen is oestrogenic in short-term assays in mice [10, 11] and tumorigenesis might be increased.

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