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Plasma cotinine levels and pancreatic cancer in the EPIC cohort study

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Abbreviations: BMI: body mass index; CI: confidence interval; EPIC: European Prospective Investigation into Cancer and Nutrition; IARC: International Agency for Research on Cancer; IRB: Institutional Review Board; MS: mass spectrometry; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone); OR: odds ratio; PAH: polynuclear aromatic hydrocarbon; UK: United Kingdom

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Smoking is an established risk factor for pancreatic cancer, previously investigated by the means of questionnaires. Using cotinine as a biomarker for tobacco exposure allows more accurate quantitative analyses to be performed. This study on pancreatic cancer, nested within the European Prospective Investigation into Cancer and Nutrition (EPIC cohort), included 146 cases and 146 matched controls. Using liquid chromatography-mass spectrometry, plasma cotinine levels were analyzed on average 8.0 years before cancer onset (5–95% range: 2.8–12.0 years). The relation between plasma cotinine levels and pancreatic cancer was analyzed with conditional logistic regression for different levels of cotinine in a population of never and current smokers. This was also done for the self-reported number of smoked cigarettes per day at baseline. Every increase of 350 nmol/L of plasma cotinine was found to significantly elevate risk of pancreatic cancer [odds ratio (OR): 1.33, 95% confidence interval (CI): 1.11–1.60]. People with a cotinine level over 1187.8 nmol/L, a level comparable to smoking 17 cigarettes per day, have an elevated risk of pancreatic cancer, compared to people with cotinine levels below 55 nmol/L (OR: 3.66, 95% CI: 1.44–9.26). The results for self-reported smoking at baseline also show an increased risk of pancreatic cancer from cigarette smoking based on questionnaire information. People who smoke more than 30 cigarettes per day showed the highest risk compared to never smokers (OR: 4.15, 95% CI: 1.02–16.42). This study is the first to show that plasma cotinine levels are strongly related to pancreatic cancer.

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Pancreatic cancer is the fifth most common cause of cancer death in Europe[1] and the fourth in the United States.[2] Apart from smoking tobacco, few risk factors have been identified for pancreatic cancer.[3] In all previous epidemiological studies, exposure assessment was based on information from questionnaires and it has been suggested that questionnaires underestimate smoking prevalence.[4] Additionally, environmental exposure to tobacco smoke is hard to determine precisely using a questionnaire.

Meta-analyses on smoking and cancer showed that smokers have an increased risk (1.7 times) of getting pancreatic cancer compared with never smokers.[3,5] The risk of pancreatic cancer increases in relation to the number of cigarettes smoked and to the lifetime consumption of cigarettes.[6–8]

Cotinine is the main metabolite of nicotine, and is used as a biomarker of exposure to tobacco.[9,10] The benefit of using cotinine is that it accurately quantifies systemic exposure to nicotine in both smokers and never smokers. However, cotinine reflects recent (1–2 days) exposure[11] and can therefore only be used to show an association for current smokers. In adult smokers, a 1 mg nicotine intake approximately corresponds to 1 smoked cigarette[2] and a blood cotinine level of 71 nmol/L.[12,13]

The use of cotinine allows for investigation of the role of environmental tobacco smoke in never smokers and in smokers accounts for different aspects of the exposure, including smoking intensity, tobacco composition, and bioavailability.[1,12] There are no previous reports describing the association between plasma cotinine levels and pancreatic cancer.

We present results from a case–control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) that aims to investigate the association between smoking and pancreatic cancer, using plasma cotinine levels as a biomarker for smoking exposure.

Material and Methods

Study population

We performed a case–control analysis on pancreatic cancer, nested within the EPIC cohort. The EPIC cohort includes 521,448 individuals from 23 centers in 10 European countries (Denmark, France, Greece, Germany, Italy, Netherlands, Norway, Spain, Sweden and United Kingdom) and has been described in detail before.[14] The study population included volunteers aged between 25 and 70 at the time of recruitment. Informed consent forms were obtained at each local center, and local ethical committees and the IARC IRB have approved the study. Questionnaire data on diet, lifestyle and personal history, anthropometric data and blood samples were collected at the time of enrolment for most of the participants.

Fasting and nonfasting blood samples of 30–40 mL were drawn in all the recruitment centers and stored at 5–10°C while protected from light and transported to local laboratories for processing and aliquoting as previously described.[14,15] The EPIC bio-repositories are centralized at IARC, and each local center stores a duplicate of its own samples. To maintain the stability and availability of the EPIC biological samples, all samples are stored at −196°C (liquid nitrogen) in specially designed storage straws, with an aliquot of 0.5 mL per straw. Samples from Sweden (2.0 mL aliquots) and Denmark (1.0 mL aliquots) are stored locally at the regional collaborating centers, in freezers at −80°C and under nitrogen vapor at a temperature of −150°C, respectively.

Pancreatic cancer diagnoses were based on population registries or a combination of active follow-up, next-of-kin information, health insurance records and pathology records. Mortality data were also obtained from cancer registries or mortality registries. All participants were followed from recruitment (1992–2000) until cancer development, death, emigration or the end of follow-up period (1992–2006). Controls were matched with cases by center, sex, length of follow-up and age (±1 month), date (±1 month) and time (±1 hr) at blood collection.

Out of 638 pancreatic cancer cases from the total cohort, 159 cases were excluded because no blood sample was available, they were diagnosed with another cancer previously, or because the cancer was a neuroendocrine type or non-malignant. Cases with missing cotinine values ($n = 27$) or missing values for covariates ($n = 30$) were excluded. Seventy-seven cases with a pancreatic cancer diagnosis within 2 years from recruitment were excluded, to prevent inclusion of people that may have changed their lifestyle because of early symptoms. Former smokers (196 cases) were excluded from the analyses because they are likely to have an increased risk of pancreatic cancer compared with never smokers, but have low-cotinine values due to the short half-life of cotinine. Three participants from Umea showed high cotinine values (>1,500 nmol/L) but were reported as never smokers. It seems plausible that these participants from Umea were *snus* users (a moist powder tobacco product commonly used in Northern Sweden[16,17]) and were therefore excluded from further analyses. One hundred forty-six cases and 146 controls were used in the analyses.

Exposure assessment

Plasma cotinine levels were analyzed for subjects included in the nested case–control study by MS-based methods[18] in the laboratory of BEVITAL AS (http://www.bevital.no) in Bergen, Norway.

Statistical analyses

The associations between plasma cotinine levels or baseline number of cigarettes smoked per day and pancreatic cancer were analyzed by conditional logistic regression. Both crude odds ratios and odds ratios adjusted for body mass index (BMI), socioeconomic status (*i.e.*, highest level of education), diabetes status and alcohol consumption at the time of recruitment—in addition to matching variables—were calculated. Possible effect modification by BMI (continuous) or alcohol consumption (categories of <5 g/day ethanol, 5–30 g/day and >30 g/day) was assessed by including a multiplicative interaction term in the model and assessing its significance.

Additionally, as continuous analyses assume linear relations, four categories of plasma cotinine levels were included in a conditional logistic regression model. Participants with cotinine levels below 55 nmol/L (±10 ng/mL) were used as a reference, as was done by Boffetta.[19] For the remaining participants ($n = 100$), tertiles based on the plasma cotinine levels of controls were created. To assess the potential effect of environmental tobacco smoke on pancreatic cancer, the relation between cotinine and pancreatic cancer was assessed in participants with cotinine levels under 55 nmol/L separately. For the analyses of baseline number of cigarettes per day, groups of current smokers that smoke less than 10 cigarettes, 10–20 cigarettes, 20–30 cigarettes and more than 30 cigarettes per day were compared with never smokers. Tests for trend were performed across categories, using median values for each category modeled as a continuous variable. Increments of 350 nmol/L cotinine and five cigarettes smoked per day at baseline were used to ensure better interpretable and comparable odds ratios. All analyses were performed using SAS 9.1. All tests were two-sided and statistical significance was assessed at the level of 0.05.

Results

Table 1 compares baseline characteristics of controls and cases. BMI, smoking status and cotinine values showed a difference between cases and controls. The results for the crude analysis of the association between plasma cotinine and pancreatic cancer are shown in Table 2. The crude odds ratio (OR) for each increase in 350 nmol/L in plasma cotinine was 1.28 [95% confidence interval (CI): 1.09–1.51] and after adjustment the OR did not materially change (OR: 1.33, 95% CI: 1.11–1.60). These ORs were comparable with the ORs for cigarettes smoked per day at baseline. The crude OR for every five cigarettes smoked per day at baseline was 1.21 (1.04–1.41) and was 1.27 (1.07–1.50) after adjustment. When multiplicative interaction terms for BMI and alcohol were

Table 1. Demographic and other characteristics of controls and cases

	Controls ($n = 147$)	Cases ($n = 147$)
Age at recruitment (years)[1]	**56.5 (8.9)**	**56.5 (8.9)**
Sex[2]		
Male	39 (26.7%)	39 (26.7%)
Female	107 (73.3%)	107 (73.3%)
Diabetes status[2]		
No diabetes	140 (95.9%)	139 (95.2%)
Diabetes	6 (4.1%)	7 (4.8%)
Body mass index (kg/m^2)[1]	25.4 (4.5)	27.0 (4.4)
Highest education[2]		
None/primary school	68 (46.6%)	66 (45.2%)
Technical/professional school	31 (21.2%)	25 (17.1%)
Secondary school	23 (15.8%)	20 (13.7%)
Longer education	24 (16.4%)	35 (24.0%)
Alcohol consumption at recruitment[2]		
Never and former drinkers	19 (13.0%)	14 (9.6%)
0–6 g alcohol/day	50 (34.3%)	57 (39.0%)
6–18 g alcohol/day	43 (29.5%)	36 (24.7%)
More than 18 g alcohol/day	34 (23.3%)	39 (26.7%)
Smoking status[2]		
Never smoker	107 (73.3%)	85 (58.2%)
Current smoker	39 (26.7%)	61 (41.8%)
Plasma cotinine level (nmol/L)[1]	268.7 (532.2)	481.5 (662.5)
Length of follow-up (years)[1]	9.7 (1.7)	6.4 (2.5)

[1]Value is displayed as "mean (standard deviation)". [2]Value is displayed as "*n* (%)".

included to assess the interaction with cotinine and cigarettes smoked per day at baseline, no interaction was found. The interaction terms of cotinine for BMI and alcohol showed *p*-values of respectively 0.94 and 0.56, where the interaction terms of cigarettes smoked at baseline were 0.87 and 0.86 (data not shown).

For the categorical analyses (Table 3), we observed an increasing trend for the ORs with increasing cotinine levels. The participants with highest cotinine values (>1187.8 nmol/L) had the highest OR, of 3.66 (1.44–9.26). No relation was found in participants with cotinine levels under 55 nmol/L [OR for every increase in cotinine by 1 nmol/L: 0.96 (0.83–1.11), data not shown]. When compared with never smokers, the risk among smokers was higher, and increased with an increasing number of cigarettes smoked. Participants who smoked more than 30 cigarettes per day showed the highest risk for pancreatic cancer compared with never smokers, OR: 4.15 (1.02–16.42).

Discussion

Principal findings

Smoking is an established risk factor for pancreatic cancer and our results show that cotinine—an objective biomarker of current smoking status—is significantly related to this type of cancer, thus strengthening the causal role of smoking. We observed a 1.33 times higher risk for pancreatic cancer for every increase of 350 nmol/L of cotinine, which approximately corresponds to five cigarettes. However, this assumes a linear relation between cotinine and pancreatic cancer, which is contradicted by the categorical analyses.

Participants with cotinine values over 664.5 nmol/L (≈9 cigarettes per day) showed increased risks, compared with the group below the threshold of 55 nmol/L, with the highest risk (OR: 3.66) for cotinine values over 1187.8 nmol/L. This value roughly corresponds to 17 cigarettes smoked per day, a number easily exceeded by smokers. Similarly, all participants that smoke more than 10 cigarettes per day at baseline according to questionnaire data show significantly increased risks of getting pancreatic cancer when compared with never smokers, with the highest risk for people who smoke more than 30 cigarettes per day (OR: 4.15). These results confirm that smoking is a risk factor for pancreatic cancer, as has been concluded by previous studies in the absence of biomarker data.[6,20–22] Odds ratios for cigarette smoking found in this study are higher than those previously reported.[20] A possible explanation may be because of additional adjustments and exclusions

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Table 2. Risk of pancreatic cancer according to plasma cotinine and smoking at baseline

	Cotinine (increment: 350 nmol/L)			No. cigarettes smoked at baseline (increment: 5 per day)		
	Odds ratio	95% CI for OR	*p*	Odds ratio	95% CI for OR	*p*
Crude	1.28	1.09–1.51	0.00	1.21	1.04–1.41	0.01
Adjusted[1]	1.33	1.11–1.60	0.00	1.27	1.07–1.50	0.01

[1]Conditional logistic regression adjusted for BMI, socioeconomic status, diabetes status and alcohol consumption.

Table 3. Risk of pancreatic cancer according to quintiles of plasma cotinine and different categories of smokers

Plasma cotinine (nmol/L)	*n* cases/ controls	Odds ratio	95% CI for OR	*p*	Self-reported smoking status	Mean plasma cotinine (nmol/L)	*n* cases/ controls	Odds ratio	95% CI for OR	*p*
0–55 (ref)	87/105	1			Never smokers	10.61	85/107	1		
>55–664.5	8/14	0.89	0.28–2.89	0.85	Smokers (<10 cigarettes/day)	520.94	10/9	2.24	0.70–7.22	0.18
664.5–1187.8	22/14	2.92	1.18–7.25	0.02	Smokers (10–20 cigarettes/day)	1202.21	24/14	3.16	1.33–7.87	0.01
>1187.8	29/13	3.66	1.44–9.26	0.01	Smokers (20–30 cigarettes/day)	1104.95	19/11	3.50	1.11–7.22	0.03
					Smokers (>30 cigarettes/day)	1443.18	8/5	4.15	1.02–16.42	0.05
P trend				0.00	P trend					0.00

Conditional logistic regression adjusted for BMI, socioeconomic status, diabetes status and alcohol consumption, participants with cotinine values below 55 nmol/L were used as a reference.

performed in this study. However it should be noted that confidence intervals are wide and a difference based on chance can not be excluded. No effect modification by BMI or alcohol consumption was found. However, power was limited to study interactions.

Strength and weakness

Strengths of this study are the large cohort size and its prospective design, which rules out selection bias. However, the large number of exclusions reduced the power of the study at detecting associations, especially for subgroup analyses.

Assessment of smoking

Cotinine was tested for its relation with pancreatic cancer as it accounts for different aspects of the exposure and because the use of self-reported smoking status was thought to underestimate the relation between smoking and pancreatic cancer. However, comparing the categorical results for cotinine and smoking status it seems arguable that self-reported smoking status is enough to establish a causal relationship and does not underestimate the relation between smoking and pancreatic cancer. An additional reason to use a biomarker and test its relation with pancreatic cancer could be to validate self-reported smoking status (a very strong correlation was found between cotinine levels and number of cigarettes smoked at baseline with a Spearman's correlation of 0.79 and a *p*-value of 0.00) or to have a reliable and objective measure of passive smoking. However, no relation between cotinine and pancreatic cancer in participants with a low-cotinine level was found.

Possible mechanism

Although we do not know in detail the mechanisms by which tobacco smoking induces pancreatic cancer, one hypothesis is related to tobacco-specific nitrosamines. In animal studies, the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces adenocarcinoma of the lung.[23] After several studies had revealed a dose-response relationship between cigarette smoking and lung cancer risk in the 1950s, various cigarette products with low tar and/or with filters were manufactured.[24] As a consequence, while in the past the smoke from cigarettes contained higher polynuclear aromatic hydrocarbon (PAH) concentrations, the smoke from the new cigarettes contains more nitrogen oxides and nitrates, precursors of *N*-nitrosamines. For example, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) has increased by 73% from late 1970s to 1995. In animals tobacco-specific nitrosamines were demonstrated to be carcinogenic for the brain, the lung and the pancreas.[23–25]

Conclusion

This study is the first to present a clear association between plasma cotinine levels and pancreatic cancer in a population of never and current smokers.

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