Methodological issues in a prospective study on plasma concentrations of persistent organic pollutants and pancreatic cancer risk within the EPIC cohort

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ABSTRACT

Background.
The use of biomarkers of environmental exposure to explore new risk factors for pancreatic cancer presents clinical, logistic, and methodological challenges that may also be relevant in research on other complex diseases.
Objectives.
First, to summarize the main design features of a prospective case-control study –nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort– on plasma concentrations of persistent organic pollutants (POPs) and pancreatic cancer risk. And second, to assess the main methodological challenges posed by associations among characteristics and habits of study participants, fasting status, time from blood draw to cancer diagnosis, disease progression bias, basis of cancer diagnosis, and plasma concentrations of lipids and POPs. Results from etiologic analyses on POPs and pancreatic cancer risk, and other analyses, will be reported in future articles.
Methods.
Study subjects were 1,533 participants (513 cases and 1,020 controls matched by study centre, sex, age at blood collection, date and time of blood collection, and fasting status) enrolled between 1992 and 2000. Plasma concentrations of 22 POPs were measured by gas chromatography - triple quadrupole mass spectrometry (GC-MS/MS). To estimate the magnitude of the associations we calculated multivariate-adjusted odds ratios by unconditional logistic regression, and adjusted geometric means by General Linear Regression Models.

Results.
There were differences among countries in subjects' characteristics (as age, gender, smoking, lipid and POP concentrations), and in study characteristics (as time from blood collection to index date, year of last follow-up, length of follow-up, basis of cancer diagnosis, and fasting status). Adjusting for centre and time of blood collection, no factors were significantly associated with fasting status. Plasma concentrations of lipids were related to age, body mass index, fasting, country, and smoking. We detected and quantified 16 of the 22 POPs in more than 90% of individuals. All 22 POPs were detected in some participants, and the smallest number of POPs detected in one person was 15 (median, 19) with few differences by country. The highest concentrations were found for *p,p'*-DDE, PCBs 153 and 180 (median concentration: 3371, 1023, and 810 pg/mL, respectively). We assessed the possible occurrence of disease progression bias (DPB) in eight situations defined by lipid and POP measurements, on one hand, and by four factors: interval from blood draw to index date, tumour subsite, tumour stage, and grade of differentiation, on the other. In seven of the eight situations results supported the absence of DPB.

Conclusions.

The coexistence of differences across study centres in some design features and participant characteristics is of relevance to other multicentre environmental studies. Relationships among subjects' characteristics and among such characteristics and design features may play important roles in the forthcoming analyses on the association between plasma concentrations of POPs and pancreatic cancer risk.

ABBREVIATIONS

BMI, body mass index; CI, confidence interval; DAG, Directed Acyclic Graph; DPB, disease progression bias; *p,p'*-DDE, dichlorodiphenyldichloroethene; *p,p'*-DDT, dichlorodiphenyltrichloroethane; EPIC, European Prospective Investigation into Cancer and Nutrition; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; PBDE, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; PeCB, pentachlorobenzene; POPs, persistent organic pollutants.

Keywords: persistent organic pollutants; biomarkers, methods; environmental epidemiology; pancreatic cancer; lipids; disease progression bias.

1. INTRODUCTION

Advancing knowledge on environmental causes of pancreatic cancer remains elusive (Amaral et al., 2012; Antwi et al., 2015; Barone et al., 2016; Kamisawa et al., 2016; Porta, 2001, 2005). This might partly be due to the difficulties that such a biologically and clinically aggressive disease poses to obtain biological specimens, to use meaningful biomarkers, to elicit accurate information from severely ill patients, and sometimes to achieve a precise anatomo-pathological diagnosis (Porta, 2001). Yet some of such clinical, logistic, and methodological challenges are also common in environmental research on other diseases (Baris et al., 2000; De Roos et al., 2005; Hoppin et al., 2000; Rylander et al., 2015; Porta et al., 1999; Vo et al., 2008; Wolff et al., 2000, 2007). Notably, biomarkers of exposure to lipophilic contaminants are prone to disease progression bias (DPB), a mechanism of reverse causation bias through which the pathophysiological progression of the disease alters body concentrations of the contaminants in blood and fatty tissues; as a consequence, disease-altered exposure estimates lack etiologic significance (Baris et al., 2000; Hoppin et al., 2000; Porta et al., 1999, 2009b, 2014; Rylander et al., 2015).

A different but related issue is the influence of fasting status at blood collection on blood concentrations of a variety of lipophilic substances (e.g., some vitamins and other nutrients, most organochlorine compounds). Another related and unresolved issue that affects many environmental etiologic studies is how to approach conceptually and analytically the concomitant confounding and mediating effects of blood lipids, fasting, and body mass index when estimating possible causal effects of such lipophilic substances (Donat-Vargas et al., 2018; Gallo et al., 2011; O'Brien et al., 2016; Rylander et al., 2015).

Diseases whose diagnostic accuracy and precision in clinical practice depend on age, gender, lifestyle or other factors (López et al., 2014; Porta, 2001, 2005; Porta et al., 1994) offer the opportunity to assess whether the diagnostic basis, and the corresponding diagnostic certainty, contribute to disease misclassification, and hence to bias causal estimates.

While prospective longitudinal designs as cohort-nested case-control studies can overcome several of the previously sketched difficulties, control of biases associated with disease progression cannot rest exclusively on such studies (Dorgan et al., 1999; Hunter et al., 1997; Lee et al., 2014; Porta et al., 2009b; Wolff et al., 1993).

We thought it was necessary and relevant to report on these methodological issues prior to analysing data from a cohort-nested case-control study on persistent organic pollutants (POPs) and pancreatic cancer risk; results from the etiologic analyses and from other analyses (such as predictors of POP concentrations in controls) will be reported in future articles.

Therefore, the objectives of the present report are two-fold: first, to summarize the main design features of a prospective case-control study –nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort– on plasma concentrations of POPs and pancreatic cancer risk; and second, to assess the main methodological challenges posed by potential associations among some characteristics and habits of study participants, fasting status, time from blood draw to cancer diagnosis, disease progression bias, basis of cancer diagnosis, and plasma concentrations of lipids and POPs.

2. METHODS AND RESULTS

2.1. Study population

We performed a case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. The EPIC cohort has been previously described in detail (Riboli et al., 2002). Briefly, 521,457 subjects (153,447 men) aged 35–70 years old were recruited between 1992 and 2000 by 23 collaborating centres from 10 European countries. Three bio-repositories from the EPIC study contributed samples for the present study: the repository from Denmark, which centralized samples from the collaborating centres of Aarhus and Copenhagen; the repository from the collaborating centre of Umeå, in Sweden; and the IARC central repository, which centralized the biospecimens of 8 countries (Germany, United Kingdom, Netherlands, Italy, Spain, Greece, France, and Norway) (Supplemental Table 1).

Over 98% of the 1,533 participants in the present study (pancreatic cancer cases plus controls) were enrolled between 1992 and 1998. They were followed until cancer diagnosis, death, migration, or the end of the follow-up period (2007, 2010 and 2014 for Denmark, IARC, and

Umeå, respectively), whichever occurred first (Supplemental Table 1). The median duration of follow-up for the study participants was 11.6 years (mean, 11.4 y; standard deviation [SD], 3.4 y).

Pancreatic cancer cases, coded as C25 (C25.0-25.3, 25.7-25.9) according to the International Classification of Diseases-Oncology (ICD-O) 3rd edition, were identified and included in the study. Exclusion criteria were: a) cases of endocrine pancreatic cancer; b) occurrence of other malignant tumours preceding the diagnosis of pancreatic cancer, except for non-melanoma skin cancer; c) cases with pancreatic cancer diagnosed during the first 2 years of blood draw (5 years for cases from Denmark); and d) 12 cases with less than 2 straws of plasma remaining available. Thus, 513 cases were included in the present study, of which 135 (26.3%) came from Denmark, 79 (15.3%) from Umeå (Sweden), and 299 from the IARC central repository (contributing countries: Germany, 13.6% of the 513 cases; United Kingdom, 11.7%; The Netherlands, 9.2%; Italy, 8.4%; Spain, 7.2%; Greece, 5.5%; France, 1.6%; and Norway, 1.2%) (Supplemental Table 1).

For each case, two control subjects alive and free of cancer at the time of diagnosis of the index case were selected using an incidence density sampling procedure (Rothman et al., 2008); only 6 cases had just one control. Thus, a total of 1020 matched controls were included. Matching factors were study centre, sex, age at blood collection (± 1 year), date (± 6 months) and time of the day (± 2 h) of blood collection, fasting status (<3 h, 3-6 h, >6 h after last meal), and, for women, use of exogenous hormones (yes, no). The age at blood collection of 11 controls differed by more than ± 1 year (up to 4.8 years) to the age of their matched case; the date of blood collection of 11 controls (4 of the previous) differed by more than ± 6 months (up to 12 months); and the time of blood collection of 84 controls differed by more than ± 2 h (up to 3.5 h) (information on time of blood collection was missing for 179 individuals from Umeå and for 27 individuals of 9 case sets from IARC repository). The fasting status of 55 controls was different from the fasting status of their matched case, and was missing for 21 participants. Finally, use of exogenous hormones of 8 controls was different from use of their matched case, and was missing for 92 women.

The EPIC study was approved by the Ethical Review Board of the International Agency for Research on Cancer (IARC, Lyon) and by the local Ethical Committees.

2.2. Main variables, and collection of blood samples

At recruitment a questionnaire collected baseline information about sociodemographic characteristics, lifestyles (especially those related to cancer aetiology, such as lifetime history of alcohol and tobacco consumption), and medical history. Validated country/centre-specific dietary questionnaires were used at baseline for recording average daily intakes over the

previous 12 months. Anthropometric measures and blood samples were taken at recruitment for most subjects. Over 80% of participants in the present study underwent blood extraction the same day of the recruitment, and 11% during the previous or the following 7 days after recruitment; only 3% of participants had their blood collected more than one year after being enrolled.

For a subset of pancreatic cancer cases information on characteristics of the tumour was also collected. Information on the pancreatic subsite of the tumour was available for 348 of the 513 cases included in the study (68%). The category 'overlapping' of tumour subsite included tumours registered as an overlapping lesion of the pancreas (C25.8, N = 18), as localised in a pancreatic duct (C25.3, N = 2), and in other specified parts of the pancreas (C25.7, N = 3). Information on the stage of the tumour was available for 177 cases (35%) as it was registered just in some centres. The EPIC classification for stage of the tumour included: in situ (none), localised (N = 51), metastatic (N = 49), metastatic regional (N = 33), and metastatic distant (N = 44). For the present study, the three last categories were joined in one category named 'metastatic'. A minimum of one basis of the cancer diagnosis was recorded for 506 cases (98.6%): 50 cases had three bases of cancer diagnosis recorded, 156 cases had two bases, and 300 cases had one basis. Only 86 cases (17%, all from IARC bio-repository) had information on the grade of the tumour: 11, 45 and 29 cases were classified as well, moderately, and poorly differentiated, respectively, while 1 case was classified as undifferentiated.

2.3. Statistical analyses

Univariate statistics were computed as customary (Armitage et al., 2002; Kleinbaum et al., 1998). To assess differences on participants' characteristics by gender, case-control status, fasting status, basis of cancer diagnosis, and concentrations of lipids and persistent organic pollutants, Student's t-test, ANOVA, Kruskal-Wallis, and Mann-Whitney's *U* tests were used. Fisher's exact test for homogeneity was applied to assess the relationship between two categorical variables. Spearman's rank correlation coefficient (ρ) was computed to evaluate correlations among pairs of POPs.

To estimate the magnitude of the associations between participants' characteristics and *a)* fasting status, and *b)* basis of cancer diagnosis, multivariate-adjusted odds ratios (ORs) and their corresponding 95% confidence intervals (CI) were calculated by unconditional logistic regression (Rothman et al., 2008). The main effects of all predictors were independently explored in base models. Final models were selected in accordance with the nature of the variables and the study objectives. Thus, final models for fasting status were adjusted for centre and time of the day of blood collection, while final models for basis of cancer diagnosis were adjusted for age at diagnosis, sex, and centre.

General Linear Regression Models (GLM) were applied to study the relationships of participants' characteristics with lipid concentrations (Armitage et al., 2002). GLM were also used to study the relation between lipid or POP concentrations in plasma samples collected during recruitment and some variables related to the disease, such as the time elapsed between blood collection and the date of pancreatic cancer diagnosis, or some characteristics of the tumour (among cases with available information). Results are expressed as adjusted geometric means (aGMs) with the corresponding 95% CIs. The Kolmogorov-Smirnov test for normality was used to check the distributions of lipid and POP concentrations, and of the time between blood collection and cancer diagnosis; as none was normal, log-transformed values were used in regression analyses. The following potential confounders were included in the final models: age, sex, body mass index (BMI), fasting status, and centre (repository in models including tumour characteristics).

We assessed associations between plasma concentrations of POPs and study and participant characteristics. POP concentrations were entered in the models as quartile categories (defined using the concentrations in controls).

To assess exposure to multiple compounds, we computed *a)* the sum of PCBs for each participant by adding plasma concentrations of all ten PCBs, and then assigning each participant to one quartile of the new variable (sum of PCBs) (Porta et al., 2012); *b)* the sum of 4 PCBs for each participant by adding plasma concentrations of 4 prevalent PCBs (congeners 118, 138, 153, and 180), and then assigning each participant to one quartile of the new variable (sum of 4 PCBs); and *c)* to compute the sum of orders of the 6 most prevalent organochlorine pesticides, each compound was categorized in quartiles and the category number of each compound was summed, producing a value ranging between 6 (when concentrations of all 6 organochlorine pesticides were in the lowest quartile) and 24 (when concentrations of all 6 compounds were in the top quartile) (Gasull et al., 2012, 2018; Porta et al., 2012). Other options were also considered; because results were similar to the three variables above, the latter were chosen for presentation.

For the 16 compounds quantified in more than 90% of individuals (see below) we calculated the number of POPs detected in each person at high concentrations (nPhc) as follows: for each subject we added the number of POPs whose plasma concentrations were equal to or greater than a selected cut-off point, as percentile 90 (P90) (the upper decile), or percentile 75 (P75) (the upper quartile) (Porta et al., 2012; Pumarega et al., 2016).

Based on historical factors related to exposure to POPs (essentially, before and after World War II) (Nøst et al., 2017; Porta et al., 2008b; Rylander et al., 2015), the following birth cohorts were defined: participants born from 1919 to 1938 (N = 817), from 1939 to 1945 (N = 503), and from 1946 to 1964 (N = 213).

The level of statistical significance was set at 0.05 and all tests were two tailed. Analyses were conducted using SPSS version 18 (SPSS, Armonk, NY, USA, 2009) and R version 3.1.3 (R Core Team, Vienna, Austria, 2015).

2.4. Baseline characteristics of study participants

At blood collection, the age of the 1,533 individuals included in the study ranged between 29 and 76 years (mean, 56.8 years). Over 58% of participants had overweight or obesity, 26% were current smokers, and 28% consumed more than 18 g of alcohol per day (Table 1).

Differences in baseline characteristics were observed by country (Supplemental Table 1). The percentage of women was significantly lower in Denmark (38%) and Germany (37%), and higher in the United Kingdom (65%) and The Netherlands (79%), while in France and Norway all participants were women (N = 24 and 18, respectively). Denmark had the highest proportion of current smokers (36%), followed by Italy (34%).

Participants from the collaborating centre of Umeå (Sweden) were younger at blood collection and had a lower BMI than participants from Denmark and the IARC central repository. As mentioned, the end of the follow-up period for participants from Umeå was in 2014, while for the rest of centres follow-up ended at least 4 years earlier (2010), and up to 9 years earlier (2005 in France). However, the period of blood collection in Umeå (1992-1996) was similar to the other centres. The age at cancer diagnosis of cases from Umeå (mean = 65.4 ± 8.7 years old) was similar to that of cases from the other centres (mean = 65.8 ± 7.6), as expected (Supplemental Table 1). For cases, the index date was the date of cancer diagnosis; for controls, the index date was the date of cancer diagnosis of the case they were matched with.

As a result, participants from Umeå:
a) had the longest follow-up (they may have been followed for up to 22 years, from 1992 to 2014), and the mean follow-up for Umeå was 15.3 years, while for the rest of centres it was 10.7 years;
b) had the longest time from blood collection to index date: mean of 12.5 years for Umeå, and 8.3 for the other centres; and
c) were younger at blood collection: mean of 52.9 years for participants from Umeå, and 57.5 for the other centres (Supplemental Table 1).

The above results are of potential relevance if the pancreatic cancer risk function was not linear over measures of time or age; e.g., if risks associated with certain exposures or subject characteristics were essentially or only apparent after 10 years since blood collection, then the characteristics of the study in Umeå would have a stronger influence in the detection of such

risks than the other countries. As we shall see later, there were also other differences by study centre.

The distribution of the time from blood collection to index date is shown in Figure 1. It was between 2 and <5 years for 14% of participants, between 5 and <10 years for 51% of participants, and ≥10 years for 35%. As mentioned, we included no cases (and thus no matched controls) with a diagnosis of pancreatic cancer within <2 years of blood draw. Only 5% of participants, all from Umeå, had such time interval ≥15 years. In multivariate models (for cases and for controls separately), repository was the only study characteristic statistically significantly associated with time from blood collection to index date, adjusting for age, sex, and BMI; the minor exception were overweight and obese controls, who had slightly longer intervals than normal weight controls: the aGM of the time from blood collection to index date (adjusted for age, sex, and centre) for obese controls was 8.7 years, 95% CI: 8.3-9.3 whereas the corresponding aGM for normal weight controls was 7.8 years, 95% CI: 7.5-8.1 (p-value = 0.008). The relationship among cases was the opposite than among controls: aGM for obese cases = 7.6 years, 95% CI: 6.9-8.4 and the aGM for normal weight cases = 8.3 years, 95% CI: 7.8-8.8 (p-value = 0.150). No other associations were observed either when subjects from Umeå were excluded.

2.5. Baseline characteristics of cases and controls

As expected, cases and controls showed no differences in the variables they were matched by. No differences between cases and controls were observed either for BMI, education, marital status, alcohol consumption, or physical activity (Table 1). In contrast, a higher proportion of cases than controls were current smokers at study entry (33% vs. 23%, respectively), and had diabetes mellitus (6% of cases vs. 3% of controls), as expected.

2.6. Basis of cancer diagnosis

We next assessed whether the diagnostic basis of the pancreatic cancer, and its corresponding potential disease misclassification, were independent or instead associated with sociodemographic, lifestyle and clinical characteristics of cases. Of the 506 cases (98.6% of all 513 cases) with information on the basis of cancer diagnosis, 382 (75.5%) were microscopically confirmed, while the remaining 124 (24.5%) were diagnosed by imaging results, laboratory tests, clinical symptoms, or physical examination (Table 2).

Significant differences in the percentage of cases with microscopic confirmation were observed across countries: more than 95% of cases from Denmark and Umeå were microscopically

confirmed, while this figure was 39% in Greece, and 22% in the United Kingdom (Supplemental Table 1).

Microscopic confirmation was higher in men than in women (83% and 68%, respectively). As expected and important, it was lower at older ages: it was about 83% in cases <60 years old, 78% in cases 60-69 years, and 68% in cases ≥70 years (Table 2). Microscopic confirmation was not related to subsite or stage of the tumour, neither to BMI. By contrast, in univariate analyses it was associated with smoking, alcohol, and physical activity. In models adjusting for age, sex and centre, only differences by age remained statistically significant. There was also a positive, monotonic, but statistically non-significant relation between education and microscopic confirmation. Age was a strong confounder of some associations with diagnostic basis. For instance, 82% of current smokers and 72% of never smokers had been diagnosed by microscopic methods; however, since current smokers were younger than never smokers (e.g., 49% vs. 34% were <55 years old, respectively), when adjusting for age the observed relation between smoking and diagnostic basis became much weaker (Table 2).

2.7. Fasting status

In descriptive analyses, differences in fasting status were observed for sex, age, study centre, time of the day of blood collection, smoking status, alcohol consumption, and physical activity. Fasting status was one of the variables displaying larger differences across study centres and countries: while a large proportion of participants from Umeå, Italy and Spain had been fasting for more than six hours (94%, 83% and 68%, respectively), the corresponding percentage for the rest of countries was <15% (except France, with 7 out of its 24 participants with >6 hours of fasting) (Supplemental Table 1). Time of blood collection was strongly associated with fasting: chances of having fasted >6 hours decreased with time from early morning to late afternoon. In Umeå over 70% of participants (165 out of 235) had been fasting for >6 hours and did not have the time of blood collection registered.

Adjusting for centre and time of blood collection, sex, age, BMI, smoking, alcohol intake or physical activity were not significantly associated with fasting status (Table 3). As expected, no relationship was observed with case-control status, since fasting status was one of the matching factors.

2.8. Lipid concentrations in plasma

Measurements of total cholesterol and triglycerides were carried out enzymatically by Abbott Architect reagents (Abbott Laboratories, Abbott Park, IL, USA) in plasma obtained at study entry (Riboli et al., 2002). Analyses were performed at the National Institute for Health and Welfare

(THL), Finland. A 400 µL straw with plasma was sent from the biorepositories to THL for lipid and POP analyses (see also below). Total lipids (TL) were calculated by the Standard formula 2, based on total cholesterol and triglycerides (Bernert et al., 2007; Phillips et al., 1989; Porta et al., 2009a).

There were no significant associations between lipid concentrations (total cholesterol, triglycerides, and TL) and case-control status (Figure 2 and Supplemental Table 2). The absence of association between lipids and case-control status was also evident when adjusting for age, sex, centre, BMI and fasting status (p-value = 0.441) (Table 4). Although these findings could be expected because of the study design (i.e., because blood samples were obtained at least 2 years before cancer diagnosis), it was methodologically warranted to confirm them.

Total lipids tended to be significantly higher in subjects with higher age and BMI. Men also had slightly higher values than women (Supplemental Table 2). Except for sex, these relationships held when models were mutually adjusted for age, sex, centre, BMI, and fasting status (p-values ≤ 0.036) (Table 4). A statistically significant association was also observed between lipid concentrations and smoking status in adjusted models: participants who reported to be current smokers had higher concentrations of triglycerides and TL (Table 4 and Supplemental Table 3). Adjusted models also show that triglycerides and TL were associated with fasting status: individuals with more than 6 hours of fasting had lower concentrations of triglycerides and of TL.

Differences in lipid concentrations were also observed by country: while participants from Umeå (Sweden) were younger, had a lower BMI, and were more often fasting (Supplemental Table 1), they had the highest mean and median concentrations of total cholesterol and total lipids (Supplemental Table 2); participants from Umeå also showed the highest concentrations of triglycerides after adjusting for age, sex, BMI and fasting status (Table 4 and Supplemental Table 3). In adjusted models, the lowest concentrations of TL were observed in participants from Spain and Greece: the aGM of TL was 736 mg/dL, 585 mg/dL and 587 mg/dL for subjects from Umeå, Spain and Greece, respectively (Table 4).

2.9. Concentrations of persistent organic pollutants (POPs)

2.9.1. Chemical analyses of POP concentrations

POP concentrations were measured in 200 µL plasma samples from the same blood collection sample obtained at study entry in which lipids were measured (Riboli et al., 2002). Analyses were performed at THL (Koponen et al., 2013). Twenty-two POPs were measured: three polybrominated diphenyl ethers (PBDEs 47, 99, 153), eight non-dioxin like polychlorinated biphenyls (PCB congeners 74, 99, 138, 153, 170, 180, 183, and 187), two dioxin like PCBs (congeners 118 and 156), and nine organochlorine pesticides or their metabolites: dichlorodiphenyltrichloroethane (*p,p'*-DDT), dichlorodiphenyldichloroethene (*p,p'*-DDE), α-

hexachlorocyclohexane (α-HCH), β-HCH, γ-HCH, pentachlorobenzene (PeCB), hexachlorobenzene (HCB), trans-nonachlor, and oxychlordane.

Pretreatment of the samples was as follows: ethanol and ^{13}C-labelled internal standards of each compound in toluene were added to samples (200 µL) in test tubes and thoroughly mixed to precipitate the proteins and equilibrate internal standards. Dichloromethane-hexane (1:4) was added for extraction followed by activated silica to bind the sample water, ethanol, and precipitate. Samples were mixed, and layers were allowed to separate. The upper dichloromethane-hexane layer was poured to a solid phase extraction cartridge (SPE cartridge) containing from bottom to top 10% $AgNO_3$ impregmented silica and a mixture of Na_2SO_4 and silica. The lower layer in the test tube was extracted again with dichloromethane-hexane, which was also poured to SPE-cartridge. Elution of SPE-cartridges was continued with dichloromethane-hexane, and the eluate was concentrated to 15-20 µL for gas chromatography - triple quadrupole mass spectrometry (GC-MS/MS) analysis. The instrument used was an Agilent 7010 GC-MS/MS system (Wilmington, DE, USA), GC column DB-5MS UI (J&W Scientific, 20m, ID 0.18 mm, 0.18 µm). Limits of detection ranged from 2 pg/mL for PCB congeners and trans-nonachlor to 16 pg/mL for *p,p'*-DDE. Limits of quantification ranged from 5 pg/mL for PCB congeners and trans-nonachlor to 40 pg/mL for *p,p'*-DDE (Supplemental Table 4). When a sample had a concentration of a compound below the detection threshold, it was assigned the mid-value of this limit; when a compound was detected but under the quantification threshold, the mid-value between detection and quantification limits was used. The THL laboratory participates three times a year in AMAP interlaboratory comparisons (Ring Test for Persistent Organic Pollutants in human serum, National Institute of Public Health, Quebec, Canada) (Koponen et al., 2013; Krauskopf et al., 2017; Vafeiadi et al., 2017). POP concentrations were individually converted to lipid-based concentrations (i.e., corrected or normalized for TL) by dividing the crude plasma POP concentration by TL (see 'Lipid concentrations' above).

The results that follow refer to the entire study population of cases and controls. The results were selected for inclusion in the present article based on their importance for the primary study on POPs and pancreatic cancer, and on the methodological challenges that the associations among POP concentrations, lipid concentrations, and other study variables and features pose for the primary study and for other studies on POPs and cancer aetiology. Therefore, as mentioned above, the sections below do not include results on POPs and pancreatic cancer risk; they also do not include results on all determinants of POP and lipid concentrations in plasma in the study subjects.

2.9.2. Percentages of detection and quantification

We detected and quantified 16 of the 22 compounds (henceforth, 'most prevalent POPs') in more than 90% of individuals (Figure 3, Table 5, and Supplemental Table 4). Seven of the 10 PCBs, as well as HCB and trans-nonachlor were detected and quantified in 100% of

participants. α-HCH and PeCB were detected in more than 80% of participants and quantified in 49% and 35%, respectively. The percentage of detection for the other 4 compounds (γ-HCH, and PBDEs 47, 99 and 153) ranged between 8% and 47%. Thus, all 22 POPs were detected in some participants, and no individual was free from POPs: the smallest number of POPs detected in one person was 15. The median number of POPs detected per person was 19, with no differences by subjects' individual characteristics, and very few by country: only Umeå in Sweden, and the United Kingdom had medians of 18 and 20 POPs detected per person, respectively. Twenty or more compounds were detected in 25% of participants; this was so in 28% of participants born in 1919–1938 and in 21% of subjects born in 1939–1964. The number of POPs quantified per person ranged between 21 and 11, and only 6% of participants had less than 16 compounds quantified.

2.9.3. Correlations among POPs

The highest Spearman's correlation coefficients (ρ) among crude or wet weight concentrations of the 16 most detected POPs were observed between pairs of PCBs; e.g., the ρ between PCB 170 and 180 was 0.985, and between PCB 138 and 153 it was 0.981). The ρ between oxychlordane and trans-nonachlor was 0.809. The ρ between *p,p'*-DDT and *p,p'*-DDE was 0.800, and between HCB and β-HCH, 0.744. The rest of correlations between pairs of organochlorine pesticides ranged from 0.159 (for β-HCH and trans-nonachlor) to 0.711 (for *p,p'*-DDT and β-HCH) (all p-values <0.001). Finally, correlation coefficients between PCBs and organochlorine pesticides ranged from 0.017 (for PCB 156 and β-HCH, p-value = 0.496) to 0.616 (for PCB 74 and oxychlordane, p-value < 0.001). When TL-corrected concentrations of POPs were analysed, ρ's were only slightly attenuated, and the results just mentioned remained virtually unaltered.

2.9.4. Concentrations of POPs

The highest concentrations were found for *p,p'*-DDE, PCBs 153 and 180 (median concentration: 3371, 1023, and 810 pg/mL, respectively). For the rest of PCBs median concentrations ranged from 66 pg/mL for PCB 74 to 635 pg/mL for PCB 138; and for the rest of organochlorine pesticides the corresponding values ranged from 55 pg/mL for oxychlordane to 393 pg/mL for HCB (Table 5). 39% of participants had one or more of the 16 most prevalent POPs at concentrations above their respective P90 (i.e., 61% of participants had each of the 16 POPs at concentrations below their respective P90). The corresponding figures for P75 were 69% and 31%. Figures were similar for TL-corrected POPs (e.g., 42% of participants had one or more of the 16 TL-corrected POPs above their respective P90). For both cut-offs (P90 and P75), the geometric means of the nPhc were 2.9 and 4.3, respectively (2.8 and 4.3 for TL-corrected POPs).

Women had statistically significant higher concentrations of HCB (median 29% higher than men's) and β-HCH (median 49% higher), while men had higher concentrations of trans-nonachlor (median 34% higher than women's) and of PCBs (except PCBs 74 and 118) (Table

5). No significant differences between men and women were observed for *p,p'*-DDT, *p,p'*-DDE and oxychlordane. In univariate analyses differences in POP concentrations according to age, BMI, fasting status, and country were also observed.

Median POP concentrations were higher in subjects with greater age and BMI; e.g., median concentrations of HCB were 1.7 times higher in the oldest group than in the youngest, and 2.5 times higher in obese participants than in normal-weight individuals (Table 5). However, as often reported in the literature (Gasull et al., 2012; Porta et al., 2008b), obese participants had lower concentrations of some PCBs (congeners 153, 156, 170, and 180) than overweight and normal-weight participants. Differences in POP concentrations were also observed by fasting status; higher median concentrations were found in individuals who had fasted more than six hours. Differences observed by sex, age, BMI and fasting status remained significant when POP concentrations were corrected by TL (Supplemental Table 5).

Participants from Spain had the highest concentrations of *p,p'*-DDT, HCB, β-HCH and PCBs 183 and 187; remarkably, their median concentration of HCB (4401 pg/mL) was more than 18 times higher than the corresponding value of participants from the United Kingdom, the country with the lowest concentrations of HCB (237 pg/mL); it was also two times higher than the corresponding value from the second country with the highest concentrations of HCB (Greece, 2187 pg/mL) (Table 5). Subjects from Italy had the highest median concentrations of oxychlordane, and of PCBs 74, 99 and 118. Germany had the highest concentrations of PCBs 153, 156, 170, and 180. The highest plasma levels of trans-nonachlor and PCB 138 were found in participants from Umeå. Subjects from Greece had the highest concentrations of *p,p'*-DDE, but also the lowest concentrations of all PCBs. The lowest concentrations of *p,p'*-DDT and *p,p'*-DDE were detected in participants from Denmark. Again, similar results were obtained when TL-corrected POP concentrations were analysed (Supplemental Table 5): the highest concentrations (in ng/g of lipid) of *p,p'*-DDT, HCB, β-HCH and PCBs 180, 183 and 187 were observed in subjects from Spain, while participants from Germany had the highest concentrations of PCBs 138, 153, 156 and 170.

2.9.5. Relation between lipid and POP concentrations

Spearman's correlation coefficients (ρ) among crude concentrations of POPs and the three lipid measures (total cholesterol, triglycerides and TL) were all positive and statistically significant; the highest values of ρ were 0.464 for oxychlordane and TL, 0.447 for trans-nonachlor and TL, and 0.412 for trans-nonachlor and triglycerides (p-values < 0.001). When models adjusting for age, sex, centre, and BMI were applied, the observed associations remained significant, with no differences according to fasting status. However, most associations between lipid and POP concentrations disappeared when concentrations of POPs corrected by TL were used; only oxychlordane and trans-nonachlor remained positively and statistically significantly associated with triglycerides and TL in adjusted models, mainly in participants with less than six hours of fasting.

2.10. Disease progression bias

The design of the present study (a prospective case–control nested within a cohort) is a valid and efficient way to cope with possible biases caused by changes in lipophilic biomarkers of exposure induced by the disease (subclinical or clinical disease); i.e., disease progression bias (DPB) (Lee et al., 2014; Porta et al., 2005, 2008a, 2009b, 2014). Moreover, to further control for potential DPB, we did not include in the study cases of pancreatic cancer diagnosed within 2 years of blood draw (5 years for cases from Denmark). Nevertheless, the effectiveness of such design features to control DPB has seldom been empirically tested. Furthermore, it is unknown whether DPB could operate more than 2 years after blood draw. Therefore, we studied the relation between lipid and POP concentrations in plasma samples collected during recruitment, and some variables related to the disease. Specifically, we studied: *a)* the relation between lipid concentrations at blood collection and the time elapsed between such collection and the date of pancreatic cancer diagnosis (please see section *Baseline characteristics of study participants*), and *b)* the relation between lipid concentrations and selected characteristics of the tumour, such as stage and subsite within the pancreas, and tumour grade (among cases with available information). We also analysed the same relationships using POP concentrations instead of lipid concentrations.

If subclinical pancreatic cancer or its precursors were increasing concentrations of lipids in plasma at the time of blood draw (e.g., through metabolic changes, weight loss and lipid mobilization from fatty tissues to blood), we would expect to observe that cases with higher lipid concentrations at blood draw (mostly, 5 to 15 years before diagnosis) were diagnosed closer to the time of blood draw than cases with lower concentrations of lipids; thus, an inverse relationship between lipid concentrations at blood draw and the interval from blood draw to cancer diagnosis would suggest the existence of DPB, which would need to be considered in the etiologic analyses (e.g., latency analyses stratified by time since blood draw would be warranted).

No such inverse association existed between total lipid concentrations of cases and time from blood collection to diagnosis, either in descriptive analyses (Figure 4) or in multivariate regression models adjusting for study centre, age, sex, BMI, and fasting status (Table 6). No inverse associations were observed either for total cholesterol and for triglycerides (results not shown). Actually, in multivariate models we observed a slightly positive, non-linear association between TL and the time interval (i.e., the opposite of the inverse association suggesting DPB): the interval for cases in the upper quartile of TL concentrations was around one year longer than for cases in the lowest TL quartile (aGM 8.6 and 7.4, respectively, Table 6). A similar positive association was observed when cases with time intervals >15 years and <5 years (only registered in Umeå and in the IARC repository, by design) were excluded. When stratifying by

fasting status, the positive association between TL and the interval was only observed in non-fasting individuals (i.e., the less reliable subgroup) (Table 6); there were no associations in cases who had fasted ≥3 hours (p-value for interaction = 0.132). Although similar results were observed in non-fasting controls, again no statistically significant associations between lipid concentrations and the time from blood collection to the index date were observed in controls. The positive associations are the opposite of the inverse associations that would suggest DPB and thus argue against the occurrence of a DPB.

If subclinical pancreatic cancer or its precursors were increasing concentrations of lipids and, thus, of POPs at the time of blood draw, we would expect that cases with higher POPs at blood draw would be diagnosed closer to the time of blood draw than cases with lower POPs; thus, an inverse relationship between POP concentrations at blood draw and the interval from blood draw to cancer diagnosis would suggest the existence of DPB. No such inverse association existed for any POP. In fact, in a few instances the association was positive: the interval from blood collection to cancer diagnosis was slightly longer in cases with higher concentrations of some POPs (both crude and TL-corrected). For instance, cases with TL-corrected concentrations of HCB in the upper quartile had such interval more than two years longer than cases in the lower quartile once adjusting for study centre, age, sex, BMI, and fasting status (aGM of the interval from blood collection to cancer diagnosis for HCB upper and lower quartiles: 9.6 and 7.3 years, respectively; p-value = 0.003) (Supplemental Table 6); this association remained statistically significant among cases who had fasted ≥3 hours. A similar association was found for the sum of PCBs 118, 138, 153 and 180 (aGM for upper quartile = 8.8 years vs. aGM for the lower quartile = 7.5 years, p-value = 0.025); this association was not present in cases who had fasted ≥3 hours (data not shown). No associations were found between the mentioned interval and other compounds, such as *p,p'*-DDT, *p,p'*-DDE and β-HCH. Although attenuated, the interval from blood collection to index date was also slightly longer in controls with higher concentrations of some POPs (both crude and TL-corrected). The null associations argue against the occurrence of a DPB, and the positive associations are the opposite of the inverse associations that would suggest DPB. Aside from reverse causation due to DPB (i.e., to disease-increased lipid concentrations), increasing concentrations of POPs over time –and, hence a higher concentration of POPs near the time of diagnosis– might also be expected if POPs were truly implicated in the development and progression of pancreatic cancer.

We also studied the relation between lipid or POP concentrations and some characteristics of the tumour and, based on previous work (Porta, 2001; Porta et al., 2007, 2008a, 2009b), we hypothesised that if subclinical pancreatic cancer were already causing subtle pathophysiologic changes at the time of blood draw (e.g., through moderate weight loss, lipid mobilisation or other metabolic changes), we would observe that cases later diagnosed with a more advanced disease (e.g., a metastatic tumour) or a more aggressive disease (e.g., a poorly differentiated tumour) would have higher lipid and POP concentrations (again, at blood draw). Among the

limited number of cases with the available information (see section *Main variables, and collection of blood samples*), tumour stage and pancreatic subsite were not associated with any of the three lipid measures (total cholesterol, triglycerides and total lipids, Table 7). These results suggest that DPB did not occur.

However, in the 85 cases with data on grading of the tumour and on adjusting variables, descriptive and multivariate analyses showed that cases with less differentiated tumours had higher concentrations of triglycerides and TL at study entry than cases diagnosed with more differentiated tumours (aGM of TL for poorly differentiated tumours = 636 vs. aGM for well differentiated tumours = 539; p-value = 0.025) (Table 7). These associations held when cases with longer times from blood extraction to cancer diagnosis (e.g., ≥10 years) were excluded. These results suggest that DPB might occur.

Similar results for the presence or absence of the associations between all three tumour characteristics and the three lipid measures were found when cases in non-fasting status were excluded from the analyses.

Finally, lower TL-corrected concentrations of some POPs (as always, at baseline, >2 years prior to the diagnosis of pancreatic cancer) were observed in cases with metastatic tumours (N = 126) than in cases with localised tumours (N = 51), again when adjusting for centre, age, BMI and fasting status. Differences were statistically significant only for the sum of PCBs 118, 138, 153, and 180, and for the sum of all PCBs: aGM of TL-corrected concentrations of all PCBs for metastatic tumours = 548 ng/g (95% CI: 503 - 597) vs. aGM for localised tumours = 679 (95% CI: 578 - 798) (p-value = 0.050). There were no differences in TL-corrected concentrations of POPs by tumour subsite. Contrary to the hypothesis supporting the existence of DPB, TL-corrected concentrations of POPs were slightly lower in cases with poorly differentiated tumours than in cases with moderately differentiated and with well differentiated tumours. These results argue against the occurrence of a DPB.

3. DISCUSSION

We observed a number of associations that may need to be considered in the upcoming analyses on the possible influence of POPs on pancreatic cancer risk. Thus, lipid concentrations were related to age, BMI, fasting status, country, and smoking; while fasting status, once adjusted for centre and time of blood collection, was related to sex and BMI. Differences among countries were observed for subjects' characteristics (as sex, smoking, alcohol consumption, physical activity, and diabetes, but not age or BMI), and for study characteristics (as year of last follow-up, length of follow-up and, hence, time from blood collection to index date, basis of cancer diagnosis, fasting status, and lipid and POP concentrations). We found no differences in lipid concentrations between pancreatic cancer cases and controls; although expected because of the study design, this fact needed confirmation.

The fact that lipid concentrations were so similar in cases and controls (Figure 2) shows the methodological progress that the present design represents with respect to previous studies on POPs and pancreatic cancer risk (Supplemental Tables 7 and 8). Other methodological characteristics and findings of previous studies are also summarized in the mentioned Tables.

Adjusted models showed increasing concentrations of total lipids with increasing age and BMI. Total lipids were also higher in smokers, in participants with less than six hours of fasting at blood collection, and in Umeå. The specific way in which the study was conducted in each country determined fasting status.

Therefore, when studying the effects of POP concentrations corrected or normalized by lipids (López et al., 2014; Porta et al., 2008a; 2009a), it may be necessary to take into account the associations observed between lipid concentrations and age, BMI, fasting status, country, and smoking; e.g., because some of the possible effects of these latter variables could partly be adjusted by the lipid correction.

Most associations between lipid and POP concentrations waned when concentrations of POPs corrected by TL were used, as it can partly be expected from the work of Phillips et al. (Bernert et al., 2007; Phillips et al., 1989).

The main traditional reason for the correction of POP concentrations by lipids is to remove differences in POP concentrations due to differences in lipid concentrations between fasting and non-fasting individuals (Bernert et al., 2007; Phillips et al., 1989; Li et al., 2013). In the present study fasting status was one of the matching factors; thus, lipid correction could be deemed unnecessary or unwarranted in conditional or matched analyses (in which, again, fasting status is one of the matching factors). However, lipid correction of POP concentrations may be preferable than adjusting by fasting if the latter is measured less accurately than plasma concentrations of lipids. If uncorrected POP concentrations are used in unconditional analyses, then fasting status and BMI may need to be included as covariates. Models with POPs uncorrected for lipids (and perhaps unadjusted for BMI as well) may be informative and valid too because lipids (and BMI changes) may be both confounders and mediators in the hypothetical causal chain between POPs and pancreatic cancer (Donat-Vargas et al., 2018; Gallo et al., 2011; O'Brien et al., 2016; Rylander et al., 2015). The main relevant causal structures (Hernán et al., 2018) are summarized in the Directed Acyclic Graphs (DAGs) shown in Supplemental Figure 1.

The specific way in which a study is conducted in each country needs to be considered. For instance, more than 90% of participants from Umeå had been fasting for >6 hours and information on time of blood collection was less frequently recorded in this centre. Thus, in the entire study population the availability of information on time of blood collection was strongly

related to fasting status. Furthermore, participants from Umeå were younger, leaner, and had higher concentrations of lipids than the average of the rest of participants.

For reasons explained above, we excluded cases diagnosed of pancreatic cancer within 2 years of blood draw. We can envision no plausible biases that such exclusion might create in the analyses on POPs and pancreatic cancer risk.

We assessed the possible occurrence of DPB in eight situations defined by lipid and POP measurements, on one hand, and by four factors: interval from blood draw to date of cancer diagnosis, tumour subsite, tumour stage, and grade of differentiation, on the other. In seven of the eight situations results argued against the occurrence of a DPB, the possible exception being that cases with less differentiated tumours had higher concentrations of triglycerides and TL at study entry than cases diagnosed with more differentiated tumours. Analyses of the eight situations were possible to different extents due to different numbers of subjects with the necessary information available. Several plausible DAGs on DPB in pancreatic cancer have previously been proposed for study designs that measure POPs and lipids at the time of pancreatic cancer diagnosis (López et al., 2014).

In both pancreatic cancer cases and controls, no inverse association was observed between total lipid concentrations and time from blood collection to diagnosis, either in descriptive analyses (Figure 4) or in multivariate models. These findings argue against the existence of DPB. The available knowledge indicates that the mechanisms of occurrence and progression (molecular, genetic and epigenetic) of exocrine pancreatic cancer years before clinical emergence of the disease do not entail metabolic and other pathophysiologic changes that alter lipophilic biomarkers (Kamisawa et al., 2016). Hence, 5-15 years before diagnosis, subclinical pancreatic cancer might be present (e.g., molecular changes) but it is implausible that it would already be causing the metabolic and other pathophysiologic changes that we hypothesize could bias the results. Nevertheless, current evidence does not rule out that higher concentrations of lipids increase risk for pancreatic cancer occurrence or progression (Di Ciaula, 2014). And, thus, higher concentrations of lipids near the time of diagnosis would not necessary occur because of DPB and reverse causation, they could reflect a true causal link. The fact is that we did not observe such association (see Table 6, particularly the more reliable rows showing results for cases with longer fasting times). We made a similar argument for timing of blood draw and POPs in section 2.10.

Also concerning DPB: among 177 cases with the available data, tumour stage was not associated with any of the three lipid measures; therefore, concentrations of lipids at blood draw were not increased by subclinical, more disseminated tumours.

Among only 85 cases with data on tumour grade, cases with less differentiated tumours had higher concentrations of triglycerides and TL at study entry than cases diagnosed of more

differentiated tumours. There were only 11 cases in the reference category of well differentiated tumours. The small numbers do not warrant the conclusion that concentrations of lipids at blood draw were already increased by subclinical poorly differentiated tumours. Future studies may consider this approach to assessing DPB.

Studies with more complete information on the pancreatic subsite of the tumour may consider assessing whether subclinical tumours in different subsites might have altered differently lipids and lipophilic substances at blood draw; e.g., because of the pathophysiologic changes that ensue when a tumour in the pancreatic head compresses the bile duct (Porta et al., 2005, 2008a, 2009b).

Cohort studies, nested case-control studies and other longitudinal designs are often most efficient to cope with DPB (Porta et al., 2014). However, biases associated with disease progression still need to be assessed in such studies (Porta et al., 2009b). First, because in some cohort studies the interval between blood draw and outcome is short for at least a subset of cases (Dorgan et al., 1999; Hunter et al., 1997; Wolff et al., 1993). Second, because such designs sometimes suffer from selection biases due to partial availability of blood samples or limited retrieval of disease-related samples (e.g., tumour tissue) (Porta, 2001). And third, because they may not have collected relevant etiologic data. Therefore, the empirical tests of DPB reported here are relevant beyond the present study.

We used a classification of diagnostic basis that will be useful to perform future etiologic analyses on POP concentrations and risk of pancreatic cancer stratified on diagnostic basis, as a proxy for diagnostic certainty; these analyses are sometimes referred to as sensitivity analyses. Over 75% of cases had been diagnosed through microscopic methods, which is a common figure in large studies and case series (Porta, 2005; Porta et al., 1994). Significant differences in the percentage of cases with microscopic confirmation were observed across countries (>95% of cases from Denmark and Umeå, <40% in other countries). Microscopic confirmation was higher in men, and in younger and more educated cases. It was not related to tumour subsite or stage, neither to other potentially important variables as BMI, smoking, alcohol, or physical activity. Thus, assessing potential biases due to diagnostic certainty and disease misclassification will be warranted in etiologic analysis not only as a general precaution, but also because diagnostic certainty could be related to exposures associated with factors as age, gender, or education (Porta, 2005).

Finally, exposure to the POPs selected for analysis was quite widespread in the study population, with substantial variability. This is a main strength of studies within EPIC (Riboli et al., 2002). All 22 POPs analysed were detected in some participants, and the lowest number detected in one person was 15. We detected and quantified 16 compounds (all but 6) in more than 90% of individuals. 42% of participants had one or more of such 16 prevalent POPs at concentrations (corrected by TL) above their respective P90. In a study based on the general

population of Catalonia (N = 919) (Porta et al., 2012), the corresponding figure was 32% (with 8 most prevalent POPs out of 19 POPs analysed). In a study based on the US general population (N >4,000) (Pumarega et al., 2016), the corresponding figure was 67%, in part, probably, because the number of POPs analysed was higher (37 most prevalent POPs out of 91 POPs analysed). For participants in the present study and in the studies in Catalonia and the US, the geometric mean of the nPhc (TL-corrected POPs; nPhc cut-off: P90) was 2.8, 2.0 and 3.4, respectively.

The observed correlations between pairs of POPs were expected (Gasull et al., 2012; Porta et al., 2008a, 2012). Correlations among certain POPs are often strong worldwide; it is a feature of human contamination by POPs that many studies must address, and so will ours.

Significant differences in plasma concentrations of several POPs (uncorrected and corrected by total lipids) were observed by age, sex, BMI, fasting status, and country. They will need to be considered in the subsequent analyses on POPs and pancreatic cancer risk.

Participants from Spain had the highest concentrations of *p,p'*-DDT, β-HCH, PCBs 183 and 187, and particularly of HCB, as also observed in previous studies (Aylward et al., 2014). Germany had the highest concentrations of some PCBs. Analyses of predictors of POP concentrations in controls will be reported in a future article.

3.1. Conclusions

The present article not only summarizes the main methodological features of the study, but it also reports a number of associations among study and subjects' characteristics that may play important roles in the forthcoming analyses on the association between plasma concentrations of POPs and pancreatic cancer risk. Notably, there were differences among countries in subjects' characteristics (as age, gender, smoking, lipid and POP concentrations), and in study characteristics (as time from blood collection to index date, year of last follow-up, length of follow-up, basis of cancer diagnosis, and fasting status). Adjusting for centre and time of blood collection, no factors were significantly associated with fasting status. Plasma concentrations of lipids were related to age, body mass index, fasting, country, and smoking. Analyses assessing disease progression bias suggested it was highly unlikely. The coexistence of differences across study centres in some design features and participant characteristics is of relevance to other multicentre studies. Associations among subjects' characteristics and between such characteristics and design features may play important roles in the forthcoming analyses on the association between plasma concentrations of POPs and pancreatic cancer risk.

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FIGURE LEGENDS

Figure 1. Proportional distribution of the time from blood collection to index date according to countries (N=1,533).

Footnote: In cases, the index date corresponds to the date of the diagnosis of pancreatic cancer, and in controls to the date of cancer diagnosis of the case matched with.

Figure 2. Distribution of plasma concentrations of total lipids (mg/dL) by case-control status (N=1,533).

Figure 3. Percentages of detection and quantification of all POPs analysed in plasma samples of the 1533 participants included in the study.

Footnote: The figures inside the bars refer to the percentage of quantification (detected and quantified). For compounds with no figure, the corresponding percentage was 100%.

Figure 4. Time from blood collection to index date according to plasma concentrations of total lipids and case-control status.

Table 1. Baseline characteristics of study participants according to case-control status.

Characteristic	Total N (%)	Cases N (%)	Controls N (%)	*p*-value [a]
Total	1533 (100)	513 (33.5)	1020 (66.5)*	
Sex				matched
Men	746 (48.7)	250 (48.7)	496 (48.6)	
Women	787 (51.3)	263 (51.3)	524 (51.4)	
Age** (years)				matched
Mean ± standard deviation	56.8 ± 7.5	56.8 ± 7.5	56.8 ± 7.6	
Median	57.7	57.6	57.7	
Birth cohort				matched
1919-1938	817 (53.3)	271 (52.8)	546 (53.5)	by age
1939-1945	503 (32.8)	172 (33.5)	331 (32.5)	
1946-1964	213 (13.9)	70 (13.6)	143 (14.0)	
Body mass index (kg/m^2)				
mean ± standard deviation	26.3 ± 4.2	26.5 ± 4.4	26.2 ± 4.1	0.204 [b]
Underweight (<18.5)	11 (0.7)	4 (0.8)	7 (0.7)	0.891
Normal weight (18.5-24.9)	611 (40.6)	197 (39.3)	414 (41.2)	
Overweight (25.0-29.9)	647 (43.0)	219 (43.7)	428 (42.6)	
Obese (≥30.0)	236 (15.7)	81 (16.2)	155 (15.4)	
Men, mean ± standard deviation	26.6 ± 3.6	26.8 ± 3.6	26.6 ± 3.5	0.535 [b]
Women, mean ± standard deviation	26.0 ± 4.7	26.2 ± 5.0	25.8 ± 4.5	0.262 [b]
Education* **				0.195
Less than primary completed	83 (5.5)	29 (5.8)	54 (5.4)	
Primary school completed	529 (35.3)	189 (38.0)	340 (34.0)	
Technical/professional school	398 (26.6)	115 (23.1)	283 (28.3)	
Secondary school	201 (13.4)	73 (14.7)	128 (12.8)	
Longer education	286 (19.1)	92 (18.5)	194 (19.4)	
Marital status				0.492
Single	67 (6.8)	26 (7.9)	41 (6.2)	
Married/living together	783 (79.1)	255 (77.5)	528 (79.9)	
Divorced/separated	73 (7.4)	22 (6.7)	51 (7.7)	
Widowed	67 (6.8)	26 (7.9)	41 (6.2)	
Smoking status				<0.001
Never	650 (42.8)	206 (40.8)	444 (43.8)	

Former	472 (31.1)	135 (26.7)	337 (33.3)	
Current	396 (26.1)	164 (32.5)	232 (22.9)	
Alcohol intake at recruitment				
(g/day), median	6.4	6.8	6.4	0.640 [c]
Never and former drinkers	141 (9.2)	45 (8.8)	96 (9.4)	0.930
>0-6 g/day	600 (39.3)	197 (38.6)	403 (39.6)	
>6-18 g/day	363 (23.8)	125 (24.5)	238 (23.4)	
>18 g/day	424 (27.7)	144 (28.2)	280 (27.5)	
Men, median	12.4	13.5	12.0	0.370 [c]
Women, median	2.6	2.4	2.6	0.946 [c]
Physical activity				0.884
Active	101 (8.0)	30 (7.1)	71 (8.4)	
Moderately active	587 (46.5)	199 (47.3)	388 (46.1)	
Moderately inactive	361 (28.6)	120 (28.5)	241 (28.6)	
Inactive	214 (16.9)	72 (17.1)	142 (16.9)	

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Table 1, continued.

Characteristic	Total N (%)	Cases N (%)	Controls N (%)	*p*-value [a]
Total	1533 (100)	513 (33.5)	1020 (66.5)*	
Diabetes mellitus				0.011
No	1323 (95.7)	431 (93.7)	892 (96.7)	
Yes	59 (4.3)	29 (6.3)	30 (3.3)	
Exogenous hormones** (women)				matched
Yes	154 (22.2)	52 (22.7)	102 (21.9)	
No	541 (77.8)	177 (77.3)	364 (78.1)	
Fasting status**				matched
Fasting (>6 hours)	455 (30.1)	152 (30.0)	303 (30.1)	
In between (3-6 hours)	295 (19.5)	102 (20.2)	193 (19.2)	
Non-fasting (<3 hours)	762 (50.4)	252 (49.8)	510 (50.7)	

* All cases have 2 matched controls, except 6 cases who have 1 matched control. Cases and controls were matched by EPIC study centre, sex, age at blood collection, date and time of blood collection, fasting status and, for women, use of exogenous hormones. **At blood collection. *** Highest educational level attained. Longer education includes university degree.
[a] Unless otherwise specified, *p* value derived from Fisher's exact test (two-tailed).
[b] Student's *t* test (two-tailed).
[c] Mann-Whitney's *U* test (two-tailed).

Table 2. Influence of characteristics of cases on the probability of having a pancreatic cancer diagnosed by
microscopic methods (vs. clinical tests and other).

		Basis of cancer diagnosis					
	Total	Microscopic methods*	Clinical tests and other**				
Characteristic	N (%)	N (%)	N (%)	*p*-value[a]	OR	(95% CI)	*p*-value[b]
Total	506 (98.6)	382 (75.5)	124 (24.5)				
Sex							
Men	246 (48.6)	204 (82.9)	42 (17.1)	<0.001	1.00	-	0.364
Women	260 (51.4)	178 (68.5)	82 (31.5)		0.76	(0.41-1.39)	
Age at diagnosis of pancreatic cancer (years)							
Median	66.8	65.9	68.7	<0.001 [c]	0.96	(0.92-0.99)	0.021
<60 years	103 (20.4)	85 (82.5)	18 (17.5)	0.014	1.00	-	0.105[d]
60-69 years	246 (48.6)	191 (77.6)	55 (22.4)		0.50	(0.23-1.11)	
≥70 years	157 (31.0)	106 (67.5)	51 (32.5)		0.47	(0.21-1.07)	
Age at blood collection (years)							
Median	57.7	57.3	60.0	<0.001 [c]	0.95	(0.92-0.99)	0.018
<50 years	73 (14.4)	60 (82.2)	13 (17.8)	0.001	1.00	-	0.389
50-54 years	117 (23.1)	99 (84.6)	18 (15.4)		0.64	(0.22-1.84)	
55-59 years	137 (27.1)	105 (76.6)	32 (23.4)		0.48	(0.18-1.26)	
≥60 years	179 (35.4)	118 (65.9)	61 (34.1)		0.47	(0.18-1.19)	
Birth cohort							
1919-1938	269 (53.2)	187 (69.5)	82 (30.5)	0.004	1.00	-	0.095[d]

1939-1945	168 (33.2)	139 (82.7)	29 (17.3)		1.54	(0.81-2.91)	
1946-1964	69 (13.6)	56 (81.2)	13 (18.8)		1.87	(0.76-4.60)	
Body mass index (kg/m^2)							
Median	25.7	25.8	25.4	0.325[c]	1.00	(0.93-1.07)	0.955
Underweight (<18.5)	4 (0.8)	3 (75.0)	1 (25.0)	0.746	–		
Normal weight (18.5-24.9)	195 (39.5)	142 (72.8)	53 (27.2)		1.00	-	0.912[e]
Overweight (25.0-29.9)	217 (43.9)	166 (76.5)	51 (23.5)		0.88	(0.46-1.69)	
Obese (≥30.0)	78 (15.8)	61 (78.2)	17 (21.8)		1.00	(0.40-2.53)	

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Table 2, continued.

		Basis of cancer diagnosis					
	Total	**Microscopic methods***	**Clinical tests and other****				
Characteristic	N (%)	N (%)	N (%)	*p*-value[a]	OR	(95% CI)	*p*-value[b]
Total	506 (98.6)	382 (75.5)	124 (24.5)				
Education							
Less than primary completed	29 (5.9)	20 (69.0)	9 (31.0)	0.782	1.00	-	0.387[d]
Primary school completed	186 (37.8)	143 (76.9)	43 (23.1)		1.31	(0.34-5.09)	
Technical/professional school	114 (23.2)	91 (79.8)	23 (20.2)		1.39	(0.31-6.29)	
Secondary school	72 (14.6)	56 (77.8)	16 (22.2)		1.53	(0.33-7.09)	
Longer education	91 (18.5)	69 (75.8)	22 (24.2)		1.86	(0.38-9.02)	
Marital status							
Single	25 (7.8)	20 (80.0)	5 (20.0)	0.114	1.00	-	0.570
Married/living together	250 (77.6)	167 (66.8)	83 (33.2)		0.37	(0.08-1.68)	
Divorced/separated	21 (6.5)	12 (57.1)	9 (42.9)		0.29	(0.05-1.73)	
Widowed	26 (8.1)	13 (50.0)	13 (50.0)		0.42	(0.07-2.66)	
Smoking status							
Never	203 (40.8)	146 (71.9)	57 (28.1)	0.059	1.00	-	0.329
Former	133 (26.7)	98 (73.7)	35 (26.3)		0.58	(0.28-1.20)	
Current	162 (32.5)	133 (82.1)	29 (17.9)		0.83	(0.40-1.72)	
Alcohol intake at recruitment							
Never and former drinkers	45 (8.8)	25 (56.8)	19 (43.2)	0.020	1.00	-	0.269
>0-6 g/day	197 (38.6)	150 (76.1)	47 (23.9)		1.54	(0.62-3.82)	
>6-18 g/day	125 (24.5)	92 (75.4)	30 (24.6)		0.71	(0.27-1.90)	
>18 g/day	144 (28.2)	114 (80.9)	27 (19.1)		1.16	(0.43-3.11)	
Physical activity							
Active	30 (7.2)	25 (83.3)	5 (16.7)	0.007	1.00	-	0.445
Moderately active	195 (47.1)	127 (65.1)	68 (34.9)		0.54	(0.15-1.91)	
Moderately inactive	119 (28.7)	82 (68.9)	37 (31.1)		0.48	(0.13-1.83)	
Inactive	70 (16.9)	59 (84.3)	11 (15.7)		0.97	(0.22-4.34)	

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Table 2, continued.

		Basis of cancer diagnosis					
	Total	**Microscopic methods***	**Clinical tests and other****				
Characteristic	N (%)	N (%)	N (%)	*p*-value[a]	OR	(95% CI)	*p*-value[b]
Total	506 (98.6)	382 (75.5)	124 (24.5)				
Diabetes mellitus							
No	424 (93.6)	338 (79.7)	86 (20.3)	0.635	1.00	-	0.347
Yes	29 (6.4)	22 (75.9)	7 (24.1)		1.82	(0.52-6.29)	
Exogenous hormones (women)							
Yes	50 (22.1)	35 (70.0)	15 (30.0)	0.502	1.00	-	0.231
No	176 (77.9)	112 (63.6)	64 (36.4)		0.51	(0.16-1.62)	
Tumour subsite							
Head of pancreas	229 (66.2)	175 (76.4)	54 (23.6)	0.372	1.00	-	0.209
Body of pancreas	58 (16.8)	47 (81.0)	11 (19.0)		0.76	(0.28-2.06)	
Tail of pancreas	36 (10.4)	27 (75.0)	9 (25.0)		1.11	(0.35-3.57)	
Overlapping	23 (6.6)	21 (91.3)	2 (8.7)		6.38	(1.06-38.3)	
Stage of the tumour							
Localised	51 (28.8)	45 (88.2)	6 (11.8)	0.372	1.00	-	0.464
Metastatic	126 (71.2)	103 (81.7)	23 (18.3)		0.57	(0.13-2.56)	

*Microscopic methods: this category includes cases with the following bases of diagnosis as the first basis, sorted from higher to lower validity: *Autopsy (N=18), Histology of primary tumour (N=144), Histology of metastasis (N=51), Histology/Cytology of primary tumour (N=96), Histology/Cytology of metastasis (N=15), and Cytology or haematology (N=58)*.

**Clinical tests and other: this category includes cases with the following bases of diagnosis as the first basis, sorted from higher to lower validity: *Specific biochemical or immunological tests (N=4), Exploratory surgery/autopsy (N=4), Endoscopy (N=4), Magnetic resonance imaging (N=2), Computerized tomography scan (N=16), Radiological examination (N=5), Clinical investigation (N=46), Clinical observation (N=34), Self-report (N=2), Death certificate only (N=7).*

OR: Odds ratio adjusted for sex, center, and (except for age at blood extraction and birth cohort) age at diagnosis of exocrine pancreatic cancer (EPC). An OR=1 indicates the reference category. An OR>1 indicates a higher probability of having the pancreatic cancer diagnosed through microscopic methods.
[a] Unless otherwise specified, *p* value derived from Fisher's exact test (two-tailed).
[b] Unless otherwise specified, *p* value derived from Wald's test.
[c] Mann-Whitney's *U* test (two-tailed).
[d] Test for linear trend (multivariate analogue of Mantel's extension test).
[e] Without participants <18.5 kg/m^2 of body mass index.

Table 3. Influence of participants' characteristics on fasting status at blood collection.*

Characteristic	aOR	(95% CI)	*p*-value
Sex			
Men	1.00	-	0.291
Women	0.77	(0.47-1.26)	
Age at blood collection (years)			
<50 years	1.00	-	0.868
50-54 years	0.82	(0.37-1.84)	
55-59 years	0.96	(0.45-2.02)	
≥60 years	1.11	(0.55-2.27)	
Birth cohort			
1919-1938	1.00	-	0.506
1939-1945	0.71	(0.40-1.26)	
1946-1964	0.95	(0.47-1.91)	

Body Mass Index (kg/m^2)			
Normal weight (18.5-24.9)	1.00	-	0.313
Overweight (25.0-29.9)	0.98	(0.57-1.69)	
Obese (≥30.0)	1.63	(0.79-3.39)	
Smoking status			
Never	1.00	-	0.918
Former	1.13	(0.63-2.01)	
Current	1.07	(0.58-1.99)	
Alcohol intake at recruitment			
Never and former drinkers	1.00	-	0.637
>0-6 g/day	0.75	(0.33-1.70)	
>6-18 g/day	0.72	(0.30-1.73)	
>18 g/day	0.56	(0.23-1.36)	
Physical activity			
Active	1.00	-	0.487
Moderately active	1.29	(0.32-5.17)	
Moderately inactive	2.04	(0.49-8.51)	
Inactive	1.63	(0.35-7.60)	
Time of blood collection**			
6:00-9:30	1.00	-	<0.001
9:31-11:20	0.26	(0.14-0.47)	
11:21-14:14	0.08	(0.03-0.20)	
14:15-20:00	<0.01	(0.00-0.03)	
Missing	0.06	(0.01-0.23)	

N = 1,512.
*Fasting >6 hours vs. fasting ≤6 hours.
aOR: Adjusted odds ratio. Each variable is adjusted for centre and time of blood collection.
An OR=1 indicates the reference category
** 179 of the 192 participants (93%) with missing information on time of blood collection were from the Umeå study centre. 165 of such 179 participants from Umeå (92%) had been fasting for >6 hours, a fact that explains why time of blood collection was less frequently recorded in this centre. All other participants (i.e., participants with available information on time of blood collection, N = 1,320) were distributed among quartiles of time of blood collection. When time of blood collection is not adjusted for study centre, the OR for the missing category is 5.2, a figure that reflects the association between missing data on time of blood collection and having fasted >6 hours in Umeå.

Table 4. Influence of participants' characteristics on total lipid concentrations (mg/dL).

Characteristic	aGM	(95% CI)	*p*-value
Case-control status			
Controls (Ref.)	642	(634-650)	
Cases	647	(636-658)	0.441
Country			
Denmark (Ref.)	635	(621-649)	
Sweden	736	(711-762)	<0.001
Germany	631	(614-649)	0.736
United Kingdom	639	(619-659)	0.747
The Netherlands	642	(620-666)	0.549
Italy	643	(618-668)	0.637
Spain	585	(563-609)	0.001
Greece	587	(562-612)	0.001
France	598	(553-646)	0.145
Norway	–	–	–
Sex			
Men (Ref.)	647	(638-657)	

Women	640	(631-650)	0.336
Age at blood collection (years)			
<50 years (Ref.)	609	(592-626)	
50-54 years	632	(619-646)	0.036
55-59 years	649	(637-662)	<0.001
≥60 years	662	(651-673)	<0.001
Birth cohort			
1919-1938 (Ref.)	659	(650-668)	
1939-1945	635	(624-646)	0.001
1946-1964	608	(591-626)	<0.001
Body Mass Index			
Normal weight (Ref.)	617	(608-627)	
Overweight	656	(646-666)	<0.001
Obese	685	(667-702)	0.005
Smoking status			
Never (Ref.)	637	(627-646)	
Former	640	(628-651)	0.689
Current	661	(648-674)	0.004
Alcohol intake at recruitment			
Never and former drinkers	652	(629-675)	
>0-6 g/day	635	(624-646)	0.193
>6-18 g/day	637	(624-650)	0.262
>18 g/day	660	(647-673)	0.550
Physical activity			
Active (Ref.)	619	(595-643)	
Moderately active	636	(626-647)	0.203
Moderately inactive	633	(620-646)	0.310
Inactive	639	(622-657)	0.185
Fasting status			
Fasting (>6 hours) (Ref.)	616	(598-635)	
In between (3-6 hours)	662	(646-678)	0.001
Non-fasting (<3 hours)	654	(642-666)	0.005

aGM: Geometric mean adjusted for sex, age (except for birth cohort), body mass index, fasting status, and centre (except for country). N = 1,487.
p-value: p-value of each category of the variable when compared with the reference group (Ref.).

Table 5. Plasma concentrations of POPs (pg/mL) detected in over 90% of study participants according to sociodemographic characteristics.*

Characteristics	p,p'-DDT	p,p'-DDE	Oxychlordane	Trans-	HCB	β-HCH	PCB 118	PCB 156
All participants	86 (48 -	3371 (1728 -	55 (38 - 82)	74 (48 -	393 (253 -	355 (201 -	151 (98 -	122 (83 -
GM and P90	94 (381)	3384 (12733)	56 (120)	76 (189)	500 (2459)	386 (1467)	149 (343)	116 (233)
Detected (%)	99.7	100	99.8	100	100	99.9	100	100
Quantified (%)	97.8	99.8	92.2	100	100	99.9	100	100
Non-	1.9	0.2	7.6	0	0	0.0	0	0
Non-detected	0.3	0	0.2	0	0	0.1	0	0
Gender								
Male	82 (46 -	3312 (1667 -	55 (38 - 82)	86 (55 -	349 (233 -	284 (176 -	144 (95 -	141 (94 -
Female	87 (49 -	3425 (1835 -	56 (37 - 82)	64 (42 -	451 (266 -	424 (233 -	156 (99 -	109 (77 -
p-value	0.138	0.348	0.961	<0.001	<0.001	<0.001	0.171	<0.001
Age at blood								
<50 years	88 (48 -	3135 (1677 -	40 (28 - 61)	49 (32 -	306 (184 -	287 (144 -	119 (77 -	94 (64 -
50-54 years	60 (35 -	2402 (1371 -	50 (35 - 70)	71 (49 -	343 (237 -	262 (157 -	131 (89 -	126 (90 -
55-59 years	82 (49 -	3170 (1693 -	57 (39 - 84)	80 (54 -	398 (268 -	332 (207 -	158 (101 -	131 (89 -
≥60 years	113 (63 -	4419 (2293 -	67.5 (46 -	86 (53 -	512 (296 -	508 (270 -	174 (111 -	130 (85 -
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Birth cohort								
1919-1938	103 (59 -	4087 (2121 -	65 (45 - 99)	87 (54 -	485 (286 -	456 (249 -	174 (112 -	133 (90 -

1939-1945	66 (38 -	2563 (1437 -	50 (36 - 72)	70 (49 -	340 (239 -	270 (174 -	134 (91 -	123 (89 -
1946-1964	82 (43 -	2779 (1448 -	38 (25 - 57)	46 (32 -	312 (167 -	290 (129 -	114 (70 -	87 (57 -
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Body Mass								
Normal range	68 (41 -	2772 (1515 -	52 (36 - 74)	69 (42 -	310 (216 -	285 (168 -	137 (87 -	125 (88 -
Overweight	95 (54 -	3766 (1974 -	57 (39 - 87)	78 (50 -	440 (278 -	382 (213 -	159 (106 -	126 (87 -
Obese	140 (76 -	5089 (2625 -	63 (44 -	81 (53 -	765 (408 -	599.5 (333 -	184 (121 -	108 (71 -
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.010
Fasting status								
Fasting (>6	99 (56 -	4216 (2076 -	57 (40 - 85)	78 (51 -	356 (242 -	358 (145 -	179 (117 -	124 (90 -
In between (3-6	83 (48 -	3511 (1640 -	54 (38 - 85)	75 (49 -	432.5 (278	393 (233 -	149 (91 -	127 (82 -
Non-fasting (<3	79 (45 -	2923 (1576 -	55 (37 - 79)	71 (46 -	384 (249.5	331 (207 -	138 (88 -	120 (82 -
p-value	<0.001	<0.001	0.192	0.011	0.010	0.002	<0.001	0.357

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Country								
Denmark	57 (37 -	2110 (1142 -	63 (45 -	106 (69 -	350 (264 -	244 (182 -	144 (91 -	139 (103 -
Sweden	63 (39 -	2509 (1455 -	57 (41 -	107 (68 -	260 (195 -	143 (98 -	173 (117 -	145 (106 -
Germany	162 (79 -	5272 (2467 -	43 (32 -	59 (41 -	743 (483 -	406 (261 -	172 (123 -	169 (132 -
United	73 (50 -	3094 (1935 -	44 (32 -	54 (39 -	237 (166 -	484 (324 -	116 (76 -	75 (61 - 99)
The	88 (53 -	3347 (1844 -	61 (45 -	51 (35.5 -	552 (296 -	394 (216 -	139 (93 -	114 (83 -
Italy	152 (89 -	5804 (3497 -	80 (59 -	80 (54 -	627 (342 -	629 (408 -	220 (123 -	122 (93 -
Spain	311 (156 -	9538 (4616 -	44 (33 -	61 (47 -	4401 (3276	2387 (1317 -	190 (134 -	88 (59 -
Greece	238 (117 -	11998 (6790 -	46 (31 -	53 (26 -	2187 (904 -	1424 (676 -	81 (55 -	31 (21 - 54)
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*The concentrations are expressed in median (percentile 25-percentile 75) pg/mL (pg/mL: parts per trillion, ppt). The second row for all participants presents the geometric mean (GM) and the 90th percentile (P90). *p*-value for Kruskal-Wallis test (two tailed).

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Table 5, continued.

Characteristics	PCB 138	PCB 153	PCB	PCB 74	PCB 99	PCB 170	PCB 183	PCB 187
All participants	635 (429 -	1023 (707	810 (577	66 (45 -	71 (46 -	370 (257	76 (49.5	192 (128
GM and P90	608 (1283)	979	790	65 (139)	69	358	73 (167)	188
Detected (%)	100	100	100	100	99.8	100	99.9	100
Quantified (%)	100	100	100	99.9	99.5	100	99.6	100
Non-	0	0	0	0.1	0.3	0	0.3	0
Non-detected	0	0	0	0	0.2	0	0.1	0
Gender								
Male	707 (491 -	1146 (803	961 (680	63 (41 -	75 (52 -	437 (304	86 (57 -	224 (151
Female	582 (376 -	929 (620 -	709 (503	70 (48 -	68 (42 -	325 (225	68 (42 -	164 (107
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Age at blood								
<50 years	544 (359 -	855 (583 -	705 (484	54 (34 -	63 (41 -	301 (216	71 (46 -	166 (106
50-54 years	650 (449 -	1037 (733	836 (610	58 (41 -	69 (44 -	385 (281	75 (49 -	190 (133
55-59 years	653 (435 -	1060 (742	840 (592	68 (47 -	69 (48 -	387 (271	74 (50 -	200 (133
≥60 years	655 (448 -	1059 (718	827 (585	81 (52 -	79 (49 -	377 (255	79 (52 -	195 (130
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.254	0.012
Birth cohort								
1919-1938	677 (465 -	1091	853 (604	78 (52 -	79 (51 -	389 (270	80 (54 -	203 (136
1939-1945	629 (432 -	1014	809 (592	58 (42 -	67 (45 -	370 (270	73 (49 -	187 (130
1946-1964	509 (324 -	777 (542	658 (458	50 (29 -	56 (36 -	290 (196	66 (38 -	155 (98 -
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Body Mass								
Normal range	620 (428 -	1014	826 (601	64 (43 -	68 (44 -	372 (268 -	73 (47 -	186 (125
Overweight	661 (452 -	1055	831 (591	68 (46 -	73 (50 -	382 (264 -	78 (54 -	197 (136
Obese	652 (425 -	1001	765 (509	72 (52 -	80 (49 -	352 (235 -	86.5 (91	203 (119
p-value	0.054	0.213	0.102	0.006	0.008	0.154	0.005	0.039
Fasting status								
Fasting (>6	708 (517 -	1100	880 (660	67 (45 -	76 (53 -	383 (293	90 (65 -	234 (159
In between (3-6	602 (406 -	1002	836 (568	66 (46 -	66 (43 -	375 (251	68 (43 -	182 (123
Non-fasting (<3	605 (404 -	986 (660	758 (541	66 (44 -	69 (44 -	355 (244	69 (46 -	171 (112
p-value	<0.001	<0.001	<0.001	0.517	<0.001	0.009	<0.001	<0.001

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Country								
Denmark	652 (499 -	1073 (804	860 (666	61 (45 -	72 (49 -	408 (315	70 (49 -	200 (148
Sweden	873 (625 -	1268 (942	901 (693	60 (40 -	84 (59 -	425 (329	98 (71 -	250 (186
Germany	814 (574 -	1346	1068 (806	72 (53 -	68 (48 -	506 (389	98 (67 -	204 (149
United	383 (282 -	621 (445 -	488 (390	79 (54 -	57 (39 -	209 (167	46 (33 -	121 (90 -
The	619 (420 -	964 (717 -	694 (535	70 (46 -	80 (51 -	339 (249	73 (49 -	139 (94 -
Italy	645 (472 -	1044 (783	888 (683	94 (64 -	96 (69 -	354 (284	84 (59 -	195 (136
Spain	655 (454 -	1051 (735	1021 (728	74 (50 -	66 (44 -	427 (299	109 (78 -	362 (251
Greece	224 (147 -	336 (222 -	234 (161	29 (18 -	31 (21 -	102 (736	29 (18.5 -	76 (45 -
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*The concentrations are expressed in median (percentile 25-percentile 75) pg/mL (pg/mL: parts per trillion, ppt). The second row for all participants presents the geometric mean (GM) and the 90th percentile (P90). *p*-value for Kruskal-Wallis test (two tailed).

Table 6. Influence of total lipid concentrations of pancreatic cancer cases on the time from blood collection to cancer diagnosis (years), in all cases and stratified by fasting status.

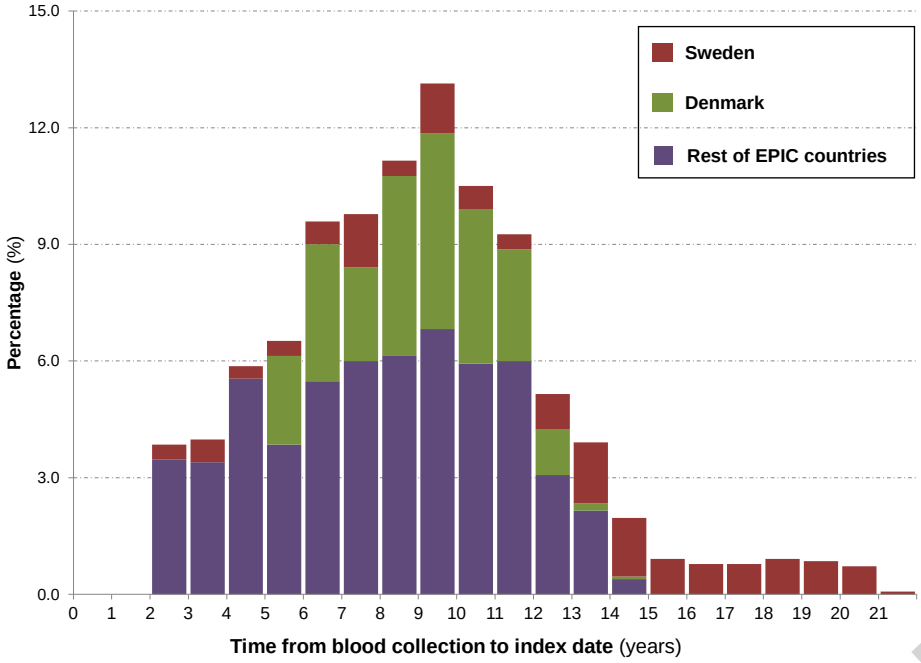
			Time from blood draw to diagnosis		
Total lipid concentrations	**N**	**(%)**	**aGM**	**(95% CI)**	***p*-value**
Total lipids (mg/dL)*	495	(96.5)			
<565.0	126	(25.5)	7.4	(6.8-8.0)	
565.0-635.7	123	(24.8)	8.3	(7.7-9.0)	0.026
635.8-733.0	126	(25.5)	8.7	(8.1-9.3)	0.003
≥733.0	120	(24.2)	8.6	(7.9-9.2)	0.009
Total lipids (mg/dL) **of cases in fasting status <3 hours**	246	(49.7)			
<565.0	70	(28.5)	6.7	(6.1-7.3)	
565.0-635.7	58	(23.6)	8.4	(7.6-9.3)	0.001
635.8-733.0	58	(23.6)	7.8	(7.1-8.7)	0.026
≥733.0	60	(24.4)	8.5	(7.6-9.4)	0.001
Total lipids (mg/dL) **of cases in fasting status between 3 and 6 hours**	99	(20.0)			
<565.0	24	(24.2)	7.1	(6.1-8.3)	
565.0-635.7	26	(26.3)	8.2	(7.1-9.6)	0.191
635.8-733.0	29	(29.3)	7.8	(6.8-9.0)	0.386
≥733.0	20	(20.2)	7.9	(6.7-9.4)	0.379
Total lipids (mg/dL) **of cases in fasting status ≥6 hours**	150	(30.3)			
<565.0	32	(21.3)	9.2	(7.6-11.1)	
565.0-635.7	39	(26.0)	8.3	(7.1-9.8)	0.434
635.8-733.0	39	(26.0)	10.4	(8.8-12.2)	0.364
≥733.0	40	(26.7)	9.7	(8.3-11.4)	0.685

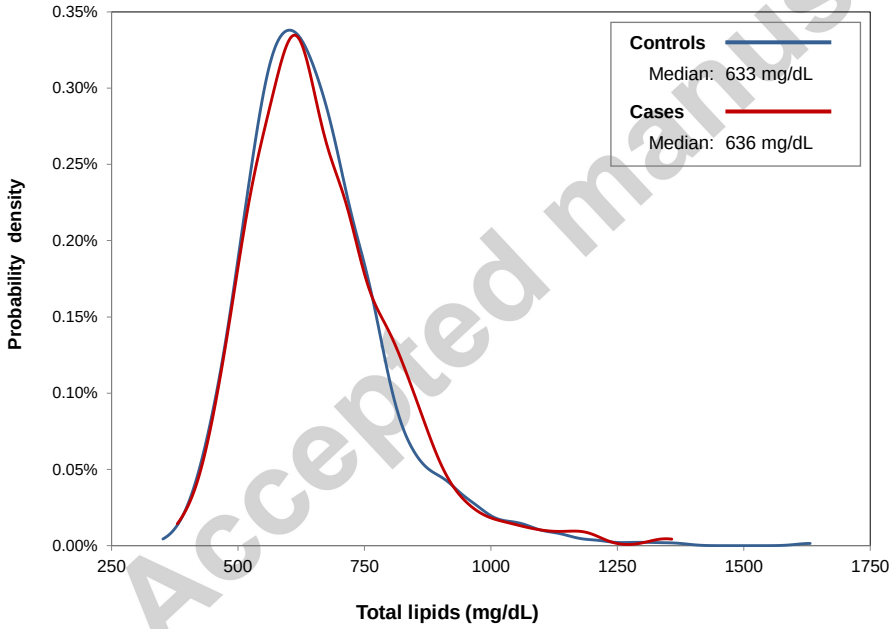
aGM: Geometric mean of the time from blood collection to the diagnosis of pancreatic cancer adjusted for age, sex, body mass index, and centre.
* further adjusted for fasting status.
p-value: p-value of each category of the variable when comparing with the reference group.

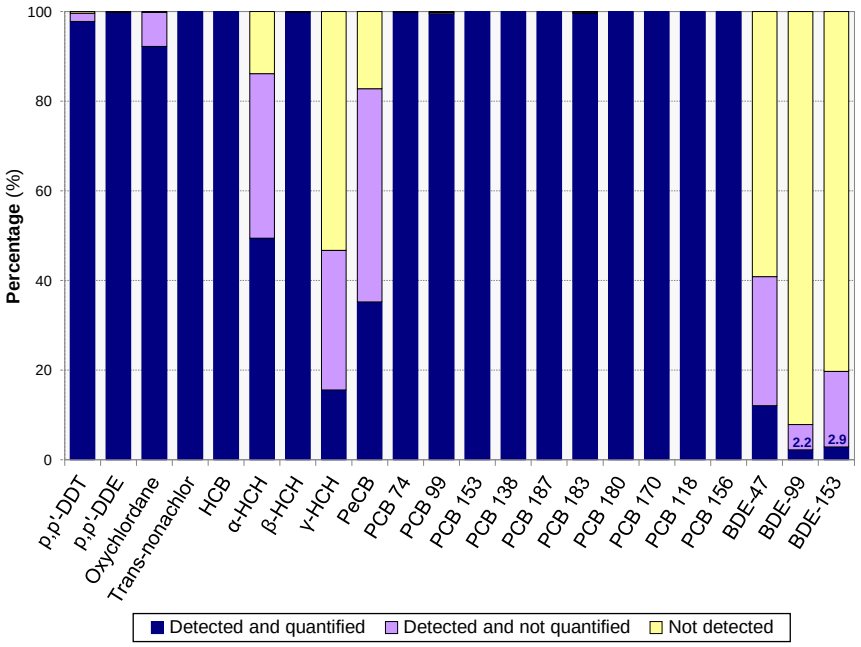
Table 7. Influence of tumour characteristics on total cholesterol, triglycerides and total lipids concentrations (mg/dL).
Multivariate General Linear Models.

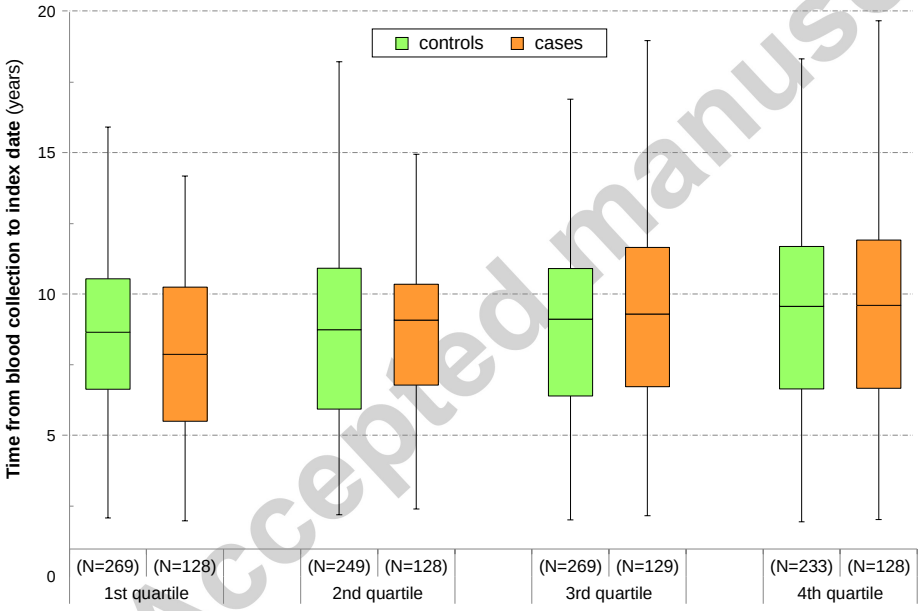
	Total		Total cholesterol			Triglycerides			Total lipids		
Tumour characteristic	N	(%)	aGM	(95% CI)	*p*-value	aGM	(95% CI)	*p*-value	aGM	(95% CI)	*p*-value
Stage of the tumour (N = 168)											
Localised	50	(29.8)	186	(175-199)		114	(94-139)		613	(568-662)	
Metastatic	118	(70.2)	200	(193-207)	0.090	119	(107-132)	0.767	650	(623-678)	0.256
Grade of the tumour (N = 84)											
Well differentiated	11	(13.1)	174	(156-194)		72	(52-98)		540	(477-610)	
Moderately differentiated	44	(52.4)	182	(172-192)	0.483	117	(100-136)	0.007	604	(568-641)	0.112
Poorly differentiated	29	(34.5)	190	(178-203)	0.182	130	(108-157)	0.002	636	(591-685)	0.025
p-trend				0.151			0.006			0.030	
Tumour subsite (N = 339)											
Head of pancreas	223	(65.8)	200	(195-204)		125	(118-134)		655	(638-672)	
Body of pancreas	58	(17.1)	195	(186-204)	0.367	118	(104-134)	0.431	636	(604-668)	0.307
Tail of pancreas	35	(10.3)	199	(188-211)	0.953	130	(110-153)	0.699	660	(619-705)	0.803
Overlapping	23	(6.8)	190	(177-204)	0.212	96	(78-118)	0.014	602	(555-652)	0.052

aGM: Geometric mean, in each of the 9 models adjusted for age, sex, body mass index, fasting status, and repository.
p-value: p-value of each category of the variable when comparing with the reference group.
p-trend: p-value derived from multivariate analogue of Mantel's extension test for linear trend.









Highlights

- There are unique methodological issues for research on environmental causes of human diseases.
- Through innovative methods, the study addressed some of such issues.
- Analyses assessing disease progression bias suggested it was unlikely in this study.
- Exposure to POPs was widespread in the study population, with substantial variability.
- Lipids were influenced by differences in study design across participating centres.
- Differences across study centres in some design features and participant characteristics are relevant for other multicentre studies.