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Influence of pre-diagnostic cigarette smoking on colorectal cancer survival: overall and by tumour molecular phenotype

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Background: Smoking is a risk factor for incident colorectal cancer (CRC); however, it is unclear about its influence on survival after CRC diagnosis.

Methods: A cohort of 706 CRC patients diagnosed from 1999 to 2003 in Newfoundland and Labrador, Canada, was followed for mortality and recurrence until April 2010. Smoking and other relevant data were collected by questionnaire after cancer diagnosis, using a referent period of '2 years before diagnosis' to capture pre-diagnosis information. Molecular analyses of microsatellite instability (MSI) status and *BRAF* V600E mutation status were performed in tumour tissue using standard techniques. Multivariate hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated with Cox proportional hazards regression, controlling for major prognostic factors.

Results: Compared with never smokers, all-cause mortality (overall survival, OS) was higher for current (HR: 1.78; 95% CI: 1.04–3.06), but not for former (HR: 1.06; 95% CI: 0.71–1.59) smokers. The associations of cigarette smoking with the study outcomes were higher among patients with $\geqslant$40 pack-years of smoking (OS: HR: 1.72; 95% CI: 1.03–2.85; disease-free survival (DFS: HR: 1.99; 95% CI: 1.25–3.19), those who smoked $\geqslant$30 cigarettes per day (DFS: HR: 1.80; 95% CI: 1.22–2.67), and those with microsatellite stable (MSS) or MSI-low tumours (OS: HR: 1.38; 95% CI: 1.04–1.82 and DFS: HR: 1.32; 95% CI: 1.01–1.72). Potential heterogeneity was noted for sex (DFS HR: 1.68 for men and 1.01 for women: P for heterogeneity = 0.04), and age at diagnosis (OS: HR: 1.11 for patients aged $<$60 and 1.69 for patients aged $\geqslant$60: P for heterogeneity = 0.03).

Conclusions: Pre-diagnosis cigarette smoking is associated with worsened prognosis among patients with CRC.

Despite the well-established connection between cigarette smoking and pre-mature mortality, $>$16% of Canadians over the age of 15 smoke. Smoking is clearly associated with malignancies in the respiratory tract (Botteri *et al*, 2008). The IARC 2009 monograph on smoking and cancer added the colorectum to the list of smoking-associated cancer sites (IARC, 2012); however, some

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recent evidence suggests that smoking is differentially associated with certain tumour molecular phenotypes of colorectal cancer (CRC), such as tumours that display microsatellite instability (MSI), *BRAF* V600E mutation, or the CpG island methylator phenotype (CIMP) (Samowitz *et al*, 2006; Curtin *et al*, 2009; Poynter *et al*, 2009; Limsui *et al*, 2010; Ogino *et al*, 2011; Nishihara *et al*, 2013).

The influence of smoking on CRC survival is inconclusive (Curtin *et al*, 2009; Limsui *et al*, 2010; Ogino *et al*, 2011). Some studies (Munro *et al*, 2006; Phipps *et al*, 2011) have suggested that cigarette use is strongly associated with reduced survival among CRC patients, whereas other studies have reported no significant differences in survival rates between smokers and never smokers with CRC (Yu *et al*, 1997; Rohan *et al*, 2000; Park *et al*, 2006). The apparent discrepancy between studies may be attributed to the long induction period of CRC (Botteri *et al*, 2008), as well as the potential for modulating effects of important prognostic variables (Newcomb *et al*, 2007; Guastadisegni *et al*, 2010; Shaukat *et al*, 2011), many of which were not accounted for in previous studies. For example, the MSI-high phenotype and the somatic *BRAF* V600E mutation are strongly associated with both smoking status (Limsui *et al*, 2010) and CRC prognosis (Samowitz *et al*, 2005; Guastadisegni *et al*, 2010; Shaukat *et al*, 2010), thus a potential interaction between smoking and these tumour phenotypes should be appreciated. However, to date, only one study has explored the potential interaction between smoking and molecular tumour phenotype on mortality among CRC patients. This study showed a prominent association between smoking and CRC-specific mortality among patients whose tumours exhibited the MSI-H phenotype (Phipps *et al*, 2011).

We investigated the association of smoking with all-cause (overall survival, OS) and disease-free survival (DFS) in an incident cohort of 750 invasive CRC patients from the province of Newfoundland and Labrador (NL), Canada. We further assessed potential interactions of smoking with sex, age at diagnosis, tumour stage at diagnosis, MSI status, and *BRAF* mutation status on mortality.

SUBJECTS AND METHODS

Study participants. A detailed description of the study cohort has been published elsewhere (Woods *et al*, 2010). In brief, participants were incident CRC patients identified through the population-based Newfoundland and Labrador Colorectal Cancer Registry (NFCCR). Eligibility criteria included patients who were newly diagnosed with pathologically confirmed, invasive CRC (ICD-9 codes: 153.0–153.9, 154.0–154.3, and 154.8 or ICD-10 codes: 18.0–18.9, 19.9, and 20.9) from 1999 to 2003, and aged 20–75 years at the time of diagnosis. Seven hundred and fifty consenting patients (64% of all eligible patients) completed and returned detailed epidemiologic questionnaires (a personal history questionnaire (PHQ), a food frequency questionnaire (FFQ), and a family history questionnaire (FHQ)). Patients were also asked to donate a blood sample and for permission to access their archived tumour tissue and medical records. Exclusions from this analysis were made if patients had unknown clinical outcome or smoking status ($n=41$), or provided insufficient information on other critical prognostic factors ($n=3$). Thus, the final cohort consisted of 706 eligible participants. Ethics approval for this study was received from the Human Investigation Committee of Memorial University of Newfoundland.

Exposure assessment and baseline information collection. The PHQ and the FFQ were administered at baseline only, with a referent period of '2 years before diagnosis' to capture pre-diagnosis information. The epidemiologic questionnaires included items regarding age, sex, marital status, education attainment, medical history, bowel screening history, physical activity, reproductive factors (women only), and alcohol and tobacco use. As baseline, participants were asked whether they had smoked at least one cigarette a day for 3 months or longer in their life. Participants who responded 'yes' were then asked about the age at which they started smoking, their usual number of cigarettes smoked per day, the duration (years/months) during which they smoked and, where applicable, the relevant information for when they quit smoking. For this analysis, cigarette smoking was represented by categories of smoking status (never, current, or former—with the referent period of 2 years before CRC diagnosis), years of smoking (none, <20, 20–29, and $\geqslant 30$), cigarettes daily (none, <20, 20–29, and $\geqslant 30$), years of abstention (non-smoker, <10, 10–29, and $\geqslant 30$), and lifetime cigarette pack-years (none, 20, 20–39, and $\geqslant 40$; calculated as the average number of cigarettes smoked per day divided by 20 and multiplied by the number of years smoked) (Zhao *et al*, 2010). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in metres. Clinical and pathologic data (e.g., tumour stage at diagnosis) were abstracted from pathology reports and medical records.

Study outcomes. The cohort was followed up for mortality and recurrence from the date of CRC diagnosis to April 2010. During this period, the FHQ was distributed to participants for the second time to collect information on additional cancer diagnosis and recurrence in their family. If a patient was deceased, then a close proxy was asked to participate (Woods *et al*, 2010). Information on vital status (i.e., death, recurrence, and metastasis) was gathered from follow-up questionnaires, local newspapers, death certificates, autopsy, pathology, radiology, surgical reports, as well as from physicians' notes. Additional data were collected from the Dr H. Bliss Murphy Cancer Care Foundation (2012). For the purposes of this analysis, OS was the primary outcome, defined as time from CRC diagnosis to death from all causes. The secondary outcome, DFS, was measured from the date of cancer diagnosis to the date of death, recurrence, or metastasis (whichever came first). Patients who were still alive or who did not have a recurrence or a metastasis by the end of the follow-up period were censored at the time of last contact.

Molecular assessment. Molecular analyses for MSI and *BRAF* V600E mutation were performed using standard protocols as described previously (Loughrey *et al*, 2007; Raptis *et al*, 2007; Campbell *et al*, 2010). Briefly, for MSI analyses, both tumour DNA and normal DNA were amplified by PCR with a panel of 10 microsatellite markers: BAT25, BAT26, BAT40, BAT34C4, D5S346, D17S250, ACTC, D18S55, D10S197, and MYCL (Raptis *et al*, 2007; Campbell *et al*, 2010). The appearance of a discordant number of bands between tumour and normal DNA was interpreted as instability (Raptis *et al*, 2007; Campbell *et al*, 2010). Tumours were classified as MSI-high if 30% or more of the repeats were unstable and MS-stable/MSI-Low if less than 30% of the repeats demonstrated instability (Phipps *et al*, 2011). Exon 15 of the *BRAF* gene, spanning the mutational hotspot c.1799T>A (p.Val600Glu), was amplified by PCR using *BRAF* V600E allele-specific primers, followed by direct automatic sequencing to verify the mutations (Loughrey *et al*, 2007). Detailed descriptions of each assay, including the primer sequences and PCR conditions, are provided in earlier studies from this cohort (Loughrey *et al*, 2007; Raptis *et al*, 2007; Campbell *et al*, 2010; Woods *et al*, 2010).

Statistical analysis. Group comparisons were performed with Dunnett's tests for continuous variables and Pearson's chi-square tests of independence for categorical variables (McCleary *et al*, 2010). The Kaplan–Meier technique was applied to graphically delineate overall and stratified survival distributions (Thrift *et al*, 2011). Proportional hazards models were used to estimate the

impact of smoking on mortality among CRC patients, while adjusting for covariates. Hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) were calculated for categories of exposure, using never smokers as the reference group. Subjects with missing data on any smoking exposure variable were only excluded for specific smoking-related variable analysis. In the selection approach of the multivariate models, we assessed an extensive list of potential confounders, including demographic variables, diet, lifestyle factors, treatment, clinicopathologic, and a series of molecular predictors. Factors were considered for inclusion in the multivariable Cox model if the log-rank test had a *P*-value of 0.2 or less in the univariate setting (Mantel, 1966).

Table 1. Selected demographic and clinicopathologic characteristics of study population, by smoking status at baseline

	Never smoker	Former smoker		Current smoker	
	No. (%)	No. (%)	*P*-value[a]	No. (%)	*P*-value[a]
Age at diagnosis (years)[b]	60.7 ± 9.6	61.1 ± 8.5	0.84	56.2 ± 9.4	0.0004
Sex					
Women	120 (60.0)	110 (29.9)		27 (36.5)	
Men	80 (40.0)	258 (70.1)	<0.0001	47 (63.5)	0.001
BMI (kg m^{-2})					
<25.0	62 (32.6)	89 (24.8)		27 (39.1)	
25.0–29.9	83 (43.7)	150 (41.8)		26 (37.7)	
⩾30	45 (23.7)	120 (33.4)	0.03	16 (23.2)	0.003
Marital status					
Single	57 (28.6)	63 (17.2)		27 (36.5)	
Married or living as married	142 (71.4)	303 (82.8)	0.002	47 (63.5)	0.21
Tumour site					
Colon	137 (68.8)	244 (66.3)		43 (58.1)	
Rectum	62 (31.2)	124 (33.7)	0.54	31 (41.9)	0.10
Tumour stage at diagnosis					
I/II	100 (50.0)	186 (50.5)		46 (62.2)	
III/IV	100 (50.0)	182 (49.5)	0.90	28 (37.8)	0.07
Alcohol drinking					
No	130 (65.0)	96 (26.1)		14 (18.9)	
Yes	70 (35.0)	272 (73.9)	<0.0001	60 (81.1)	<0.001
Family history of CRC					
No	178 (89.0)	327 (88.9)		66(89.2)	
Yes	22 (11.0)	41 (11.1)	0.96	8 (10.8)	0.96
Reported screening procedure					
No	176 (88.0)	316 (85.9)		69 (93.2)	
Yes	24 (12.0)	52 (14.1)	0.48	5 (6.8)	0.21
Reported chemoradiotherapy					
No	142 (71.0)	306 (83.2)		60 (81.1)	
Yes	58 (29.0)	62 (16.8)	0.001	14 (18.9)	0.09
MSI status					
MSS/MSI-L	171 (89.1)	312 (90.2)		51 (78.5)	
MSI-H	21 (10.9)	34 (9.8)	0.68	14 (21.5)	0.03
BRAF mutation status					
Wild type	164 (88.2)	289 (88.7)		58 (90.6)	
V600E mutant	22 (11.8)	37 (11.3)	0.87	6 (9.4)	0.59

Abbreviations: BMI = body mass index; CRC = colorectal cancer; MSI = microsatellite instability; MSI-H = microsatellite instability-high; MSS/MSI-L = microsatellite stable/microsatellite instability-low.

[a]*P*-values are for the significance of the Dunnett's test for continuous variables and of the chi-square test for categorical variables.

[b]Continuous variables presented as mean ± s.d. (standard deviation).

Table 2. Hazard rate ratios associated with overall and disease-free colorectal cancer survival for cigarette smoking exposures

	Overall survival				Disease-free survival			
	No. of events[a]/ No. at risk[b]	Overall CRC HR (95% CI)[c]	Colon cancer HR (95% CI)[c]	Rectal cancer HR (95% CI)[c]	No. of events[a]/ No. at risk[b]	Overall CRC HR (95% CI)[c]	Colon cancer HR (95% CI)[c]	Rectal cancer HR (95% CI)[b]
Cigarette status								
Non-smoker	90/200	1.00	1.00	1.00	97/200	1.00	1.00	1.00
Ever-smoker	248/506	1.25 (0.84–1.88)	1.52 (0.91–2.54)	0.90 (0.45–1.82)	272/505	1.30 (0.90–1.88)	1.57 (0.97–2.50)	0.87 (0.46–1.63)
Former	151/368	1.06 (0.71–1.59)	1.46 (0.87–2.45)	0.80 (0.38–1.67)	172/367	1.21 (0.83–1.77)	1.50 (0.93–2.43)	0.82 (0.43–1.57)
Current	33/74	1.78 (1.04–3.06)	2.34 (1.01–5.45)	1.23 (0.52–2.93)	36/74	1.69 (0.99–2.84)	2.03 (0.95–4.33)	1.11 (0.48–2.55)
Cigarette years								
<20	52/117	1.11 (0.66–1.87)	1.11 (0.56–2.18)	1.17 (0.50–2.72)	58/117	1.26 (0.77–2.03)	1.29 (0.67–2.45)	1.02 (0.49–2.16)
20–29	53/107	1.13 (0.67–1.89)	1.50 (0.79–2.83)	0.73 (0.29–1.81)	57/107	1.22 (0.75–2.00)	1.61 (0.87–2.99)	0.63 (0.28–1.43)
⩾30	110/226	1.28 (0.83–1.97)	1.45 (0.85–2.48)	0.97 (0.45–2.10)	122/225	1.53 (1.01–2.34)	1.73 (1.03–2.93)	1.01 (0.52–1.99)
P trend[d]		0.05	0.09	0.62		0.08	0.02	0.76
Cigarettes daily								
<20	75/186	0.95 (0.60–1.52)	1.16 (0.66–2.05)	0.70 (0.30–1.62)	83/185	0.88 (0.64–1.22)	1.03 (0.70–1.52)	0.65 (0.31–1.38)
20–29	99/193	1.38 (0.88–2.17)	1.47 (0.82–2.63)	1.13 (0.53–2.41)	105/193	1.05 (0.77–1.44)	1.17 (0.79–1.72)	0.85 (0.43–1.70)
⩾30	45/77	1.41 (0.79–2.54)	1.93 (0.92–4.06)	0.83 (0.31–2.19)	53/77	1.80 (1.22–2.67)	2.12 (1.26–3.57)	1.66 (0.73–3.77)
P trend[d]		0.12	0.02	0.95		0.21	0.15	0.36
Pack-years of smoking								
<20	117/257	0.96 (0.61–1.50)	1.06 (0.60–1.87)	0.86 (0.40–1.87)	126/257	1.03 (0.67–1.57)	1.20 (0.70–2.08)	0.72 (0.36–1.43)
20–39	67/135	1.30 (0.81–2.07)	1.43 (0.79–2.57)	1.02 (0.45–2.31)	73/134	1.37 (0.88–2.13)	1.67 (0.95–2.92)	0.87 (0.41–1.83)
⩾40	64/114	1.72 (1.03–2.85)	2.08 (1.11–3.87)	1.07 (0.43–2.66)	73/114	1.99 (1.25–3.19)	2.45 (1.34–4.46)	1.26 (0.57–2.75)
P trend[d]		0.08	0.06	0.47		0.07	0.03	0.48
Years of abstention[e]								
⩾30	28/69	1.10 (0.63–1.95)	1.13 (0.56–2.29)	1.08 (0.41–2.87)	31/69	1.21 (0.71–2.05)	1.21 (0.62–2.37)	1.27 (0.53–3.03)
10–29	66/162	1.26 (0.79–2.00)	1.49 (0.86–2.58)	0.67 (0.27–1.64)	75/162	1.36 (0.88–2.10)	1.76 (1.02–3.04)	0.75 (0.36–1.55)
<10	32/77	1.28 (0.74–2.20)	1.49 (0.76–2.93)	0.87 (0.33–2.34)	37/76	1.42 (0.85–2.36)	1.77 (0.92–3.41)	0.87 (0.40–1.91)
P trend[d]		0.03	0.06	0.42		0.03	0.05	0.47

Abbreviations: BMI = body mass index; CI = confidence interval; CRC, colorectal cancer; HR = hazard rate ratios.
[a]Events are defined as deaths for overall survival and death, recurrence, or metastasis (whichever occurred earliest) for disease-free survival.
[b]Subjects with missing data on any smoking exposure variables are excluded but for specific smoking-related variable analysis only.
[c]Cox proportional hazard model adjusted for sex, age at diagnosis, stage at diagnosis, BMI, marital status, alcohol consumption, intake of fruits, family history, reported screening procedure, reported chemoradiotherapy, and MSI status, where appropriate.
[d]Linear trend tested by modelling the ordinal variables of exposure as a continuous variable.
[e]Excludes current smokers.

Only terms that entered the model at $P<0.1$, or altered the effect estimates by 10% or more, or improved the fit of the models were retained for the final models (Thrift *et al*, 2011). The final list of potential confounders included in the model was based on both backwards selection and the literature, including sex, age at diagnosis, BMI, stage at diagnosis, marital status, alcohol consumption, fruit intake, family history of CRC, reported chemoradiotherapy, and MSI status. The proportional hazards assumption was verified by checking the parallelism of the Kaplan–Meier curves and by testing the statistical significance of time-dependent covariates when included in the model (Statistical Consulting Group, 2012). Evidence of linear trends was tested by modelling ordinal variables of exposure as a continuous variable in a linear regression (Woodward, 2005; Zhao *et al*, 2010). Potential interactions were evaluated by including interaction terms between smoking and respective stratification variable in the model with the Wald test. Statistical significance was conducted at two-sided $P<0.05$. All calculations were performed with the SAS software version 9.2 (SAS Institute Inc, Cary, NC, USA).

RESULTS

By the end of the follow-up period, there was a maximum of 10.9 years of observation and 338 deaths from all causes. At baseline, 506 patients were ever smokers and 200 patients were non-smokers. Among those with available molecular data, MSI-high was observed in 11.4% ($n=69$) of 603 tumours. The *BRAF* V600E mutation was found in 65 (11.2%) of 576 CRCs.

Baseline characteristics by smoking status. Current smokers were slightly younger, leaner, consumed more alcohol, more likely to be men, and showed a greater proportion of MSI-high tumours compared with never smokers (21.5% *vs* 10.9%, $P=0.03$) (Table 1). Similarly, most former smokers were men, married or living as married, and reported greater alcohol consumption and less chemoradiotherapy use relative to never smokers.

Pre-diagnostic smoking and mortality. Current smoking (HR: 1.78; 95% CI: 1.04–3.06) was statistically significantly associated

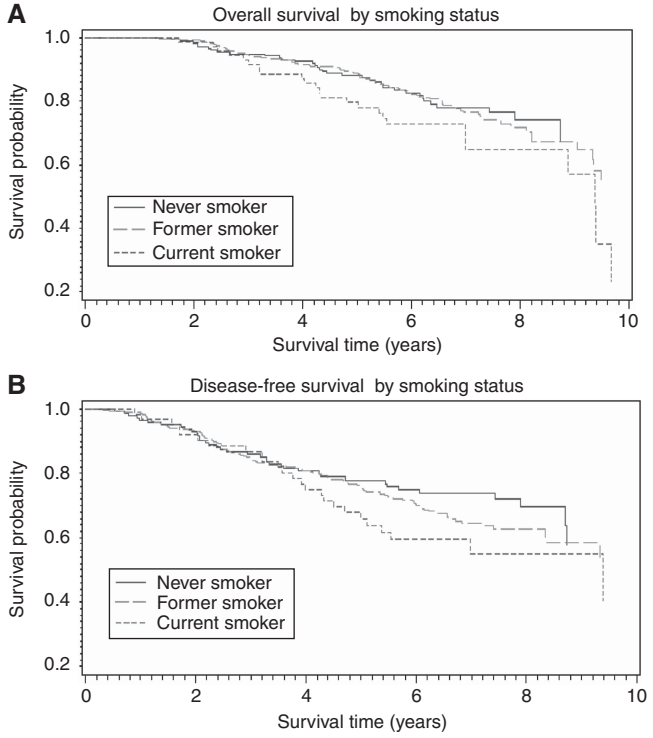

Figure 1. Survival curves for (**A**) overall survival and (**B**) disease-free survival by smoking status. Adjusted for sex, age at diagnosis, stage at diagnosis, BMI, marital status, alcohol consumption, family history, reported chemoradiotherapy, and MSI status.

with higher risk of all-cause mortality in multivariable models (Table 2; Figure 1). The higher risks of mortality from smoking persisted in more detailed definitions of the exposure, including pack-years, cigarettes daily, and years of smoking, although, for the latter two variables, the risk estimates of being in the highest quartile of exposure did not quite attain statistical significance at the 0.05 level. Moreover, there was a stepwise gradient of decreasing risk of mortality with increasing years of abstention for former smokers (P for trend = 0.03). Similarly, when DFS was the outcome, the HRs were elevated in the groups who had a smoking history of $\geqslant$30 years (HR: 1.53; 95% CI: 1.01–2.34), individuals who smoked $\geqslant$30 cigarettes per day (HR: 1.80; 95% CI: 1.22–2.67), and those with $\geqslant$40 pack-years of smoking (HR: 1.99; 95% CI: 1.25–3.19). When the data were stratified by tumour site, smoking was associated with worse prognosis for patients diagnosed with colon cancer, but not for rectal cancer (Table 2).

Interactions between smoking and demographic or tumour characteristics. The multivariable models were repeated for smoking status between strata defined by demographic and tumour characteristics (Table 3). The P-values for heterogeneity were statistically significant between strata of sex for DFS ($P = 0.04$) and age at diagnosis for OS ($P = 0.03$). More specifically, the risk of mortality associated with ever smoking seemed limited to men (DFS: HR: 1.68; 95% CI: 1.16–2.44) and to patients aged $\geqslant$60 years at CRC diagnosis (OS: HR: 1.69; 95% CI: 1.20–2.40). Although the interaction terms were not statistically significant, the impacts of smoking on mortality were more marked for patients diagnosed at earlier disease stages (OS: HR: 1.83; 95% CI: 1.07–3.14 and DFS: HR: 1.70; 95% CI: 1.04–2.78) than for those diagnosed at advanced stages (OS: HR: 1.19; 95% CI: 0.87–1.62 and DFS: HR: 1.16; 95% CI: 0.86–1.57), and for patients with microsatellite stable/MSI-low (MSS/MSI-low) tumours (OS: HR: 1.38; 95% CI: 1.04–1.82 and DFS: HR: 1.32; 95% CI: 1.01–1.72) than for those with cancers exhibiting MSI-high (OS: HR: 1.04; 95% CI: 0.28–3.95 and DFS: HR: 1.24; 95% CI: 0.37–4.14).

DISCUSSION

We examined smoking status and gradients of smoking duration/intensity in relation to OS and DFS in a cohort of over 700 CRC patients. Pre-diagnostic smoking was associated with higher risk of all-cause mortality and poorer DFS. Evidence suggests that the association between smoking and decreased survival was restricted to patients diagnosed with colon, and not rectal, cancer. Our results are consistent with the findings from a recent study in Washington State (Phipps *et al*, 2011). Those authors reported that CRC patients who currently smoke have a significantly higher disease-specific (HR: 1.30; 95% CI: 1.09–1.74) and all-cause (HR: 1.51; 95% CI: 1.24–1.83) mortality than non-smokers. These authors also reported higher associations of smoking with mortality for patients diagnosed with colon cancer than for rectal cancer. Likewise, a study from the UK (Munro *et al*, 2006) on a cohort of CRC patients receiving curative surgery found a significantly worse cause-specific survival in active smokers compared with non-smokers (HR: 2.55; 95% CI: 1.40–4.64). Notably, current smoking, but not former smoking, was associated with poorer survival.

There are several biologic mechanisms that may explain the higher mortality among CRC patients who smoke. First, tobacco smoking may mutate the *GSTM1* gene, resulting in impaired detoxification of tobacco carcinogens (McCleary *et al*, 2010); these carcinogens may exert growth promoting effects to residual tumour cells, either through resistance to chemotherapy or through promotion of angiogenesis (Ye *et al*, 2005; Munro *et al*, 2006). Smoking may also induce aberrant promoter DNA methylation, thus silencing regulatory genes (e.g., *ECAD*, *p16*, *MGMT*, and *DAPK*) in tumor progression (Russo *et al*, 2005). Possible explanations for the differential associations by subsite include the higher concentration of tobacco carcinogens in the colon and the longer contact time of tobacco constituent-carrying feces with the colon, where they are mainly stored, than the rectum (Batty *et al*, 2008).

In this study, cigarette smoking was associated with decreased survival only among male patients. It is important to note that the prevalence of smoking in men is higher than in women in Newfoundland and Labrador (Statistics Canada, 2013); and it is plausible that we were underpowered to detect smaller associations in women.

Our findings suggest that smoking has a negligible impact on survival for those diagnosed with advanced-stage disease, perhaps because patient outcomes are inherently poor for this patient population irrespective of smoking status. Intriguingly, smoking was observed to be significantly associated with poorer survival in patients diagnosed with early-stage disease, providing further support for the recommendation that newly diagnosed patients with less advanced CRC should consider immediate smoking cessation (Kobrinsky *et al*, 2003).

Smoking is strongly associated with specific somatic molecular alterations (e.g., MSI-high, CIMP, and the *BRAF* V600E mutation) (Curtin *et al*, 2009; Poynter *et al*, 2009; Limsui *et al*, 2010; Wish *et al*, 2010; Ogino *et al*, 2011; Nishihara *et al*, 2013). As these alterations are also related to OS, it is important to evaluate the influence of smoking on survival stratified by molecular features of tumour. However, we do not have CIMP status in this study. Our study is among the first to assess possible interactions between MSI status, *BRAF* V600E mutation status, and smoking on both OS and DFS for CRC patients. We found that ever smoking was associated with higher risk of mortality among patients diagnosed with MSS/MSI-low tumours, whereas smoking had little impact on patients diagnosed with MSI-high tumours (Table 3).

Table 3. Overall and disease-free colorectal cancer survival in relation to cigarette smoking by sex, age at diagnosis, stage at diagnosis, MSI, and BRAF V600E mutation status

	Never smoker		Ever smoker		
	Events[a]/ at risk	HR[b] (95% CI)	Events[a]/ at risk	HR[b] (95% CI)[a]	*P* for heterogeneity[c]
Overall survival					
Sex					
Men	36/80	1.00	183/350	1.65 (1.12–2.44)	
Women	54/120	1.00	65/156	1.16 (0.77–1.76)	0.12
Age at diagnosis					
<60	37/85	1.00	94/217	1.11 (0.72–1.71)	
⩾60	53/115	1.00	154/289	1.69 (1.20–2.40)	0.03
Stage at diagnosis					
I/II	21/100	1.00	79/241	1.83 (1.07–3.14)	
III/IV	69/100	1.00	169/265	1.19 (0.87–1.62)	0.14
MSI status					
MSS /MSI-L	82/171	1.00	220/420	1.38 (1.04–1.82)	
MSI-H	5/21	1.00	11/51	1.04 (0.28–3.95)	0.24
BRAF mutation status					
Wild type	72/164	1.00	197/397	1.15 (0.75–1.77)	
V600E mutant	13/22	1.00	27/54	1.65 (0.42–6.53)	0.42
Disease-free survival					
Sex					
Men	38/80	1.00	202/350	1.68 (1.16–2.44)	
Women	59/120	1.00	70/155	1.01 (0.69–1.48)	0.04
Age at diagnosis					
<60	41/85	1.00	108/217	1.11 (0.74–1.66)	
⩾60	56/115	1.00	164/288	1.61 (1.15–2.26)	0.08
Stage at diagnosis					
I/II	25/100	1.00	92/240	1.70 (1.04–2.78)	
III/IV	72/100	1.00	180/265	1.16 (0.86–1.57)	0.10
MSI status					
MSS /MSI-L	89/171	1.00	239/419	1.32 (1.01–1.72)	
MSI-H	5/21	1.00	16/51	1.24 (0.37–4.14)	0.82
BRAF mutation status					
Wild type	78/164	1.00	216/396	1.23 (0.83–1.82)	
V600E mutant	14/22	1.00	30/54	1.45 (0.44–4.82)	0.52

Abbreviations: BMI, body mass index; CI = confidence interval; HR = hazard rate ratio; MSI = microsatellite instability; MSI-H = microsatellite instability-high; MSI-L = microsatellite instability-low; MSS = microsatellite stable; .

[a]Events are defined as deaths for overall survival and death, recurrence, or metastasis (whichever occurred earliest) for disease-free survival.

[b]Cox proportional hazard model adjusted for sex, age at diagnosis, stage at diagnosis, BMI, marital status, alcohol consumption, and MSI status, where appropriate.

[c]*P* for heterogeneity is the significance of interaction term between smoking and respective stratification variable, calculated from the Wald test.

MSI-high tumours generally have a more favourable prognosis relative to MSS/MSI-low tumours, independent of stage, grade, and other prognostic variables (Guastadisegni *et al*, 2010). To our knowledge, only one previous study on smoking and CRC survival has considered potential effect modification by molecular phenotypes of tumour (Phipps *et al*, 2011). Phipps *et al* (2011) reported a prominent association between smoking and disease-specific mortality in CRC patients with MSI-H tumours (HR: 3.83; 95% CI: 1.32–11.11). The reason for this discrepancy in results is unclear, but may relate to population differences or chance. These findings underscore the need for large, collaborative Molecular Pathological Epidemiology (MPE) studies of smoking and CRC aetiology to better understand the potential heterogeneous nature of smoking and colorectal carcinogenesis.

This study has both strengths and limitations. The relatively large sample size allowed us to perform stratified analyses. The availability of detailed information on personal, clinicopathologic, and molecular characteristics also allowed us to assess potential confounders, effect modifiers, and sources of potential heterogeneity. Limitations to this study include a lack of information on the cause of death for all deceased participants. The observed differences in OS and DFS could be deaths from causes other than CRC. However, the cause of death, classified according to the ICD codes, was obtained for 200 of 338 deceased patients in this cohort. Of these, the majority (86%) had died from CRC, which is in line with other studies (Jones *et al*, 2007; Riihimäki *et al*, 2012). Second, smoking is self-reported by respondents from the distant past, which leaves open the potential for recall bias; however, recent studies have generally shown strong agreement between smoking behaviours when reported over similar, and longer, time spans to the current study (Brigham *et al*, 2010). Self-reported cigarette consumption has been shown to be accurate in current smokers but may be under-reported in some never smokers (Martinez *et al*, 2004; McCleary *et al*, 2010); such misclassification should be non-differential and therefore bias the study results towards the null (McCleary *et al*, 2010). In addition, cigarette use after diagnosis was not updated in this study; hence, we were unable to assess the potential impacts of post-diagnosis changes in smoking habits on survival. Regardless, we were interested in the effect of pre-diagnosis exposures on survival among CRC patients. Finally, this study additionally involved analyses of associations stratified by tumour subtypes, thus increasing the probability of false positive findings committing a type I error due to multiple testing (Ogino *et al*, 2011); therefore, some results in this study should be taken as merely suggestive of potential biological or clinical associations. This underscores the need for our results to be confirmed in future large MPE studies (Ogino and Stampfer, 2010).

In conclusion, pre-diagnosis cigarette smoking was independently predictive of worse survival after CRC diagnosis. Results from this prospective, population-based study underscore the importance of cultivating healthy lifestyle habits. This study presents preliminary results concerning potential interactions between smoking, clinicopathologic features, tumour molecular phenotype, and CRC survival. Confirmation of these findings is needed in other large studies and further analyses using tobacco-specific DNA adducts as quantitative measurements of exposure are warranted.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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