Inflammation marker and risk of pancreatic cancer: a nested case–control study within the EPIC cohort

**BACKGROUND:** Established risk factors for pancreatic cancer include smoking, long-standing diabetes, high body fatness, and chronic pancreatitis, all of which can be characterised by aspects of inflammatory processes. However, prospective studies investigating the relation between inflammatory markers and pancreatic cancer risk are scarce.

**METHODS:** We conducted a nested case–control study within the European Prospective Investigation into Cancer and Nutrition, measuring prediagnostic blood levels of C-reactive protein (CRP), interleukin-6 (IL-6), and soluble receptors of tumour necrosis factor-α (sTNF-R1, R2) in 455 pancreatic cancer cases and 455 matched controls. Odds ratios (ORs) were estimated using conditional logistic regression models.

**RESULTS:** None of the inflammatory markers were significantly associated with risk of pancreatic cancer overall, although a borderline significant association was observed for higher circulating sTNF-R2 (crude OR = 1.52 (95% confidence interval (CI) 0.97–2.39), highest vs lowest quartile). In women, however, higher sTNF-R1 levels were significantly associated with risk of pancreatic cancer (crude OR = 1.97 (95% CI 1.02–3.79)). For sTNF-R2, risk associations seemed to be stronger for diabetic individuals and those with a higher BMI.

**CONCLUSION:** Prospectively, CRP and IL-6 do not seem to have a role in our study with respect to risk of pancreatic cancer, whereas sTNF-R1 seemed to be a risk factor in women and sTNF-R2 might be a mediator in the risk relationship between overweight and diabetes with pancreatic cancer. Further large prospective studies are needed to clarify the role of proinflammatory proteins and cytokines in the pathogenesis of exocrine pancreatic cancer.

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Evidence is accumulating that systemic low-grade chronic inflammation in addition to local inflammation in the pancreas is involved in the pathogenesis of pancreatic cancer (Farrow and Evers, 2002; Whitcomb, 2004; McKay et al., 2008). Research findings pointing to this direction include the documented relationship of pancreatic cancer risk with chronic pancreatitis (Raimondi et al., 2010), as well as with smoking (Lynch et al., 2009; Vriezing et al., 2010), pre-existing and long-standing diabetes mellitus (Huxley et al., 2005), and excess weight (Genkinger et al., 2010), all of which are known or suggestive determinants of low-grade inflammatory states (Whitcomb, 2004; Kolb and Mandrup-Poulsen, 2005; Hotamisligil, 2006; Goncalves et al., 2011).

Even though the mechanisms by which chronic inflammation leads to carcinogenesis are not fully understood, it is generally accepted that inflammation results in repeated DNA damage and in the accumulation of genetic defects (McKay et al., 2008). However, proinflammatory cytokines and growth factors are also released in response to the tumour, making it difficult to distinguish between cause and effect in the inflammatory processes (McKay et al., 2008).

Circulating C-reactive protein (CRP) concentration, an acute-phase protein produced in the liver, is increased in pancreatic cancer patients (Barber et al., 1999; Moses et al., 2009; Mroczko et al., 2010), most likely as part of the systemic inflammatory response to the tumour. Interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) are upregulating factors of CRP and have also been shown to be increased in pancreatic cancer patients (Barber et al., 1999; Ebrahimi et al., 2004; Moses et al., 2009; Talar-Wojnarowska et al., 2009; Mroczko et al., 2010). Prospectively, increased levels of CRP have inconsistently been associated with pancreatic cancer risk. To our knowledge, prospective studies on the association of IL-6, TNF-α, or its receptors with risk of pancreatic cancer are lacking.

We measured prediagnostic concentrations of CRP, IL-6, and soluble TNF receptors (sTNF-R1 and R2) in blood samples of 455 primary exocrine pancreatic cancer cases and 455 individually matched controls within the Prospective Investigation into Cancer and Nutrition (EPIC) as possible reflections of either pancreatic cancer or a metabolic risk factor potentially increasing pancreatic cancer risk by aggravating pancreatic inflammatory disease.

**Materials and Methods**

**Study population**

The European Prospective Investigation into Cancer and Nutrition (EPIC) is a large cohort study conducted in 23 centres in ten European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Spain, Sweden, and the United Kingdom). Detailed descriptions of study design, population, and baseline data collection of the cohort can be found elsewhere (Haftenberger et al., 2002; Riboli et al., 2002). Briefly, about 370,000 women and 150,000 men were enrolled between 1992 and 2000. Participants provided information on dietary habits and lifestyle factors, and in addition, weight, height, and waist and hip circumferences were measured at baseline. Each participant provided informed consent, and the local ethical review committees approved the EPIC cohort study as well as the current project.

**Blood sample collection and storage**

In the seven EPIC core countries (France, Germany, Greece, Italy, the Netherlands, Spain, and the United Kingdom), blood samples were collected at baseline, based on a standardized protocol and aliquoted in plastic straws (plasma, serum, erythrocytes, and buffy coat for DNA). The aliquoted specimens were then stored in a central biorepository in liquid nitrogen (−196°C). In Sweden, all samples were stored locally in freezers at −70°C and in Denmark in nitrogen vapour (−150°C). In this study, Norway was excluded because blood samples were only recently collected and very few pancreatic cancer cases have been diagnosed after blood donation.

**Follow-up for cancer incidence and vital status**

In six of the participating countries (Denmark, Italy, the Netherlands, Spain, Sweden, and the United Kingdom), follow-up of cancer cases was based on population registries. In the other three countries (France, Germany, and Greece), a combination of methods was used including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin. In all EPIC centres, data on vital status are collected from mortality registries at the regional or national level, which is combined with health insurance data (France) or data collected by active follow-up (Greece). Cases reported in this study were all diagnosed up to the latest dates of complete follow-up, which was between December 2002 and 2005, depending on the study centre. For Germany, Greece, and France, the end of follow-up was the last known contact, date of diagnosis, or date of death, whichever came first.

**Selection of cases and control subjects**

Up to December 2006, follow-up has led to the identification of 578 incident cases of non-endocrine pancreas cancer that were coded according to ICD-10 (C25.0–25.3, 25.7–25.9), and for 455 of these cases blood specimens were available. Exclusion criteria were occurrence of other malignant tumours preceding the diagnosis of pancreatic cancer, except for non-melanoma skin cancer. Of the 455 cases, 334 (76%) were microscopically confirmed and the remaining 24% were diagnosed by imaging results, physical examination, or clinical symptoms. Most tumours occurred in...
the head of the pancreas (42%), followed by body (7%) and tail (5%), while the rest of the tumours were of unknown localisation. For each case, one control subject was selected, that was alive and free of cancer at the time the index case was diagnosed, using an incidence density sampling procedure. All identified cases were matched with one control by centre, sex, age at blood collection (±3 years), date of blood donation (±3 months), time of blood donation (±2 h), fasting status (<3 h, 3–6 h, >6 h after last meal), and use of hormones (oral contraceptive pill, hormone, or oestrogen replacement therapy).

Laboratory assays
Plasma (in Scandinavian samples) and serum concentrations of CRP were measured by multiplex immunoassays using the Fluorokine MAP Obesity Base Kit (R&D Systems Inc., Minneapolis, MN, USA). Interleukin-6 and sTNF receptors were measured by enzyme linked immune sorbent assays using the Quantikine kit (R&D Systems Inc.). The total amount of free receptor plus the total amount of receptor bound to TNF is measured using this method. All measurements were performed in our specialised immunoassay laboratory of the Division of Cancer Epidemiology (German Cancer Research Center, Heidelberg, Germany). Samples of cases and matched controls were analysed within the same analytical batch. Intra-batch and inter-batch coefficients of variation were 6.6 and 10.8% for IL-6, 3.6 and 4.1% for sTNF-R1, 5.5 and 11.0% for sTNF-R2, and 10.3 and 11.6% for CRP. Units of IL-6 are expressed as pg per ml, of sTNF receptors as ng per ml, and of CRP as mg per litre. One batch during the sTNF-R2 measurements did not perform well and, therefore, 70 subjects were excluded due to technically invalid results (all from Malmö, Sweden).

Statistical analysis
Case and control differences across baseline characteristics were assessed by paired t-tests (continuous variables) or by generalised McNemar’s Test (categorical variables). Spearman’s partial rank correlation coefficients (r) adjusted for age, sex, and EPIC recruitment centre were used to assess the strength of associations between waist circumference, waist–hip ratio, BMI, glycated haemoglobin (HbA1c), and inflammatory markers, as well as for the correlation between the inflammatory markers.

Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for pancreatic cancer at different serum levels of IL-6, sTNF receptors, and CRP were calculated by conditional logistic regression models, using the exposure assessments of the matched case–control sets. Continuous measurements of the inflammatory markers were log2 transformed to achieve approximate normality. In this scale, a unit increase corresponds to a doubling of the expected value. Odds ratios were log2 transformed to achieve normality. In this scale, a unit increase corresponds to a doubling of the expected value. Odds ratios were log2 transformed to achieve normality. In this scale, a unit increase corresponds to a doubling of the expected value.

RESULTS
Baseline characteristics of pancreatic cancer cases and matched control subjects are shown in Table 1. Mean age at recruitment into the initial cohort was 58 years and mean age of cases at pancreatic cancer diagnosis was 63 years, resulting in mean follow-up time of 5.3 years for cases (range 0–13). Female pancreatic cancer cases had a significantly higher BMI and waist circumference than corresponding controls, but no difference in waist–hip ratio was observed. For men, however, no significant difference for any of the anthropometric measures comparing cases and controls was seen. A higher percentage of cases currently smoked compared with controls (31% vs 22%). At baseline, cases also reported more often to be diabetic and/or had HbA1c levels >6.5% compared with controls (14% vs 8%). However, these results are not mutually adjusted and serve descriptive purposes only.

Among controls, sTNF-R1 and sTNF-R2 showed a high degree of correlation. The correlation of circulating CRP levels with IL-6, sTNF-R1, and sTNF-R2 concentrations was relatively high with Spearman’s rank correlation coefficients up to 0.44. Waist circumference, BMI, and waist–hip ratio correlated moderately with CRP and IL-6, and to lesser extent with sTNF-R1 but not with sTNF-R2 concentrations (Table 2). Participants with diabetes (self-reported at baseline and/or HbA1c >6.5%) and those who...
smoked had higher levels of CRP and IL-6 than non-diabetics (Table 2). Mutual adjustments for smoking categories and/or BMI resulted in unaltered (diabetes) or stronger associations (smoking, data not shown).

The potential confounders or effect modifiers overweight (OR = 1.05 (95% CI 1.01–1.08), per 5 BMI units), smoking (OR = 1.84 (95% CI 1.30–2.60), current vs never), and diabetes (OR = 1.74 (95% CI 1.12–2.71)) were associated with risk of pancreatic cancer in our study.

Pancreatic cancer risk tended to be increased with higher levels of sTNF-R2 (crude OR = 1.52 (95% CI 0.97–2.39) comparing highest with lowest quartiles, P-trend over quartiles = 0.07), but these associations were not significant at the 5% level, and BMI and smoking adjustments attenuated the risks of pancreatic cancer (Table 3). Elevated CRP (crude OR = 1.36 (95% CI 0.92–2.01), P-trend = 0.26), IL-6 (OR = 1.30 (95% CI 0.84–2.00), P-trend = 0.61), and sTNF-R1 levels (OR = 1.23 (95% CI 0.78–1.94), P-trend = 0.23) showed no significant association with risk of pancreatic cancer. Adjustments for HbA1c levels and mutually for the other inflammatory markers in addition to BMI and smoking categories attenuated risk estimates for elevated levels of inflammatory markers closer to 1.0 (data not shown). Exclusion of subjects with CRP levels above 10 mg l⁻¹ (as this is more likely an indication for an acute rather than a chronic inflammatory state) had no effect on the association between CRP levels and pancreatic cancer risk (data not shown). Women tended to be at increased pancreatic cancer risks for higher CRP or sTNF receptor levels, and specifically so for sTNF-R1, although risk

| Variable | Cases (n = 455) | Controls (n = 455) | P-value* |
|----------|----------------|------------------|----------|
| Women, n (%) | 235 (52) | 235 (52) | matched |
| Age at recruitment (year), mean (range) | 58 (30–76) | 58 (30–76) | matched |
| Age at diagnosis (year), mean (range) | 63 (37–82) | | |
| Follow-up (year), mean (range) | 5.3 (0–13) | | |
| BMI (kg m⁻²), mean ± s.d. | | |
| Male | 26.8 ± 3.6 | 26.7 ± 3.7 | 0.7 |
| Female | 26.5 ± 5.0 | 25.2 ± 4.3 | 0.002 |
| Waist-hip ratio, mean ± s.d. | | |
| Male | 0.95 ± 0.06 | 0.95 ± 0.06 | 0.6 |
| Female | 0.82 ± 0.07 | 0.81 ± 0.06 | 0.09 |
| Waist circumference (cm), mean ± s.d. | | |
| Male | 96.3 ± 99 | 96.7 ± 102 | 0.7 |
| Female | 84.4 ± 12.5 | 81.2 ± 10.7 | 0.001 |
| Smoking status, n (%) | | |
| Never | 162 (36) | 198 (44) | < 0.001 |
| Former | 145 (32) | 151 (33) | |
| Current | 143 (31) | 101 (22) | |
| Unknown | 5 (1) | 5 (1) | |
| Alcohol intake at recruitment (g per day), mean ± s.d. | | |
| Male | 21 ± 26 | 23 ± 31 | 0.9 |
| Female | 9 ± 13 | 8 ± 11 | |
| Fasting status, n (%) | | |
| Fasting (≥6 h) | 118 (26) | 113 (25) | |
| In between (3–6 h) | 78 (17) | 78 (17) | |
| Non-fasting (<3 h) | 177 (39) | 183 (40) | |
| Unknown | 82 (18) | 81 (18) | |
| Diabetes status, n (%) | | |
| Self-reported diabetes at recruitment | 33 (8) | 19 (4) | 0.05 |
| Subjects HbA1c ≥6.5% | 54 (12) | 29 (6) | 0.006 |
| Self-reported diabetes or HbA1c ≥6.5% | 59 (14) | 34 (8) | 0.01 |
| Unknown | 18 (4) | 17 (4) | |
| CRP (mg l⁻¹), geometric mean (95% CI) | | |
| Men | 1.12 (0.97–1.29) | 1.08 (0.94–1.25) | 0.8 |
| Women | 1.24 (1.08–1.42) | 0.97 (0.84–1.12) | 0.02 |
| IL-6 (pg ml⁻¹), geometric mean (95% CI) | | |
| Men | 1.79 (1.63–1.96) | 1.69 (1.52–1.89) | 0.6 |
| Women | 1.58 (1.43–1.74) | 1.44 (1.31–1.59) | 0.3 |
| sTNF-R1 (ng ml⁻¹), geometric mean (95% CI) | | |
| Men | 1.31 (1.28–1.37) | 1.36 (1.32–1.41) | 0.3 |
| Women | 1.39 (1.34–1.44) | 1.32 (1.28–1.36) | 0.003 |
| sTNF-R2 (ng ml⁻¹), geometric mean (95% CI) | | |
| Men | 2.31 (2.23–2.40) | 2.28 (2.20–2.37) | 0.5 |
| Women | 2.43 (2.35–2.51) | 2.33 (2.26–2.40) | 0.04 |

Abbreviations: CI = confidence interval; s.d. = standard deviation. Note: matching factors were EPIC recruitment centre, sex, age at blood collection, date of blood donation, time of blood donation, fasting status, and use of hormones (in women). *P-values for continuous variables were based on paired t-tests; P-values for categorical variables were based on generalised McNemar’s tests.
estimates were inconsistently significant between categorical and continuous analyses and between crude and BMI and smoking-adjusted models (Table 3).

Tests for heterogeneity of continuous sTNF receptors, adjusted for matching factors, resulted in statistically significant differences in pancreatic cancer risk by median BMI, diabetes, and smoking status, but not by median waist circumference, waist–hip ratio or median age. Compared with never smokers, risks in former and current smokers were elevated, albeit not statistically significant. Diabetics (P-interaction = 0.001) and subjects with a BMI above the median (P-interaction = 0.04) had a significantly higher risk of pancreatic cancer with elevated levels of sTNF-R2 than non-diabetic participants.

### Table 2

| Covariate | CRP | IL-6 | sTNF-R1 | sTNF-R2 |
|-----------|-----|------|---------|---------|
| IL-6      | 0.44 (0.35–0.53) | 0.29 (0.18–0.39) | 0.27 (0.16–0.37) | 0.40 (0.30–0.49) |
| sTNF-R1   | 0.29 (0.18–0.39) | 0.33 (0.22–0.42) | 0.23 (0.12–0.33) | 0.29 (0.18–0.39) |
| sTNF-R2   | 0.27 (0.16–0.37) | 0.33 (0.22–0.42) | 0.23 (0.12–0.33) | 0.29 (0.18–0.39) |
| BMI       | 0.40 (0.30–0.49) | 0.29 (0.18–0.39) | 0.17 (0.06–0.28) | 0.17 (0.06–0.28) |
| Waist     | 0.32 (0.22–0.42) | 0.31 (0.20–0.41) | 0.21 (0.10–0.31) | 0.21 (0.10–0.31) |
| WHR       | 0.23 (0.13–0.34) | 0.25 (0.14–0.35) | 0.16 (0.04–0.26) | 0.16 (0.04–0.26) |
| Hba1c     | 0.16 (0.05–0.27) | 0.09 (0.02 to 0.20) | 0.10 (0.01 to 0.21) | 0.10 (0.01 to 0.21) |
| Diabetes   | 1.37 (1.08–1.74) | 1.49 (1.06–2.11) | 1.41 (1.04–1.85) | 1.30 (0.42–4.02) |
| Smoking    | 1.30 (1.10–1.54) | 1.36 (1.06–1.74) | 1.60 (0.72–3.58) | 0.97 (0.43–2.16) |
| Sex        | 1.07 (0.95–1.21) | 1.21 (1.02–1.44) | 1.79 (0.97–3.11) | 0.99 (0.50–1.71) |
| Age        | 0.13 (0.03–0.23) | 0.18 (0.08–0.28) | 0.30 (0.20–0.39) | 0.32 (0.22–0.41) |

Abbreviations: CRP = C-reactive protein; IL-6 = interleukin-6; sTNF-R1 and sTNF-R2 = soluble tumour necrosis factor receptors. 1 and 2 = body mass index; waist = waist circumference; WHR = waist–hip ratio; Hba1c = glycated haemoglobin; age = age at recruitment. *For continuous covariates, Spearman's partial rank correlation coefficients were applied. For categorical covariates we used logistic regression. Both methods were performed in controls and adjusted for age, sex, and EPIC recruitment centre if not stated otherwise. **Diabetic (Hba1c > 6.5% or self-reported diabetes at baseline) vs non-diabetic participants. ¹Current vs never smokers. ²Men vs women, adjusted for age and EPIC recruitment centre. ³Adjusted for sex and EPIC recruitment centre.

### Table 3

| Quartiles | CRP | IL-6 | sTNF-R1 | sTNF-R2 |
|-----------|-----|------|---------|---------|
| 1         | 0.02–0.51 | 0.52–1.04 | 1.05–2.05 | 2.06–3.407 |
| 2         | 112/112 | 112/112 | 112/112 | 112/112 |
| 3         | 1.30 (0.88–1.94) | 1.45 (1.00–2.10) | 1.36 (0.92–2.01) | 1.08 (0.99–1.18) |
| 4         | 1.25 (0.83–1.88) | 1.20 (0.80–1.79) | 1.02 (0.66–1.57) | 1.01 (0.92–1.11) |
| No. of cases/controls (total 449/449) | 86/106 | 123/106 | 108/107 | 107/105 |
| 1         | 1.38 (0.76–2.52) | 0.98 (0.56–1.70) | 1.23 (0.68–2.41) | 0.91 (0.80–1.15) |
| 2         | 1.39 (0.75–2.58) | 0.95 (0.52–1.66) | 1.09 (0.58–2.04) | 1.00 (0.88–1.13) |
| 3         | 1.15 (0.67–1.97) | 2.14 (1.27–3.59) | 1.44 (0.85–2.47) | 0.11 (1.02–1.31) |
| 4         | 1.19 (0.67–2.11) | 1.65 (0.92–2.98) | 0.99 (0.54–1.81) | 0.10 (0.82–1.18) |
| 1         | 0.16–0.94 | 0.95–1.57 | 1.58–2.65 | 2.66–9.66 |
| 2         | 86/106 | 123/106 | 108/107 | 107/105 |
| 3         | 1.45 (0.79–1.96) | 1.29 (0.85–1.93) | 1.30 (0.84–2.00) | 1.09 (0.95–2.16) |
| 4         | 1.29 (0.86–1.94) | 0.97 (0.62–1.51) | 1.01 (0.64–1.61) | 0.79 (0.85–1.16) |
| Men, crude | 2.02 (1.11–3.48) | 1.73 (0.92–3.26) | 1.36 (0.70–2.64) | 1.13 (0.86–1.29) |
| Adjusted for smoking, BMI | 1.88 (1.00–3.51) | 1.51 (0.75–3.04) | 1.21 (0.60–2.45) | 1.00 (0.80–1.05) |
| Women, crude | 1.10 (0.65–1.87) | 1.01 (0.58–1.75) | 1.29 (0.72–2.33) | 0.12 (0.92–1.36) |

Abbreviations: CI = confidence interval; No. = number; CRP, IL-6, and sTNF receptor concentrations on continuous scales were log2 transformed. Smaller number of subjects due to missing laboratory values. ¹Crude P-interaction over quartiles, for CRP = 0.03, IL-6 = 0.2, sTNF-R1 = 0.09, sTNF-R2 = 0.8. Body mass index and smoking-adjusted P-interaction, for CRP = 0.03, IL-6 = 0.2, sTNF-R1 = 0.1, sTNF-R2 = 0.9. ¹¹Quartile cut-points were based on the distribution of controls. ²P-trend test was based on median values of each quartile. ³Logistic regression conditioned on matching factors (EPIC recruitment centre, sex, age at recruitment, date at entry in the cohort, time between blood sampling and last consumption of foods and drinks, hormone use). Adjusting variables in further model: smoking (former smokers adjusted for quitting smoking (Mean = 0.04–0.16)).
non-diabetics or subjects with lower than median BMI, respectively (Figure 1B). Adjusting subgroup analyses for BMI, smoking categories, HbA1c levels, and/or mutually for inflammatory markers attenuated the risk estimates to non-significance (data not shown). Interestingly, higher circulating CRP and IL-6 levels tended to be related to increased pancreatic cancer risk in thinner subjects, although ORs and tests for interaction were not statistically significant (Figure 1C and D).

**DISCUSSION**

In our nested case–control study of 455 pancreatic cancer subjects and 455 individually matched controls, higher circulating levels of sTNF-R2, but not of sTNF-R1, CRP, and IL-6 levels, tended to be positively associated with the risk of pancreatic cancer. Stratification by sex revealed significantly increased pancreatic cancer risks in women for higher sTNF-R1 levels. Positive associations between sTNF-R2 and pancreatic cancer seemed to be likely for diabetic subjects, those with a higher BMI, and possibly also for smokers.

In the acute-phase response to tissues damage, infection, inflammation, or malignant neoplasia, CRP is increasingly produced by hepatocytes, predominantly under control by IL-6. C-reactive protein binds to damaged cell membranes or apoptotic cells, forming an aggregate that activates the complement pathway, resulting in the phagocytosis of the damaged cells and in increased proinflammatory pathophysiological effects. C-reactive protein, therefore, reflects ongoing inflammation and/or tissue damage and functions as a proinflammatory mediator. In this context, it may not only be a marker of a disease, but it may also contribute to pathogenesis (Pepys and Hirschfield, 2003). In several small hospital-based case–control studies, CRP levels were significantly higher in pancreatic cancer cases compared with chronic pancreatitis patients or controls (Barber et al, 1999; Moses et al, 2009; Mrozcko et al, 2010). In addition, elevated levels of CRP were associated with a poor prognosis in pancreatic cancer patients (McKay et al, 2008). Prospectively, no association was observed in a Greek study with 14 pancreatic cancer cases (Trichopoulos et al, 2006), whereas a weak decrease in pancreatic cancer risk with an OR of 0.94 (95% CI 0.89–0.99) was seen among 311 cases in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) cohort of male Finish smokers (Douglas et al, 2010). The same authors did not find an association in the Ovarian Cancer Screening Trial (PLCO) or in combined analyses of
both cohorts. Our results are in line with the prospective Greek and PLCO study showing no association of CRP with risk of pancreatic cancer.

No prospective study has been conducted so far to assess the association of circulating TNF-α, its soluble receptors, or IL-6 levels with risk of pancreatic cancer, both upregulators of CRP. Tumour necrosis factor-α is a proinflammatory cytokine produced by many cell types, including cancer cells, upon exogenous noxious stimuli. The effects of TNF-α are mediated mainly by two receptors, TNF-R1 and TNF-R2, which also circulate in soluble forms upon shedding. Tumour necrosis factor receptor activation leads to induction of genes involved in inflammation and cell survival, resulting in the activation of nuclear factor-κB (NF-κB). However, if NF-κB activation is inadequate, apoptosis is mediated via accumulation of reactive oxygen species as a late response to TNF-α. This cytokine, thus, is not only involved in maintenance of the immune system, but also in pathological processes such as malignant diseases. The majority of cell types and tissues express both receptor types (Balkwill, 2006), and among colon cancer patients it has been shown that the concentrations of sTNF-Rs correlate with the stage of disease as tumour cells have a greater tendency than non-malignant cells to shed forms of their cell surface proteins (Aderka, 1996). Soluble TNF receptors can serve as TNF antagonists, carrier proteins of TNF, slow release reservoirs for TNF, and stabilisers of TNF bioactivity. It is not known, however, whether the two soluble receptors have distinct or similar functions (Aderka, 1996), and based on this, we cannot explain why we observed a potential increase in pancreatic cancer risk for elevated sTNF-R2 but not for sTNF-R1. It might be, however, that sTNF-R2 has a more prominent role in pancreatic cancer development. This aspect needs to be explored in functional studies. So far, TNF-α and/or the soluble receptors have been assessed in hospital-based case–control studies with pancreatic cancer patients, observing either higher levels of TNF-α/soluble TNF receptors among pancreatic cancer subjects than among controls (healthy volunteers or chronic pancreatitis patients (Barber et al, 1999; Talar-Wojnarowska et al, 2009)), or no difference in serum levels (Ebrahimi et al, 2004). To our knowledge, our nested case–control study within the prospective EPIC cohort study is the first to address the association of STNF receptors with risk of pancreatic cancer, and we observed a non-significant increase in pancreatic cancer risk for sTNF-R2 than sTNF-R1, and which was attenuated after adjustment for smoking status, BMI, and HbA1c levels or diabetes status. It is unclear why we found a difference in risk between men and women with elevated risks for increasing levels of sTNF-R1 in women only.

As with TNF-α, pancreatic cancer patients’ IL-6 concentrations have shown to be higher than in healthy controls in hospital-based case–control studies (Barber et al, 1999; Ebrahimi et al, 2004; Moses et al, 2009; Mroczko et al, 2010). In contrast to these observations, in our prospective study we did not find elevated pre-diagnostic IL-6 concentrations in subjects who became pancreatic cancer cases later in time compared to non-cancer controls (Table 1). IL-6 is synthesised by most cell types in response to stimulation from TNF-α and IL-1, and indirectly regulates cell proliferation and apoptosis through its activation of other factors. Therefore, IL-6 has a role in chronic inflammation, which may enhance cancer development (Hodge et al, 2005). However, due to the small number of prospective studies so far investigating the relationship of IL-6 with cancer, a recent published review concluded that it is yet impossible to determine whether IL-6 is causally related to cancer (Heikkilä et al, 2008). It has been shown in a wide range of studies that CRP, IL-6, TNF-α, and TNF receptor levels vary by body weight, with higher levels among overweight or obese compared with normal weight subjects, and with decreasing levels during weight loss (Himmerich et al, 2006; Forsythe et al, 2008). Furthermore, compared with never smokers, cigarette smokers also have significantly higher levels of CRP and IL-6, and possibly also of TNF receptors (Fernandez-Real et al, 2003). Finally, subclinical systemic inflammation has been reported in type 2 diabetes (Kolb and Mandrup-Poulsen, 2005), including elevated levels of the aforementioned and evaluated parameters in our study. In our study, elevated levels of CRP, IL-6 and sTNF-R1 correlated with excess weight and, in addition, higher CRP and IL-6 levels were associated with smoking and diabetes.

Furthermore, overweight, smoking, or diabetic participants at baseline were at increased pancreatic cancer risk. This risk was even stronger if overweight or diabetic participants had elevated levels of sTNF-R2, even though this marker was not correlated with BMI or associated with diabetes in controls. This can be interpreted as sTNF-R2 being a mediator of the relationship between overweight and/or diabetes and pancreatic cancer. A similar scenario is likely for sTNF-R1, but our results do not clearly support this hypothesis (Figure 1A). In contrast, stratification by median BMI, diabetes, or smoking status resulted in similar weak risk estimates for elevated CRP and IL-6 concentrations. It seems as if, regardless of the presence of a putative pancreatic cancer risk factor (overweight, diabetes, and smoking), these inflammatory markers are not associated with pancreatic cancer risk themselves. In addition, they also do not appear to be in the causal chain between risk factor and cancer.

Some strengths and limitations of our study should be mentioned. Although a single measurement of a biomarker, as assessed in our study, could result in random misclassification, CRP, IL-6, and sTNF receptors have been shown to be reliably measured over time (Gu et al, 2009; Clendenen et al, 2010). A major strength of our study is that questionnaire data and blood samples were collected prospectively around the same time point, prior to pancreatic cancer diagnosis, which reduces the possibility of reverse causation bias to some extent. In addition, pancreatic cancer risk seemed to be stronger for elevated sTNF receptor levels among subjects with longer follow-up times. A limitation of our study is that information on pancreatic or liver disorders, on inflammatory diseases, or on use of anti-inflammatory drugs was not recorded for most of the EPIC centres; therefore, controlling for these potential confounders was not possible. Consequently, we cannot exclude the possibility that the observed suggestive increased pancreatic cancer risk among individuals with elevated sTNF-R2 levels might partly be due to chronic pancreatitis or impaired liver function, for example. Furthermore, number of subjects in specific subgroups were rather small; thus, we cannot rule out that results obtained from these analyses are chance findings. Further large prospective studies are needed to verify our results in the respective subgroups with sufficient power to detect significant risk associations.

CONCLUSION

Prospectively, CRP and IL-6 do not seem to play a role in our study with respect to risk of pancreatic cancer, whereas sTNF-R1 seemed to be a risk factor in women and sTNF-R2 might be a mediator in the risk relationship between overweight and diabetes with pancreatic cancer. In order to clarify the role of proinflammatory proteins and cytokines in the pathogenesis of exocrine pancreatic cancer, more prospective studies in large settings are needed, controlling for the potential bias of other conditions and stratifying by sex.

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