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Frits H.M. van Osch[a,e], Charlotte G.G.M. Pauwels[b,d], Sylvia H.J. Jochems[a,e], Ranti Fayokun[h], Nicholas D. James[e,g], D. Michael A. Wallace[e], Kar-keung Cheng[f], Richard T. Bryan[e], Frederik J. van Schooten[b] and Maurice P. Zeegers[a,c,e]

Cigarette smoking is a major risk factor for bladder cancer (BC); however, the impact of cigarette content remains unclear. This study aims to investigate tar, nicotine and carbon monoxide (TNCO) yields of different filtered cigarettes in relation to BC risk. From the Bladder Cancer Prognosis Programme 575 non-muscle-invasive bladder cancer (NMIBC) cases, 139 muscle-invasive bladder cancer (MIBC) cases and 130 BC-free controls with retrospective data on smoking behaviour and cigarette brand were identified. Independently measured TNCO yields of cigarettes sold in the UK were obtained through the UK Department of Health and merged with the Bladder Cancer Prognosis Programme dataset to estimate the daily intake of TNCO. BC risk increased by TNCO intake category for NMIBC cases (P <0.050 in all multivariate models), but only for the daily intake of tar for MIBC cases ($P = 0.046$) in multivariate models. No difference in risk was observed between smokers of low-tar/low-nicotine and high-tar/high-nicotine cigarettes compared with never smokers, either for NMIBC ($P = 0.544$) or MIBC ($P = 0.449$). High daily intake of TNCO additionally increases the risk of both NMIBC and MIBC compared with low daily intake. However, as there is no difference in BC risk between low-tar/low-nicotine and high-tar/high-nicotine cigarette smokers, it remains unclear whether smoking behaviour or TNCO yield of cigarettes explains this association. *European Journal of Cancer Prevention* **28:40–44**

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[a]Department of Complex Genetics and Epidemiology, [b]Department of Pharmacology and Toxicology, NUTRIM School for Nutrition and Translational Research in Metabolism, [c]Department of Complex Genetics and Epidemiology, CAPHRI School for Public Health and Primary Care, Maastricht University, Maastricht, [d]Centre for Health Protection, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, [e]Institute of Cancer and Genomic Sciences, [f]Department of Public Health and Epidemiology, University of Birmingham, [g]Department of Urology, University Hospital Birmingham, NHS Foundation Trust, Birmingham and [h]Department of Medicine, Centre for Pharmacology and Therapeutics, Division of Experimental Medicine, Imperial College London, London, UK

Correspondence to Frits H.M. van Osch, MSc, Department of Complex Genetics, PO Box 616, 6200 MD Maastricht, The Netherlands
Tel: +31 043 388 2012; e-mail: f.vanosch@maastrichtuniversity.nl

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Background

Bladder cancer (BC) ranks fifth in the list of the most common cancers in western countries and active smoking is indicated as its most common risk factor together with occupational exposure to carcinogenic chemicals and some diet-related factors (Al-Zalabani *et al.*, 2016; Antoni *et al.*, 2017). The impact of cigarette smoking has been quantified in a large number of studies and a recent meta-analysis showed that current smokers have a three-fold increased risk of developing BC compared with never smokers (van Osch *et al.*, 2016).

The relation between the amount of smoking and the risk of cancer has been investigated extensively, and is mostly characterized by smoking duration in years, smoking intensity in cigarettes per day or pack years (an amalgamation of duration and intensity). However, the type of cigarette or cigarette composition is taken into account less often. Therefore, the evidence on the impact of different types of cigarettes, with respect to the composition of the cigarette smoke, on BC risk remains weak. Previous studies have shown lower BC risks for filter versus nonfilter cigarette smokers and also when comparing blond tobacco with black tobacco (Howe *et al.*, 1980; Vineis *et al.*, 1984; Clavel *et al.*, 1989; García-Closas *et al.*, 2005). Two observational studies quantified BC risk for different intakes of tar and nicotine, of which one showed a linearly increasing trend in risk related to the amount of tar and nicotine and the other study showed no association between BC risk and cumulative tar intake (Castelao *et al.*, 2001; Zeegers *et al.*, 2002). By introducing the filter tip, which changed cigarette design but not necessarily the contents, smoking-related mortality has decreased moderately (Tang *et al.*, 1995), although there are studies indicating that the levels of carcinogens in contemporary cigarettes might have become higher (Baris *et al.*, 2009). Nevertheless, it remains unclear whether differences in cigarette content are related to meaningful differences in BC risk at the population level. Therefore, we calculated the levels of tar, nicotine and carbon monoxide (TNCO) in mainstream smoke in a

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UK-based cohort study and aimed to investigate whether these levels influence BC risk.

Methods

Study population

This case–control study was carried out within the framework of the West Midlands Bladder Cancer Prognosis Programme (BCPP), an ongoing BC patient cohort study carried out in multiple centres in the West Midlands, UK. Further details of the BCPP are described elsewhere (Zeegers *et al.*, 2010). In summary, the study population included 1544 adult individuals who were referred to one of the participating urology centres because of symptoms indicative of BC (predominantly haematuria). Of these 1544 individuals, 1008 patients were diagnosed with non-muscle-invasive bladder cancers (NMIBC), 275 patients were diagnosed with muscle-invasive bladder cancer (MIBC) patients and 205 individuals were subsequently diagnosed as free from any form of cancer after histological tests at the urology clinic and recruited as controls. In addition, 57 patients were diagnosed with other primary cancers (e.g. prostate cancer) or had missing data on important staging data and thus could not be confirmed to have BC (Fig. 1).

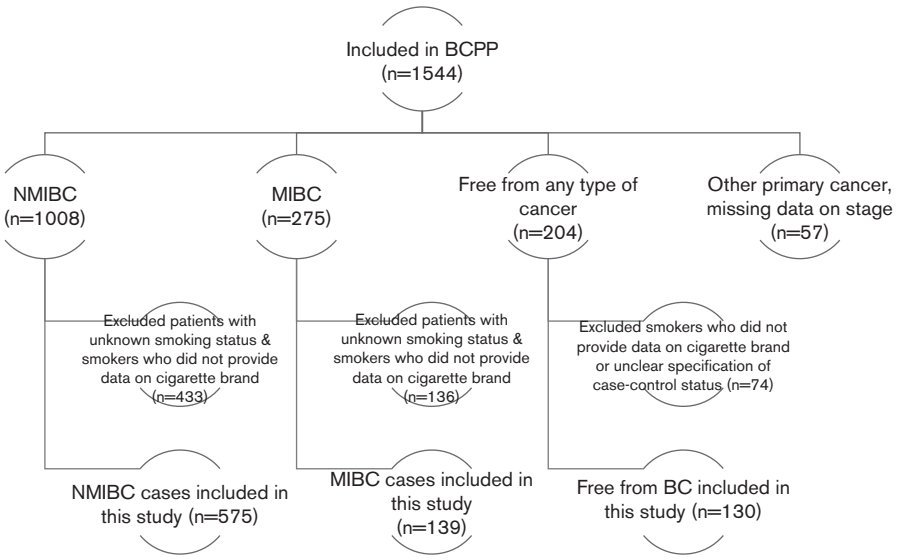
Cases and controls who did not provide data on cigarette brand and smoking status were excluded from analysis. Of the 205 potential controls, 130 had a clear specification of control status and provided data on smoking status and cigarette brand. Of these 130 controls, 34 had benign papillomas, 25 had a normal urothelium, 24 had cystitis and 20 had urothelial inflammation. In addition, for 27 BCPP participants in the control group, the urologist did not provide a description apart from 'no bladder cancer present' (Fig. 1). All participants received a baseline questionnaire including questions to assess demographic characteristics, occupation and retrospectively characterize smoking and dietary behaviour.

Tar, nicotine and carbon monoxide data from the UK department of health

In the UK, an approved and accredited laboratory appointed by the UK Department of Health periodically and independently analyses the yields of tar, nicotine and carbon monoxide (CO) in smoke of random samples of cigarette brands sold in the UK according to the International Organisation for Standardisation (ISO) standards (Legislation, 2002). This examination verifies the TNCO yields declared on cigarette packs by manufacturers and ensures that the TNCO yields of cigarettes on the UK market do not exceed the maximum allowed levels as set out in the relevant tobacco regulation (10 mg/cigarette for tar, 1 mg/cigarette for nicotine and 10 mg/cigarette for CO). This is a legal obligation in all Member States of the EU and is set out in the UK in the Tobacco Products (Manufacture, Presentation, Presentation and Sale) (Safety) Regulations 2002 (Statutory Instrument 3041) (Legislation, 2002). For tar, measurements were made in line with ISO 4387 and for nicotine and CO, ISO 10315 and ISO 8454 were used, respectively, with the accuracy of measurements determined by ISO 8243 (International Organization for Standardization ISO, 2016).

By combining these data with the filter cigarette brand(s) currently or previously smoked in BCPP and the number

Fig. 1



Flow chart of case and control selection from bladder cancer prognosis. BC, bladder cancer; BCPP, Bladder Cancer Prognosis Programme; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle-invasive bladder cancer.

Table 1 Baseline characteristics of non-muscle-invasive bladder cancer cases, muscle-invasive bladder cancer cases and bladder cancer-free controls

	NMIBC ($n=575$)	MIBC ($n=139$)	BC-free ($n=130$)
Age at diagnosis (95% CI)	68.0 (67.1–68.8)	70.1 (68.2–71.9)	65.2 (63.0–67.5)
Sex (male/female)	442/133	99/40	91/39
Smoking status			
Never	127	31	59
Former	299	67	45
Current	149	41	26

BC, bladder cancer; CI, confidence interval; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle-invasive bladder cancer.

of cigarettes smoked per day, the daily intake of TNCO was estimated. Intake is a proxy measure for absolute TNCO exposure as it is an estimation of the amount of TNCO that reaches the lungs, which is also influenced by smoking behaviour (e.g. puff volume and whether the cigarette is smoked completely). Patients who smoked brands that were not in the UK Department of Health database were either excluded (88 out of 602, 15%) or the TNCO values were based on the original packaging as determined by the manufacturer (40 out of 602, 7%).

Statistical analysis

From the BCPP questionnaire data, the daily TNCO intake was estimated by multiplying the amount of cigarettes smoked per day (smoking intensity) with the TNCO levels. On the basis of these TNCO levels, cigarettes were classified as either low tar/low nicotine (tar <9 mg/cigarette, nicotine <0.9 mg/cigarette) or high tar/high nicotine (tar ≥ 9 mg/cigarette, nicotine ≥ 0.9 mg/cigarette). Odds ratios (OR) and 95% confidence intervals (CI) estimating BC risk were calculated using logistic regression models. Potentially confounding factors included in multivariate analyses were restricted to age, sex and smoking duration. Ideally, smoking intensity would also be included as a possible confounder, but this was not possible because of collinearity issues because smoking intensity is used to estimate the daily TNCO intake. Furthermore, data on occupation were sparse in controls ($n=2$ for controls, $n=186$ for NMIBC cases); thus, occupational exposure could not be included as a covariate. Tests for linear dose–response trends in ORs between TNCO intake categories were performed by comparing logistic regression models with categorical variables for TNCO intake to models with a continuous variable for TNCO intake using likelihood-ratio tests.

Results

After exclusion of cases and controls in the analysis because of missing data on cigarette brand or the number of cigarettes smoked per day 575 NMIBC, 139 MIBC and 130 BC-free participants were included in the analysis. Figure 1 summarizes the inclusion of participants for this case–control study recruited from the BCPP participants. Table 1 shows the baseline characteristics of the NMIBC, MIBC and BC-free controls who were included in the analysis.

Table 2 shows linearly increasing dose–response relationships between daily tar, nicotine and CO intake and NMIBC risk compared with never smokers in both adjusted and unadjusted models ($P<0.05$ in all models). The adjusted logistic regression models show mitigated associations compared with the unadjusted model. The highest OR was observed in the highest intake category for tar (OR: 3.00; 95% CI: 1.36–6.63), although the 95% CI was wide.

The results were similar when looking at MIBC risk, albeit the 95% CIs were wider because of the smaller number of MIBC cases (Table 3). Furthermore, the only increasing trend in a multivariate model was observed for daily tar intake ($P=0.046$), where the highest OR was 2.88 (95% CI: 1.10–7.55).

Furthermore, there does not seem to be a meaningful difference in BC risk between smokers of low-tar/low-nicotine cigarettes and smokers of high-tar/high-nicotine cigarettes ($P=0.544$ for NMIBC and $P=0.449$ for MIBC). In addition, smokers of low-tar/low-nicotine cigarettes did not smoke more filter cigarettes than high-tar/high-nicotine cigarette smokers on a daily basis ($P=0.516$, data not shown).

Discussion

This study is the first to investigate all TNCO levels from cigarettes in relation to BC risk within a single study sample. Our results confirm the findings of another study, indicating a linearly increasing dose–response relationship for daily tar and nicotine intake. In addition, we showed a similar association with the daily CO intake (Zeegers *et al.*, 2002). Another study investigating cumulative tar intake did not show any association with BC risk (Castelao *et al.*, 2001). Our results indicate that especially the highest daily intake categories of TNCO values are associated with an increased risk of BC compared with the lower categories. Tar in cigarette smoke is associated with cancer risk because of its high concentration of polycyclic aromatic hydrocarbons, oxidants and free radicals, which all play an important role in inducing DNA damage, possibly leading up to carcinogenesis (Van Schooten *et al.*, 1997; International Association for Reseach on Cancer, 2010).

The results might be driven by the number of cigarettes smoked and to a lesser extent by TNCO values of cigarettes as we did not observe any meaningful differences in BC risk between smokers of low-tar/low-nicotine and high-tar/high-nicotine cigarettes, although this analysis was underpowered because of the low number of controls smoking low-tar/low-nicotine cigarettes ($n=7$).

Table 2 Adjusted and unadjusted odds ratios estimating non-muscle-invasive bladder cancer risk for daily tar, nicotine and carbon monoxide intake and cigarette type comparing ever smokers with never smokers

	Cases in cohort	Controls in cohort	OR (95% CI) crude	OR (95% CI) multivariate adjusted model*
Never smoker	127	59	1.00 (reference)	1.00 (reference)
Ever smoker	448	71	2.93 (1.97–4.36)	2.14 (1.11–4.11)
Tar (mg/day)				
< 100	130	30	2.01 (1.22–3.33)	1.57 (0.78–3.15)
100 ≤ 200	154	21	3.41 (1.96–5.91)	2.73 (1.23–6.03)
> 200	161	19	3.94 (2.23–6.94)	3.00 (1.36–6.63)
P-value for linear trend			< 0.001	0.007
Nicotine (mg/day)				
< 5	70	18	1.81 (0.99–3.30)	1.48 (0.69–3.18)
5 ≤ 10	93	16	2.70 (1.46–4.99)	2.02 (0.90–4.55)
10 ≤ 15	113	15	3.50 (1.88–6.51)	2.71 (1.15–6.41)
> 15	169	21	3.74 (2.16–6.47)	2.85 (1.32–6.19)
P-value for linear trend			< 0.001	0.030
CO (mg/day)				
< 50	68	16	1.97 (1.06–3.69)	1.62 (0.73–3.56)
50 ≤ 100	71	14	2.36 (1.23–4.52)	1.69 (0.74–3.83)
100 ≤ 150	103	14	3.42 (1.81–6.47)	2.76 (1.15–6.61)
> 150	203	26	3.63 (2.17–6.05)	2.75 (1.30–5.84)
P-value for linear trend			< 0.001	0.034
Ever smoker cigarette type				
Low tar/low nicotine	52	7	3.45 (1.48–8.05)	2.80 (0.97–8.06)
High tar/high nicotine	396	64	2.87 (1.91–4.32)	2.14 (1.11–4.12)
P-value			0.667	0.544

CI, confidence interval; CO, carbon monoxide; OR, odds ratio.
*Adjusted for age, sex and smoking duration.

Table 3 Adjusted and unadjusted odds ratios estimating muscle-invasive bladder cancer risk for daily tar, nicotine and carbon monoxide intake and cigarette type comparing ever smokers with never smokers

	Cases in cohort	Controls in cohort	OR (95% CI) crude	OR (95% CI) multivariate adjusted model*
Never smoker	31	59	1.00 (reference)	1.00 (reference)
Ever smoker	108	71	2.90 (1.71–4.91)	1.82 (0.79–4.21)
Tar (mg/day)				
< 100	33	30	2.09 (1.08–4.04)	1.31 (0.52–3.28)
100 ≤ 200	28	21	2.54 (1.24–5.18)	1.42 (0.51–3.99)
> 200	44	19	4.41 (2.21–8.80)	2.88 (1.10–7.55)
P-value for linear trend			< 0.001	0.046
Nicotine (mg/day)				
< 5	19	18	1.89 (0.92–4.37)	1.30 (0.48–3.50)
5 ≤ 10	19	16	2.26 (1.02–5.00)	1.26 (0.43–3.70)
10 ≤ 15	19	15	2.41 (1.08–5.39)	1.34 (0.43–4.20)
> 15	48	21	4.35 (2.22–8.52)	2.75 (1.07–7.11)
P-value for linear trend			< 0.001	0.105
CO (mg/day)				
< 50	18	16	2.14 (0.96–4.77)	1.40 (0.51–3.83)
50 ≤ 100	17	14	2.31 (1.01–5.30)	1.19 (0.39–3.60)
100 ≤ 150	12	14	1.63 (0.67–3.95)	0.96 (0.29–3.16)
> 150	58	26	4.25 (2.25–8.01)	2.60 (1.03–6.56)
P-value for linear trend			< 0.001	0.061
Ever smoker cigarette type				
Low tar/low nicotine	13	7	3.53 (1.27–9.77)	2.69 (0.73–9.84)
High tar/high nicotine	95	64	2.83 (1.64–4.84)	1.80 (0.77–4.18)
P-value			0.265	0.449

CI, confidence interval; CO, carbon monoxide; OR, odds ratio.
*Adjusted for age, sex and smoking duration.

Although smokers of low-tar/low-nicotine cigarettes did not smoke more cigarettes than high-tar/high-nicotine cigarette smokers, they might have altered their smoking behaviour (e.g. larger puff volume or more puffs) to increase nicotine intake (Scherer, 1999), as has been observed in other studies. Our estimates of daily TNCO intake might be confounded by this compensation behaviour, but could not be corrected for as detailed smoking behaviour data were not collected.

Furthermore, the controls were selected from the BCPP cohort in which all participants were under suspicion of BC at inclusion. Therefore, the control group included individuals with chronic urothelial inflammation (Michaud, 2007) and benign papilloma (including some inverted papillomas) (Picozzi *et al.*, 2013), which could be considered risk factors for BC development. Hence, the presented ORs are probably underestimated because our control group is more similar to the case group than a

hypothetical, completely healthy control group because of the presence of these risk factors.

Conclusion

High daily intake of TNCO increases NMIBC risk compared to low daily intake. However, it remains unclear whether smoking behaviour or cigarette type causes this association. More research with larger sample sizes is needed to corroborate these results and to shed light on whether smoking behaviour outplays cigarette content in determining BC risk.

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Conflicts of interest

There are no conflicts of interest.

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