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Serum transforming growth factor- β 1 and risk of pancreatic cancer in three prospective cohort studies

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Abstract

Purpose—Clinically evident chronic pancreatitis is a strong risk factor for pancreatic cancer. A small Japanese cohort study previously reported that pre-diagnostic serum transforming growth factor- β 1 (TGF- β 1) concentration, a potential marker of subclinical pancreatic inflammation, was associated with higher risk of pancreatic cancer. We further explored this association in a larger prospective study.

Methods—Serum TGF- β 1 concentrations were measured in pre-diagnostic samples from 729 pancreatic cancer cases and 907 matched controls from a cohort of Finnish male smokers (the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study) and two cohorts of U.S. men and women, the Cancer Prevention Study-II (CPS-II) and the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. Multivariable-adjusted odds ratios (ORs) were estimated using conditional logistic regression.

Results—Overall, serum TGF- β 1 concentration was not associated with a clear increase in pancreatic cancer risk (OR=1.36, 95% confidence interval (CI), 0.98-1.88 for highest vs. lowest quintile, p trend = 0.20). However, this association differed significantly by follow-up time ($p=0.02$). Serum TGF- β 1 concentration was not associated with risk during the first 10 years of follow-up, but was associated with higher risk during follow-up after 10 years (OR=2.13, 95% CI 1.23-3.68 for highest vs. lowest quintile, p trend=0.001). During follow-up after 10 years, serum TGF- β 1 was associated with higher risk only in the ATBC cohort, although most subjects were from ATBC during this time period and statistical evidence for heterogeneity across cohorts was limited ($p=0.14$).

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Conclusions—These results suggest that high serum TGF- β 1 may be associated with increased risk of pancreatic cancer although a long follow-up period may be needed to observe this association.

Keywords

pancreatic cancer; epidemiology; serum transforming growth factor-beta; cohort study

Introduction

Pancreatic cancer is a leading cause of cancer death and pancreatic cancer incidence and mortality rates are increasing in both the U.S. [1] and the European Union [2]. However, much about its etiology is unclear. Established common risk factors include smoking, diabetes, and obesity [3]. Identifying biomarkers associated with risk of pancreatic cancer may provide insights into its etiology.

One biomarker, serum transforming growth factor- β 1 (TGF- β 1) concentration, was associated with increased risk in a small case-control study nested within a Japanese cohort which included 85 cases of pancreatic cancer (OR=2.5, 95% CI 0.9-6.9 for highest vs. lowest quartile, p trend = 0.04) [4]. TGF- β 1 is a protein that plays an important role in many biological processes including cell proliferation and immune regulation [5]. TGF- β 1 signaling is important in pancreatic carcinogenesis and can have either tumor suppressive or tumor promoting effects depending on the setting [6,7]. However, it is unclear what mechanism explains the association between serum TGF- β 1 concentration and increased risk of pancreatic cancer observed in the Japanese cohort study [4].

One possible mechanism is that individuals with high serum TGF- β 1 are more likely to have pancreatic inflammation and therefore to be at increased risk of pancreatic cancer. This possibility is supported by evidence that clinically diagnosed chronic pancreatitis is associated with both elevated serum TGF- β 1 [8,9] and strongly increased risk of pancreatic cancer [10]. Although the role of subclinical pancreatic inflammation in pancreatic cancer etiology is less clear, it is plausible that subclinical pancreatic inflammation could also increase both serum TGF- β 1 and risk of pancreatic cancer.

We examined the association between serum TGF- β 1 concentration and subsequent risk of developing pancreatic cancer using cases and matched controls from 3 large prospective studies from the U.S. [11,12] and Finland [13]. Combining these studies provided a large study size for replicating the association observed in the Japanese cohort study [4] and for examining results stratified by smoking and time since blood draw.

Material and Methods

Study design and population

This analysis combined data from nested case-control analyses within three cohort studies, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) [13] and two U.S. cohorts, the Cancer Prevention Study-II (CPS-II) [12], and the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial [11]. The ATBC study included approximately

29,000 Finnish male smokers who provided a blood sample at enrollment between 1985 and 1988. The CPS-II study, enrolled in 1992-93, included a subset of approximately 40,000 men and women who provided a blood sample between 1998 and 2001. The screening arm of the PLCO study included approximately 77,000 men and women who provided a blood sample at enrollment between 1993 and 2001. Each cohort collected information on medical and lifestyle factors by questionnaire. All participants provided informed consent at blood draw. The ATBC and PLCO studies were approved by the National Cancer Institute institutional review board and the CPS-II study was approved by the Emory University institutional review board.

Case ascertainment varied between cohorts. In ATBC, cases were identified through the Finnish Cancer Registry [14]. Cases in CPS-II and PLCO were identified as previously described using linkage with state cancer registries and the National Death Index and self-reports subsequently verified with medical records or through linkage with state cancer registries [12,15]. Cancers were defined as in an earlier analysis [15] and included incident primary pancreatic adenocarcinoma but not endocrine pancreatic cancers, which are rare and may have a different etiology. Cases were diagnosed between blood draw and the end of the follow-up period, which was up to 23 years in ATBC, up to 8 years in CPS-II, and up to 15 years in PLCO. This analysis includes 729 cases, 348 cases from ATBC diagnosed from between 1986 and 2009, 90 cases from CPS-II diagnosed between 1999 and 2006, and 291 cases from PLCO diagnosed between 1995 and 2010.

One control was randomly selected for each case in the ATBC and CPS-II cohorts. In the PLCO cohort, two controls were randomly selected for each case diagnosed during approximately 1995-2005 and one control for each case diagnosed thereafter. All controls were alive and free from pancreatic cancer on the date their matched case was diagnosed. Controls were matched to cases on age at blood draw (± 5 years), date of blood draw (± 30 days for ATBC and within 2-3 month blocks for PLCO and CPS-II), sex, and race.

Measurement of serum TGF- $\beta 1$

Serum TGF- $\beta 1$ concentrations were measured in Dr. Michael Pollack's laboratory at the Lady Davis Institute for Medical Research in Montreal using an enzyme-linked immunosorbent assay (ELISA) with reagents from R&D Systems (Minneapolis, MN). The laboratory was blinded to case-control status and matched case and control samples were assayed within the same laboratory batch (plate). Approximately 10% of the samples in each of the 49 laboratory batches were blinded replicate quality control samples. Based on measurements from these quality control samples and a variance components estimation procedure [16], the estimated overall (intrabatch and interbatch) coefficient of variation was 9.5%.

Statistical analysis

Odds ratios (OR) were estimated using conditional logistic regression modeling, conditioned on matched set. All models also adjusted for age at blood draw, body mass index (BMI) (15- <20, 20- <22.5, 22.5- <25, 25- <27.5, 27.5- <30, 30- <32.5, 32.5 kg/m², and unknown), diabetes, and smoking status (never, former quit 15 years ago, former quit <15 years ago,

current, and unknown). Further adjustment for intake of fat, red meat, and alcohol had negligible influence on results and these factors were not included in the final model. Serum TGF- β 1 concentration was categorized into sex-specific quintiles based on the distribution among the combined control population. Serum TGF- β 1 was also examined as a continuous variable, with results presented in units of 1 standard deviation (8.1ng/ml for men and 7.4ng/ml for women).

We evaluated potential interactions by stratified analysis and by modeling a multiplicative interaction between a continuous variable for TGF- β 1 and variables for sex, smoking status (never, former, current), cohort, and follow-up time (continuous variable for time since blood draw). Analyses stratified by smoking status were based on an unconditional logistic regression model adjusted for BMI and the matching factors of age, sex, and cohort. Due to small numbers, analyses within current smokers were adjusted for BMI using only a three level variable (<25, 25-<30, 30) and excluded participants with unknown BMI. Because the association with risk of pancreatic cancer appeared to vary by follow-up time, results are presented both overall and stratified by follow-up time.

Results

Cases and controls were predominantly white, with a median age of approximately 62 years at blood draw (Table 1). Median time from blood draw to diagnosis was 2.9 years in CPS-II, 7.7 years in PLCO, and 11.4 years in ATBC. The ATBC cohort included only men who were current smokers, whereas the U.S. cohorts (CPS-II and PLCO) included both men and women and had a low prevalence of current smoking (< 10% of controls). TGF- β 1 concentrations were slightly higher in CPS-II than in ATBC or PLCO. Compared to controls with low TGF- β 1, those with high TGF- β 1 were slightly younger and more likely to be obese (Table 2), but appeared generally similar with respect to smoking status, diabetes, and intake of alcohol and dietary fat.

In overall analyses, the highest quintile of TGF- β 1 was associated with a marginally significantly higher risk of pancreatic cancer compared to the lowest quintile (OR = 1.36, 95% CI 0.98-1.88, $p = 0.06$) (Table III). However, there was no evidence of a trend across the first four quintiles.

The association between TGF- β 1 and risk of pancreatic cancer differed significantly by follow-up time ($p=0.02$, based on continuous measures of TGF- β 1 and follow-up time), with considerably higher risk observed during follow-up occurring 10 or more years after blood draw (OR = 2.13, 95% CI 1.23-3.68 for highest vs. lowest quintile, p trend = 0.001), but not earlier during follow-up (Table 3). This increase in risk appeared to be limited to the top quintile, with no clear trend in risk in the bottom four quintiles. The association with TGF- β 1 appeared similar during follow-up from 10 to < 15 years and follow-up after 15 years, although numbers were limited (results not shown). TGF- β 1 was associated with higher risk only in the ATBC cohort and among current smokers (who were predominantly from ATBC), although p -values for interactions by cohort ($p=0.08$) and smoking status ($p=0.14$) were not statistically significant. In analyses stratified by follow-up time, there was no apparent interaction between TGF- β 1 and cohort during either the first 10 years of follow-up

($p = 0.76$), or during follow-up occurring after 10 years ($p = 0.14$). Within ATBC, the risk associated with serum TGF- β 1 appeared to vary by follow-up time ($p=0.06$) and was most strongly associated with risk during follow-up after 10 years (OR = 2.74, 95% CI 1.45-5.20 for highest vs. lowest quintile of TGF- β 1).

Discussion

In this large prospective study including 729 cases of pancreatic cancer, serum TGF- β 1 was not clearly associated with risk in overall analyses, but was associated with approximately two-fold increased risk of developing pancreatic cancer during follow-up occurring 10 or more years after blood sample collection. Although both our study and the only other study to examine this association, a Japanese cohort including 85 cases [4], found some evidence of a positive association between serum TGF- β 1 and pancreatic cancer, the timing of the associations differed. We did not observe any association during the first 10 years of follow-up, whereas the Japanese study observed an association despite having a maximum of approximately 12 years of follow-up.

It is important to understand the source of serum TGF- β 1 in order to understand potential biological mechanisms that might explain an association with risk of pancreatic cancer. Serum TGF- β 1 is largely a measure of TGF- β 1 contained within platelets that is released into serum after blood collection as a result of platelet degranulation [17,18]. Serum TGF- β 1 is therefore relatively strongly correlated with platelet count [19]. In contrast, serum TGF- β 1 is not correlated with plasma TGF- β 1 [17] and would not be expected to correlate with tissue exposure to circulating extra-cellular TGF- β 1 except in settings where TGF- β 1 is released from activated platelets.

TGF- β 1 and/or other factors simultaneously released from activated platelets could nonetheless plausibly promote pancreatic carcinogenesis. Aggregation of activated platelets has been observed in areas of pancreatic inflammation [20,21], and growth factors released from platelets, including TGF- β 1 and platelet-derived growth factor (PDGF), may play an important role in activating pancreatic stellate cells (PSCs) [21]. Increasing evidence indicates that activated PSCs promote pancreatic cancer [22]. Therefore, higher serum TGF- β 1 levels (reflecting either higher platelet count or more TGF- β 1 per platelet), could be associated with higher risk of pancreatic cancer as a result of greater PSC activation. Studies of breast and colon cancer cell lines also suggest that exposure to platelet-derived TGF- β 1 may alter the phenotype of cancer cells and increase their invasiveness [23]. In addition, a recent study of pancreatic cancer cell lines indicates that exposure to platelets can activate biological pathways important in pancreatic cancer cell proliferation and survival (PI3K/Akt and MEK/Erk), and that this activation is specifically due to platelet derived TGF- β 1 [24].

Alternatively, elevated serum TGF- β 1 may be associated with increased risk of pancreatic cancer because it is a marker for subclinical chronic pancreatitis. Clinically evident chronic pancreatitis, like other chronic inflammatory conditions, can cause elevated platelet counts [25], and has been associated with higher serum TGF- β 1 [8,9]. In addition, clinically evident chronic pancreatitis has been associated with approximately 6-fold higher risk of pancreatic cancer [10]. Few, if any, individuals in our analysis are likely to have had clinically evident

chronic pancreatitis, as this condition is rare. However, it is possible that even subclinical chronic pancreatitis increases both serum TGF- β 1 and risk of pancreatic cancer. It should be noted that markers of systemic inflammation, including circulating concentrations of IL-6 and C-reactive protein, have not been clearly associated with risk of pancreatic cancer in previous prospective studies [15,26,27].

It is unclear why, in our study, high serum TGF- β 1 was associated with increased risk only during follow-up after 10 years. One possibility is that an association earlier in follow-up was obscured by effects of undiagnosed pancreatic cancer on serum TGF- β 1 concentrations (reverse causation). Serum TGF- β 1 was associated with higher BMI among controls in this study (Table 2) as well as in a cross-sectional analysis of a Japanese cohort [28], and pancreatic cancer cases often lose weight due to latent disease several years prior to diagnosis. Therefore, pre-diagnosis weight loss could have resulted in decreased serum TGF- β 1 levels at the time of blood collection among cases diagnosed during the first 10 years of follow-up. It also can be speculated that undiagnosed pancreatic tumors lower circulating platelet levels, and therefore serum TGF- β 1 levels, through a mechanism involving enlargement of the spleen. Both benign and malignant pancreatic tumors can cause obstruction of the splenic vein, located just behind the pancreas, resulting in enlargement of the spleen [29]. In turn, enlargement of the spleen can lower circulating platelet counts as a result of increased platelet sequestration within the spleen [30–32]. An alternative possibility is that very long-term pancreatic inflammation lasting many years may be required to increase risk of pancreatic cancer and serum TGF- β 1 measured a relatively short time before diagnosis may not be meaningfully associated with long-term pancreatic inflammation. Finally, the association observed during follow-up after 10 years could be a result of chance, despite its low p-value ($p=0.001$ for trend).

The association between serum TGF- β 1 and higher risk of pancreatic cancer in this analysis was driven by results from the ATBC cohort; no association was observed within the PLCO or CPS-II cohorts. This apparent difference in results by cohort could be due to differences in the distribution of follow-up time between cohorts. During the first 10 years of follow-up, there was no suggestion that serum TGF- β 1 was associated with pancreatic cancer risk within any of the individual cohorts, including ATBC. During follow-up occurring after 10 years, serum TGF- β 1 was associated with higher risk of pancreatic cancer. However, most cases during this time period were from the ATBC cohort, with a more limited number from PLCO and none from CPS-II. During follow-up after 10 years, the point estimate for TGF- β 1 was greater in ATBC (OR=1.44 per SD, 95% CI 1.17-1.78) than in PLCO (OR=1.12 per SD, 95% CI 0.74-1.71), although this difference was not formally statistically significant ($p=0.14$ for heterogeneity by cohort).

Strengths of this analysis include its prospective design and relatively large size, which enabled us to examine risk by smoking status and across a wide range of follow-up time. A notable limitation is that serum TGF- β 1 was measured at only one time point, and therefore is an imperfect measure of long-term serum TGF- β 1 concentrations. In addition, the increase in risk associated with TGF- β 1 observed during follow-up after 10 years could be a result of chance and requires replication. It also remains to be determined if the increase in risk associated with TGF- β 1 observed during follow-up after 10 years is widely

generalizable. It was not possible to examine if, during follow-up after 10 years, serum TGF- β 1 was associated with higher risk among smokers not from the ATBC cohort, among women, or among nonsmokers. However, in the earlier Japanese cohort study [4], the association between serum TGF- β 1 and higher risk of pancreatic cancer was driven primarily by result among nonsmokers.

Our results suggest high serum TGF- β 1 may be associated with increased risk of pancreatic cancer although a long follow-up period may be needed to observe this association. Further research examining associations of serum TGF- β 1 and other potential markers of subclinical pancreatic inflammation with pancreatic cancer risk may be useful in clarifying the role and timing of subclinical pancreatic inflammation in pancreatic carcinogenesis.

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Table 1

Selected baseline characteristics of cases and controls by study cohort

Characteristic	ATBC		CPS-II		PLCO		All cohorts combined	
	Cases (N = 348)	Controls (N = 348)	Cases (n = 90)	Controls (N = 90)	Cases (n = 291)	Controls (N = 469)	Cases (n = 729)	Controls (N = 907)
Age at blood draw (yrs) ^a	57 (53-61)	57 (54-61)	70 (67-73)	70 (67-73)	65 (61-69)	66 (61-69)	62 (56-67)	63 (57-67)
Age at diagnosis (yrs) ^{a,b}	69 (64-73)	69 (64-73)	73 (69-77)	73 (69-77)	72 (68-76)	71 (68-76)	71 (66-75)	71 (66-75)
Time from blood draw to diagnosis ^a	11.4 (7.1-16.4)		2.9 (1.6-4.6)		7.7 (4.5-10.0)		8.3 (4.2-12.2)	
Serum TGF- β 1 (ng/ml) ^a								
Male	26.1 (20.6-34.1)	25.0 (19.9-30.9)	28.1 (22.5-33.9)	31.3 (25.6-36.7)	26.9 (21.7-32.6)	26.5 (21.9-31.5)	26.9 (21.3-33.4)	26.1 (21.1-31.5)
Female	—	—	32.6 (27.7-39.1)	32.8 (27.0-37.1)	26.5 (21.5-32.1)	28.3 (22.7-31.6)	28.6 (22.8-33.6)	28.9 (23.7-32.9)
Male sex ^c	100.0	100.0	52.2	52.2	61.5	62.9	78.7	76.1
Race/ethnicity ^c								
White	100.0	100.0	96.7	97.8	90.0	90.2	95.6	94.7
Black	—	—	—	—	3.1	3.0	1.2	1.5
Asian	—	—	1.1	1.1	5.2	5.1	2.2	2.8
Other	—	—	2.2	1.1	1.7	1.7	0.9	1.0
Cigarette smoking status ^c								
Never	—	—	50.0	48.9	38.5	47.5	21.5	29.4
Former	—	—	44.4	50.0	43.3	44.6	22.8	28.0
Current	100.0	100.0	5.6	1.1	18.2	7.9	55.7	42.6
Body mass index (kg/m ²) ^c								
<25	35.6	38.2	46.7	33.3	33.7	35.0	36.2	36.1
25.0-30	47.1	43.7	38.9	43.3	42.6	45.6	44.3	44.7
30	17.2	18.1	14.4	20.0	22.7	19.0	19.1	18.7
Unknown	—	—	—	3.3	1.0	0.4	0.4	0.6
Body mass index ^a	26.2 (23.9-28.5)	26.2 (23.8-29.0)	25.5 (23.3-27.7)	26.4 (23.3-29.3)	26.6 (24.1-29.8)	26.6 (24.0-28.8)	26.2 (23.9-29.1)	26.4 (23.9-28.9)
Diabetes mellitus ^c	5.7	5.7	14.4	8.9	12.4	8.1	9.5	7.3
Alcohol intake (g/day) ^a	10.8 (2.4-27.8)	10.7 (2.8-25.0)	3.7 (0.5-11.2)	2.3 (0.2-11.7)	1.5 (0.3-12.4)	1.0 (0.3-9.7)	6.1 (0.5-20.0)	3.8 (0.4-16.5)

Characteristic	ATBC		CPS-II		PLCO		All cohorts combined	
	Cases (N = 348)	Controls (N = 348)	Cases (n = 90)	Controls (N = 90)	Cases (n = 291)	Controls (N = 469)	Cases (n = 729)	Controls (N = 907)
Total fat intake (g/day) ^a	116.6 (93.8-142.0)	116.4 (93.8-147.1)	52.8 (41.0-71.3)	54.3 (43.1-68.0)	62.0 (43.7-83.9)	62.4 (44.8-89.3)	86.5 (58.2-122.0)	82.5 (53.3-116.0)
Saturated fat intake (g/day) ^a	49.7 (37.4-62.3)	49.4 (37.0-62.7)	17.1 (13.3-23.4)	18.0 (13.1-21.4)	21.1 (14.3-28.8)	20.7 (14.7-30.5)	31.3 (19.0-50.1)	28.3 (17.8-46.3)

^aMedian (interquartile range: 25%–75%).

^bAge at diagnosis for controls is age at diagnosis of their matched case.

^cPercentage.

Table 2

Selected baseline characteristics of control participants by sex-specific quintile of serum TGF- β 1 concentration^a

Characteristic	Sex-specific quintile of serum TGF- β 1 concentration ^b				
	Q1	Q2	Q3	Q4	Q5
Mean serum TGF- β 1 (ng/ml)					
Male	15.6	22.1	26.2	30.4	38.2
Female	18.3	25.3	29.2	32.3	38.3
Mean age at blood draw (yrs)	66	64	64	64	63
Mean age at diagnosis (yrs) ^c	73	72	72	71	70
Male sex (%)	73.6	73.7	79.2	76.1	78.9
Race/ethnicity (%)					
White	96.7	96.3	95.7	92.2	91.8
Black	0.6	1.2	0.9	2.8	2.5
Asian	2.2	0.6	2.2	4.4	4.4
Other	0.6	1.8	1.1	0.6	1.3
Cigarette smoking status (%)					
Never	31.9	30.5	28.6	29.4	25.8
Former	23.7	27.5	28.9	26.6	31.4
Current	44.4	42.0	42.4	43.9	42.8
Body mass index (kg/m ²) (%)					
<25	40.0	37.7	40.5	34.3	28.0
25.0-<30	46.0	47.8	41.7	44.2	45.8
30	14.0	13.9	16.0	21.0	26.1
Unknown	0.0	0.6	1.8	0.6	0.0
Diabetes (%)	7.7	6.3	6.8	6.1	8.9
Mean alcohol intake (g/day) ^d	9.9	10.7	15.9	9.9	12.7
Mean total fat intake (g/day) ^d	80.8	87.7	85.2	80.6	86.2
Mean saturated fat intake (g/day) ^d	30.6	32.8	32.4	29.8	32.7

^aMeans and percentages standardized for cohort.^bSex-specific quintiles were <19.7, 19.7-<24.6, 24.6-<28.2, 28.2-<32.8, and 32.8 ng/ml for men and <22.6, 22.6-<27.4, 27.4-<30.5, 30.5-<33.7, and 33.7 ng/ml for women.

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Age at diagnosis for controls is age at diagnosis of their matched case.
In the CPS-II cohort, nutritional variables were assessed at enrollment several years before blood draw, while all other variables reflect status at the time of blood draw.

Table 3

Risk of pancreatic cancer by TGF- β 1 concentration, overall and stratified by follow-up time, study population and smoking status

	Follow-up period ^a						10 years					
	Overall			<5 years			5 - <10 years			10 years		
	Cases	Controls	OR 95% CI	Cases	Controls	OR 95% CI	Cases	Controls	OR 95% CI	Cases	Controls	OR 95% CI
Overall population^b												
Q1	147	181	1.00 (ref)	32	47	1.00 (ref)	44	62	1.00 (ref)	71	72	1.00 (ref)
Q2	146	181	1.10 (0.79-1.54)	48	58	1.30 (0.67-2.52)	50	52	1.42 (0.79-2.56)	48	71	0.73 (0.41-1.29)
Q3	122	182	0.98 (0.70-1.36)	50	54	1.52 (0.80-2.88)	42	77	0.82 (0.46-1.45)	30	51	0.61 (0.33-1.15)
Q4	120	181	0.88 (0.63-1.22)	33	64	0.73 (0.38-1.42)	42	67	0.81 (0.47-1.42)	45	50	0.98 (0.55-1.75)
Q5	194	182	1.36 (0.98-1.88)	58	76	1.04 (0.55-1.96)	50	61	1.27 (0.70-2.30)	86	45	2.13 (1.23-3.68)
<i>p-trend</i>	–	–	0.20	–	–	0.45	–	–	0.64	–	–	0.001
Per SD	729	907	1.07 (0.97-1.19)	221	299	0.93 (0.77-1.12)	228	319	0.95 (0.79-1.16)	280	289	1.34 (1.12-1.61)
By study population^b												
ATBC												
Q1	73	84	1.00 (ref)	14	20	1.00 (ref)	14	14	1.00 (ref)	45	50	1.00 (ref)
Q2	67	80	0.95 (0.59-1.53)	19	13	2.19 (0.69-6.96)	12	14	0.65 (0.18-2.31)	36	53	0.77 (0.40-1.46)
Q3	51	52	1.15 (0.68-1.92)	13	5	4.75 (0.95-23.8)	15	12	0.97 (0.30-3.13)	23	35	0.79 (0.38-1.63)
Q4	55	70	0.94 (0.59-1.51)	8	15	0.47 (0.14-1.51)	14	18	0.60 (0.20-1.76)	33	37	1.07 (0.55-2.09)
Q5	102	62	1.98 (1.25-3.14)	15	16	1.22 (0.36-4.13)	18	15	1.14 (0.39-3.34)	69	31	2.74 (1.45-5.20)
<i>p-trend</i>	–	–	0.02	–	–	0.26	–	–	0.75	–	–	0.0003
Per SD	348	348	1.18 (1.02-1.37)	69	69	0.82 (0.58-1.16)	73	73	0.94 (0.67-1.34)	206	206	1.44 (1.17-1.78)
PLCO/CPS-II^{b,c}												
Q1	74	97	1.00 (ref)	18	27	1.00 (ref)	30	48	1.00 (ref)	26	22	1.00 (ref)
Q2	79	101	1.16 (0.72-1.88)	29	45	0.89 (0.36-2.16)	38	38	1.61 (0.80-3.23)	12	18	0.69 (0.17-2.76)
Q3	71	130	0.81 (0.51-1.30)	37	49	0.94 (0.41-2.14)	27	65	0.72 (0.36-1.42)	7	16	0.29 (0.07-1.25)
Q4	65	111	0.76 (0.47-1.22)	25	49	0.61 (0.25-1.51)	28	49	0.80 (0.40-1.59)	12	13	0.67 (0.17-2.65)
Q5	92	120	0.93 (0.58-1.51)	43	60	0.73 (0.31-1.70)	32	46	1.15 (0.54-2.45)	17	14	1.12 (0.32-3.92)
<i>p-trend</i>	–	–	0.54	–	–	0.48	–	–	0.64	–	–	0.59
Per SD	381	559	0.95 (0.82-1.11)	152	230	0.92 (0.72-1.17)	155	246	0.94 (0.74-1.20)	74	83	1.12 (0.74-1.71)
<i>p-heterogeneity</i>	–	–	0.08	–	–	–	–	–	–	–	–	–

	Overall						< 5 years						5 - <10 years						10 years					
	Cases			OR 95% CI			Cases			OR 95% CI			Cases			OR 95% CI			Cases			OR 95% CI		
	Cases	Controls		OR	95% CI		Cases	Controls		OR	95% CI		Cases	Controls		OR	95% CI		Cases	Controls		OR	95% CI	
By smoking status^d																								
Never																								
Q1	30	53		1.00	(ref)		11	11		1.00	(ref)		10	31		1.00	(ref)		9	11		1.00	(ref)	
Q2	36	50		1.14	(0.60-2.16)		16	22		0.65	(0.21-2.04)		13	19		2.10	(0.73-6.00)		7	9		0.77	(0.19-3.09)	
Q3	30	57		0.80	(0.42-1.55)		15	22		0.41	(0.13-1.33)		13	26		1.85	(0.64-5.33)		2	9		0.25	(0.04-1.51)	
Q4	30	52		0.87	(0.45-1.68)		9	22		0.25	(0.07-0.90)		14	24		1.76	(0.64-4.87)		7	6		1.26	(0.30-5.34)	
Q5	31	52		0.80	(0.41-1.57)		13	24		0.26	(0.08-0.89)		12	23		1.96	(0.65-5.89)		6	5		1.23	(0.24-6.32)	
<i>p-trend</i>	–	–		0.71			–	–		0.064			–	–		0.27			–	–		0.62		
Per SD	157	264		0.96	(0.78-1.19)		64	101		0.72	(0.50-1.03)		62	123		1.23	(0.85-1.76)		31	40		1.14	(0.68-1.92)	
Former																								
Q1	36	38		1.00	(ref)		4	14		1.00	(ref)		18	14		1.00	(ref)		14	10		1.00	(ref)	
Q2	31	44		0.69	(0.36-1.35)		12	21		2.07	(0.50-8.55)		16	15		0.81	(0.28-2.31)		3	8		0.23	(0.04-1.35)	
Q3	27	61		0.45	(0.24-0.87)		17	22		2.91	(0.76-11.2)		8	36		0.17	(0.06-0.51)		2	3		0.36	(0.04-3.35)	
Q4	29	48		0.59	(0.31-1.15)		13	24		2.16	(0.54-8.60)		12	18		0.47	(0.16-1.39)		4	6		0.46	(0.08-2.53)	
Q5	41	61		0.63	(0.33-1.19)		21	35		2.10	(0.53-8.27)		13	18		0.48	(0.17-1.38)		7	8		0.63	(0.15-2.61)	
<i>p-trend</i>	–	–		0.09			–	–		0.50			–	–		0.07			–	–		0.43		
Per SD	164	252		0.84	(0.68-1.03)		67	116		1.12	(0.80-1.56)		67	101		0.73	(0.51-1.04)		30	35		0.81	(0.49-1.36)	
Current																								
Q1	80	90		1.00	(ref)		16	22		1.00	(ref)		16	17		1.00	(ref)		48	51		1.00	(ref)	
Q2	78	86		1.01	(0.66-1.56)		20	14		1.94	(0.74-5.07)		20	18		1.08	(0.41-2.82)		38	54		0.74	(0.42-1.32)	
Q3	64	61		1.15	(0.72-1.83)		18	9		2.66	(0.93-7.61)		21	14		1.53	(0.58-4.04)		25	38		0.70	(0.36-1.33)	
Q4	61	80		0.86	(0.54-1.34)		11	17		0.91	(0.33-2.52)		16	25		0.64	(0.25-1.64)		34	38		0.96	(0.52-1.77)	
Q5	122	69		1.93	(1.26-2.96)		24	17		2.04	(0.81-5.14)		25	20		1.26	(0.51-3.15)		73	32		2.42	(1.35-4.31)	
<i>p-trend</i>	–	–		0.02			–	–		0.75			–	–		0.70			–	–		0.0007		
Per SD	405	386		1.18	(1.03-1.35)		89	79		1.05	(0.80-1.38)		98	94		0.94	(0.70-1.27)		218	213		1.37	(1.14-1.66)	
<i>p-heterogeneity</i>	–	–		0.14			–	–		–			–	–		–			–	–		–		

^ap=0.024 for heterogeneity by follow-up time.^bOdds ratios calculated using conditional logistic regression conditioned on age, sex, race, cohort, and date of blood draw, and adjusted for age, smoking, body mass index and diabetes.

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Sex-specific quintiles were <19.62, 19.62–<24.41, 24.41–<28.23, 28.23–<32.82, and 32.82 ng/ml for men and <22.64, 22.64–<27.37, 27.37–<30.53, 30.53–<33.75, and 33.75 ng/ml for women. Standard deviations (SD) were 8.10 ng/ml for men and 7.35 ng/ml for women.

^c Analyses combine the two US cohorts (PLCO and CPS-II) which were similar in demographics and calendar era.

^d Odds ratios calculated using unconditional logistic regression adjusted for age, sex, body mass index, diabetes, and cohort, with the same sex-specific quintiles and SD values as in analyses of the overall population. Participants with unknown body mass index excluded. Results within the strata of current smokers with < 5 years follow-up could not be adjusted for cohort due to small numbers.