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Association between levels of C-reactive protein and leukocytes and cancer: Three repeated measurements in the Swedish AMORIS study

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Abstract

Objective—To study levels of C-reactive protein (CRP) and leukocytes, as inflammatory markers, in the context of cancer risk.

Methods—From the Apolipoprotein MOrtality RISk (AMORIS) study, we selected 102,749 persons with one measurement and 9,273 persons with three repeated measurements of CRP and leukocytes. Multivariate Cox proportional hazards regression was applied to categories of CRP (<10, 10-15, 15-25, 25-50, >50 g/L) and quartiles of leukocytes. An Inflammation-based Predictive Score (IPS) indicated whether someone had CRP levels >10mg/L combined with leukocytes $>10\times10^9$/L. Reverse causality was assessed by excluding those with <3, 5, or 7 years of follow-up. To analyze repeated measurements of CRP and leukocytes the repeated IPS (IPS_r) was calculated by adding the IPS of each measurement.

Results—In the cohort with one measurement, there was a positive trend between CRP and cancer, with the lowest category being the reference: 0.99 (0.92-1.06), 1.28 (1.11-1.47), 1.27 (1.09-1.49), 1.22 (1.01-1.48) for the 2nd to 5th categories, respectively. This association disappeared when excluding those with follow-up <3, 5 or 7 years. The association between leukocytes and cancer was slightly stronger. In the cohort with repeated measurements the IPS_r

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was strongly associated with cancer risk: 1.87 (1.33-2.63), 1.51 (0.56-4.06), 4.46 (1.43-13.87) for IPS_r =1, 2, and 3, compared to IPS_r =0. The association remained after excluding those with follow-up <1 year.

Conclusions and impact—Our large prospective cohort study adds evidence for a link between inflammatory markers and cancer risk by using repeated measurements and ascertaining reverse causality.

Keywords

cancer; C-reactive protein; leukocytes; Sweden

Introduction

C-reactive protein (CRP), a marker of acute-phase inflammatory response, has been suggested to be useful for early detection of cancer. A recent meta-analysis using 14 prospective studies of circulating CRP and any incident cancer, comprising 3,957 cancer cases, showed that a log unit increase in CRP was associated with a 1.1-increase in overall cancer risk (1). Inflammation-associated oxidative damage could initiate carcinogenesis which causes inactivating mutations in tumour-suppressor genes or post-translational modifications in proteins involved in DNA repair or apoptotic control. Tumour progression can also be facilitated by inflammatory cytokines, enzymes, and transcription factors inhibiting apoptosis and promoting the growth and proliferation of cancer cells (1). However, it is also possible that the immune response of the host is a consequence of the tumour growth itself (2). Nevertheless, the evidence for whether there is an association between CRP and cancer risk remains inconclusive, mainly due to a lack of large-scale studies in which CRP is measured prospectively (3). The largest prospective study today is based on a total of 10,408 individuals from the Danish general population, of whom 1,624 developed cancer. In this study, an increased risk of overall cancer and of lung cancer was associated with elevated levels of CRP in cancer-free individuals (4).

Since CRP is an acute phase-protein, repeated measurements and other markers of inflammation could potentially be more informative in predicting cancer risk in the context of inflammation. To our knowledge no prospective study has yet conducted an analysis of more than two repeated measurements of CRP and only one study assessed CRP in parallel with leukocytes (5). Infiltration of leukocytes is part of the inflammatory process associated with cancer (6) as it has been shown that lymphocytes naturally acquire the ability to recognise cancer cells, however they cannot control cancer growth (7). Moreover, congenital and acquired immunodeficiencies have been associated with cancer development indicating that lymphocytes also have an active protective role in surveillance against cancer (8). Leukocytes appear at sites of infection, chronic irritation and inflammation at different times after tissue injury and they are involved in the control of infection as well as in tissue remodelling (9-10). In a prospective cohort study including 143,748 women aged 50-79 years, a statistically significant positive association was found between leukocytes and bladder, colorectal, endometrial, and lung cancer risk when comparing the fourth with the first quartile (11). Another prospective cohort study including 4,831 subjects aged 43 to 86 years found a 2.8-fold increased risk for lung cancer when comparing the upper tertile with the lowest tertile of leukocyte counts (12).

We examined possible associations between CRP, leukocytes and cancer risk in a prospective cohort study of 102,749 persons of whom 6,913 were diagnosed with cancer. In a subgroup of 9,273 persons we analyzed the association between CRP and leukocytes in three repeated measurements and cancer risk.

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Methods

Study population and data collection

The Central Automation Laboratory (CALAB) database (1985-1996), includes laboratory measurements obtained from 351,487 men and 338,101 women, mainly from the greater Stockholm area (Sweden). All individuals were either healthy individuals referred for clinical laboratory testing as part of a general health check-up or outpatients referred for laboratory testing. No individuals were inpatients at the time their blood samples were taken and none were excluded due to disease symptoms or because of treatment. Apart from the information on blood testing, no personal data were included in the CALAB database (13). This database was linked to several Swedish national registries such as the National Cancer Register, the Hospital Discharge Register, the Cause of Death Register, the consecutive Swedish Censuses during 1970-1990, and the National Register of Emigration by using the Swedish 10-digit personal identity number to provide information on socio-economic status (SES), vital status, cancer diagnosis, and emigration. This linkage of national registers to the CALAB database is called the AMORIS study and it has been described in detail elsewhere (13-19). This study complied with the Declaration of Helsinki, and the ethics review board of the Karolinska Institute approved the study.

For the analysis of one measurement of CRP and cancer risk we used all 102,749 persons aged 20 years or older whose levels of CRP and leukocytes were measured at baseline and did not die or were not diagnosed with cancer within three months after their measurement. Follow-up started at time of measurement. For the repeated measurement analysis, we used a sub-cohort of all 9,273 persons aged 20 years or older, whose levels of CRP and leukocytes were measured three times within a time frame of five years and with a minimum of nine months between each measurement. These restrictions were set to avoid confounding by indication (e.g. if an infection was found at the first measurement, people might have had repeated measurements taken within the next few months). Follow-up started at time of the third measurement. Nobody in either cohort was diagnosed with benign neoplasms or cancer before the last measurement. In each cohort, follow-up time ended at time of event (i.e. cancer diagnosis), death from any cause, emigration, or end of follow-up (31 December 2002), whichever occurred first.

The following information was obtained from the CALAB database: CRP (mg/L), leukocytes (10^9/L), age at measurement, and gender. All other information was retrieved from the national registries. SES was obtained from the Censuses and is based on occupational groups and allows classification of gainfully employed subjects into manual workers and non-manual employees, below designated as blue-collar and white-collar workers (20). The quantitative determination of CRP was done with an established turbidimetric assay (reagents from Orion Diagnostics, Finland) using fully automated multichannel analyzers (an AutoChemist-PRISMA, New Clinicon, Stockholm, Sweden, 1985-1992) and DAX 96, Technicon Instruments Corporation, Tarrytown, NY, USA, 1993-1996). High sensitive CRP was not available at any time of the period of blood sampling collection (1985-1996)(21). Leukocytes were counted with routinely used haematology analyzers (Coulter^R STKS Haematology System from Coulter Corporation, Hialeah, FL). Total imprecision calculated by the coefficient of variation was <2.7% at leukocytes level 10×10^9/L and 12% at CRP level 40mg/L. All methods were fully automated with automatic calibration and accredited laboratory facilities (14).

Data analysis for the cohort with one measurement of CRP and leukocytes

Multivariate Cox proportional hazards regression was used to investigate the log-transformation of leukocytes and quartiles of leukocytes (<5.27, 5.25-6.30, 6.30-7.60,

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>7.60) as well as five categories of CRP (<10, 10-15, 15-25, 25-50, >50 g/L) in relation to cancer risk. Due to the non-high sensitivity of CRP measurements this biomarker was not analysed as a continuous variable. All models took into account age, SES, gender, and history of circulatory disease (ICD10: I00-I99) prior to measurement. A test for trend was conducted by using assignment to categories as an ordinal scale. The analysis was also repeated for CRP and leukoctyes categorized according to their clinical cut-off of 10 mg/L and 10×10^9/L(22). Moreover, an Inflammation-based Predictive Score (IPS) was devised based on levels of CRP as well as leukocytes to take into account the variability of the acute phase-protein CRP. Study subjects were given a score of one when they had abnormal values of both CRP and leukocytes according to their clinical cut-offs (CRP>10mg/L and leukocytes $>10\times10^9$/L)(22-23) and a score of zero otherwise. A stratified analysis was conducted by gender and history of circulatory disease. The five most common cancers among Swedish men (prostate, lung, colon, bladder, and other skin cancers) and women (breast, colon, cervix, lung, and melanoma) were studied separately (24). To assess the effect of reverse causation, three sensitivity analyses were conducted in which all persons with follow-up time <3, 5, and 7 years were excluded (n=3,459, 6,173, and 20,398, respectively). As no information on smoking, a possible confounder for the association between inflammation and cancer, was available in the current study, another sensitivity analysis was conducted in which all smoking-related cancers (lung, bladder, head and neck – ICD7: 162, 163, 181, 140-149) were excluded (n=939).

Data analysis for the cohort with three measurements of CRP and leukocytes

To take into account the three repeated measurements and the variability of the acute phase-protein CRP, we developed a repeated score for CRP and leukocytes, according to their clinical cut-off, as well as IPS (CRP_r, $leukocytes_r$, and IPS_r, respectively). The three repeated scores ranged from 0 to 3 and were calculated by adding the score of each repeated measurement. The same multivariate Cox proportional hazards regression analysis as conducted for single measurements was used to investigate CRP_r, $leukocytes_r$, and IPS_r in relation to cancer risk. The adjustment for age was based on age at time of the third measurement. To assess the effect of reverse causation, a sensitivity analysis was conducted in which all persons with follow-up time <1 year were excluded (n=219). Due to the smaller sample size of this cohort a shorter exclusion time than for the cohort with one measurement was chosen. Moreover, at time of the third measurement everyone had been free of cancer for at least 18 months since the first measurement. A similar sensitivity analysis excluding smoking-related cancers was conducted to assess the possible effect of smoking.

All analyses were conducted with Statistical Analysis Systems (SAS) release 9.1.3 (SAS Institute, Cary, NC).

Results

Results for the cohort with one measurement of CRP and leukocytes

A total of 13,631 persons (14.22%) had high levels of CRP (>10mg/L) in the group free of cancer compared to 1,368 persons (19.79%) in the group who developed cancer during follow-up, whereas a total of 5,452 persons (5.69%) had high levels of leukocytes ($>10\times10^9$/L) in the group free of cancer compared to 519 persons (7.51%) in the group who developed cancer during follow-up. Participant characteristics are shown in Table 1.

Multivariate adjusted hazard ratios for incident cancer showed an increased incidence by CRP categories > 15mg/L, with the lowest category being the reference: 1.28 (1.11-1.47), 1.27 (1.09-1.49), and 1.22 (1.01-1.48), for the 3rd to 5th categories, respectively (P-value for trend: <0.001). Excluding those with follow-up time <3, 5 or 7 year resulted in null-findings.

Compared to the overall results, excluding smoking-related cancers resulted in slightly attenuated hazard ratios for the association between CRP and cancer risk. The association between leukocytes and cancer turned out to be slightly stronger and showed statistically significant findings for the log-transformation as well as the quartiles and the clinical cut-off of leukocytes (e.g. HR for log unit increase in leukocytes: 1.47 (95%CI: 1.34-1.61)). Sensitivity analyses did not alter the association between leukocytes and cancer, however the strength of the associations attenuated (e.g. HR for log unit increase in leukocytes when excluding smoking-related cancers: 1.29 (1.18-1.42)). The IPS score was statistically significantly associated with risk of cancer for the main analysis as well as the sensitivity analyses (Table 2).

A stratified analysis showed no clear differences in hazard ratios by gender or history of circulatory disease (results not shown). A cancer site-specific analysis for the five most common Swedish male and female cancers showed only statistically significant findings for CRP and incident male lung cancer: 1.20 (1.00-1.44), 2.02 (1.48-2.77), 2.09 (1.47-2.99), 1.58 (0.96-2.99), for the 2^{nd} to 5^{th} categories, respectively (P-value for trend: <0.001) (Table 3). The same observation was made when using the clinical cut-off of CRP (HR: 1.75 (95%CI: 1.43-2.14). Adjustment for respiratory disease (ICD10:J00-99), as a proxy for smoking, did not alter these findings (results not shown). Leukocytes and IPS were also positively associated with male lung cancer risk, moreover the association was also observed for female lung cancer (eg. HR IPS=1: 2.82 (95%CI: 1.39-5.71)) (Table 3). Finally, a difference in risk for colon cancer was observed between men and women. When further investigating this risk by gender in stratified analyses of inflammatory markers, we did not find any significant differences (results not shown).

Results for the cohort with three measurements of CRP and leukocytes

A total of 875 persons developed cancer during follow-up. A larger proportion of persons diagnosed with cancer had values of CRP and leukocytes above the clinical cut-off at all three measurements, than of those who did not develop cancer (e.g. at the third measurement, 8.00% of persons diagnosed with cancer had leukocytes > 10^9/L versus 5.11% of those without cancer). All participant characteristics are shown in Table 4. The multivariate adjusted hazard ratios for different values of CRP_r, $leukocytes_r$, and IPS_r showed a positive trend (eg. HR for IPS_r: 1.87 (1.33-2.63), 1.51 (0.56-4.06), 4.46 (1.43-13.87) for IPS_r=1, 2, and 3, compared to IPS_r=0). The sensitivity analyses in which those with short follow-up or with smoking-related cancer were excluded, slightly attenuated these findings (Table 5).

Discussion

In the present study we found evidence for an association between elevated levels of CRP and leukocytes, and risk of cancer overall. Specifically, a single measurement of CRP or leukocytes was associated with an increased risk for lung cancer. Combining CRP with leukocytes or using repeated measurements of CRP and leukocytes strengthened the association with overall cancer risk, even after excluding those with a smoking-related cancer or those with short follow-up.

Inflammation and cancer

The hypothesis of a causal link between chronic inflammation and cancer has been studied for several decades, but the precise underlying molecular and cellular mechanisms causing cancer and stimulating tumour growth remain unresolved (25) (9). Experimental studies have shown that tumour cells produce various cytokines and attract a diverse leukocyte population that is capable of producing different mediators of cell killing such as tumour-

necrosis factor (TNF)-α, interleukins, and interferons (9). This is, for instance, shown in mouse models where growing intestinal tumour burden coincided with significantly increased levels of inflammatory cytokines IL-9, IL-6, and IL-17 (25). IL-6 is a strong inducer of acute-phase response, which can result in elevation of acute-phase proteins such as CRP. It has been speculated that CRP may have significant pro-inflammatory effects because of its capacity to activate the complement in order to exacerbate tissue infection. However, an occasional high CRP value can also relate to minor and subclinical infections, inflammation or trauma while a moderately increased CRP value may reflect subclinical pathologies (10). The plasma half-life of CRP is about 19 hours and is constant under all conditions of health and disease, so that circulating CRP concentration directly reflects the intensity of the pathological process stimulating CRP production. When the stimulus for increased production ceases, the circulating CRP concentration also falls rapidly (10). Leukocytes, on the other hand, have often been studied as markers of systematic inflammation in the context of cancer survival (22).

Following an increasing number of experimental studies suggesting a link between inflammation and cancer, more observational studies have been conducted to look at a link between markers of inflammation, such as CRP and leukocytes, and risk of cancer. The most recent observational study on CRP and cancer risk focused on lung cancer in a nested case-control study of 592 lung cancer patients and 670 controls matched on age, sex, entry year, follow-up time, and smoking. Comparing the fourth quartile (≥5.6 mg/L) with the first quartiles (<1.0 mg/L) resulted in a significant positive association between elevated CRP levels and risk of lung cancer (26). This association between CRP and lung cancer was also observed in the largest published observational study on CRP and incident cancer. In this Danish prospective cohort of 10,408 individuals baseline CRP >3mg/L versus <1mg/L was associated with multivariate smoking adjusted hazard ratios of 1.3 for overall cancer and 2.2 for lung cancer(4). In another prospective cohort study of 4,831 participants, it was found that those with leukocyte counts in the upper tertile were 2.81 times more likely to develop lung cancer as those with counts in the lowest tertile (12). Despite these findings, a meta-analysis carried out by Heikillä and co-workers showed that several studies did not find any association between elevated CRP levels and incident cancer and suggested that reverse causation might bias the observed associations (1). Our study in AMORIS is probably the first that is large enough to exclude a sufficiently long period of early follow-up without losing statistical power.

One measurement of CRP and leukocytes

Our study results confirm that reverse causation can affect the association between CRP and incident cancer: excluding those with <3 years of follow-up resulted in null-findings. However, a weak association was still apparent when using the clinical cut-off of CRP suggesting that those with CRP>10mg/L are indeed at increased risk for developing cancer. As we used non-(hs)CRP, we could not specify strata <10mg/L. Despite the association between dichotomized CRP and cancer, male lung cancer was the only neoplasm for which we could observe a strong association with increasing levels of CRP. These findings are consistent with what has been shown previously in Dutch and Danish prospective cohort studies (2, 4). In contrast to these studies, we did not use (hs)CRP measurements. Smoking may drive the association with male lung cancer. However, adjustment for lung disease (ICD-10: J00-J99), as a proxy for smoking, did not alter the findings. Our sensitivity analysis in which we excluded smoking-related cancer attenuated the associations, but despite the strong link observed with lung cancer in Table 3, a weak association remained between inflammatory markers and overall cancer risk. This suggests an association between inflammation and cancer over and above the influence of smoking habits. Despite the positive findings in several other studies for elevated levels of CRP and risk of colon and

stomach cancer, our findings in the AMORIS study only found an association between log(leukocytes) and male and female colon cancer risk (2-3, 27). Combining men and women or combining stomach and colon cancer did not alter the findings.

Even though the association between CRP and incident cancer was rather weak, a combination with leukocytes resulted in a statistically significant positive finding that remained in the sensitivity analyses. By using leukocytes as another marker to indicate systemic inflammation, we tried to exclude elevated CRP levels due to acute infections. From our findings it can be seen that defining those with elevated CRP and elevated leukocytes as the risk group is more predictive for cancer risk than only CRP levels. Nevertheless, the small increase in hazard ratios suggests that levels of CRP and leukocytes are more interesting in the context of cancer etiology rather than for clinical use in cancer risk prediction.

Three measurements of CRP and leukocytes

The HR for IPS of 1.37, when using one measurement, became much stronger when using three repeated measurements of IPS (HR: 4.46). It can be observed from our findings that the association with cancer became stronger for both CRP_r and $leukocytes_r$, as well as IPS_r. By choosing a minimum interval time of nine months between measurements, we excluded those who had a strong indication of infection at the time of their first measurement and likely oversampled those who are more health-aware and go for annual check-ups. Since we do not know how the association between markers of inflammation and cancer risk differs between those who are healthy and those who are burdened with more comorbidities, we cannot know how the oversampling is affecting our results. From the sensitivity analyses one can see that part of the association between CRP, leukocytes, and cancer risk is driven by smoking-related cancers. Nevertheless, after excluding these smoking-related cancer the statistically significant trends remained for repeated CRP, leukocytes, and IPS.

Strengths and limitations

The major strength of this analysis lies in the large number of persons with prospective measurements of CRP and leukocytes in AMORIS, all measured at the same clinical laboratory. Use of national health registers provided complete follow-up for each person as well as detailed information on cancer diagnosis, time of death, and emigration. Furthermore, assessment of both exposures (CRP and leukocytes measurement) and outcome (cancer) were conducted in an accurate manner. In addition, we were able to take into account within-person variation because CRP was measured three times in a cohort of 9,273 persons. The AMORIS population was selected by analysing blood samples from health check-ups in non-hospitalized individuals. During the study period the all cause mortality was about 14% lower in the AMORIS population than in the general population of Stockholm county when taking age, gender, and calendar year into account (28). This healthy cohort effect does not affect the internal validity of our study and it is also likely to be minor since it has been shown that the AMORIS cohort is similar to the general working population of Stockholm county in terms of SES and ethnicity. A limitation of this study is that information on other commonly measured markers for inflammation such as hsCRP or IL-6 was not available, moreover CRP and leukocytes are non-specific markers of inflammation. In the AMORIS study it was not possible to study hsCRP because at the time of blood sampling and analysis (1985-1996) assay methods for plasma proteins had limited sensitivity so that CRP concentrations <10mg/L could not be measured precisely (i.e. non-hsCRP) and the cut-off of 10 mg/L was widely accepted as the upper limit of the health-associated reference range (29). To our knowledge no study has investigated the effect of using hsCRP instead of non-hsCRP in the context of inflammation and cancer risk, but it is likely that low grade inflammation is not captured by using this cut-off resulting in an

underestimation of the association between CRP and cancer. However, the cut-off value of 10 mg/L is thought to be satisfactory for the purpose of medical events such as ischemic necrosis (29) and has been used in several other studies looking into the association between CRP and cancer diagnosis and prognosis (30-31). Furthermore, we did not have information on other possible confounders such as smoking habits or obesity. By excluding smoking-related cancers, our sensitivity analysis addressed this limitation and showed that there was still an association between inflammation and cancer. Obesity is associated with a state of low-grade chronic inflammation, characterized by infiltrating macrophages within adipose tissue and elevated concentrations of pro-inflammatory molecules (32-33). To date it is unclear whether inflammation is an intermediate on the pathway between obesity and cancer or whether obesity is confounding the association between inflammation and cancer. As our study focused on the association between inflammation as a marker of any disease or abnormality, we believe that residual confounding due to lack of information on BMI is minor. Finally, no information was available on tumour stage and CRP genotypes (34).

Conclusions

By replicating our findings for one measurement of CRP and leukocytes in a cohort with three repeated measurements of CRP and leukocytes and by assessing reverse causality in a very large prospective cohort study, our findings provide additional evidence for a link between markers of inflammation and cancer risk. As this link is not yet well understood, the current observations call for experimental studies assessing the association between markers of inflammation and the processes they are reflecting in the context of cancer development.

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Table 1

Descriptive characteristics by cancer status for the cohort with one measurement of CRP and leukocytes.

	No cancer N=95,836 (93.27%)	Cancer N=6,913 (6.73%)
	n(%)	n(%)
Mean Age (years) (SD)	47.31 (16.31)	61.00 (13.01)
Gender		
Men	40347 (42.10)	3182 (46.03)
Women	55489 (57.90)	3731 (53.97)
SES		
White collar	31818 (33.20)	2530 (64.66)
Blue collar	40099 (41.48)	2512 (36.34)
Not gainfully employed/Missing	23919 (24.96)	1871 (27.06)
Circulatory disease prior to CRP measurement		
Yes	8327 (8.69)	1089 (15.75)
Mean follow-up time (years) (SD)	9.74 (2.96)	5.90 (3.69)
CRP (mg/l)		
Mean (SD)	6.21 (13.24)	7.19 (13.20)
<10	82205 (85.78)	5545 (80.21)
10-15	8900 (9.29)	908 (13.13)
15-25	2060 (2.15)	194 (2.81)
25-50	1587 (1.66)	159 (2.30)
>50	1084 (1.13)	107 (1.55)
Leukocytes (10^9/l)		
Mean (SD)	6.62 (2.03)	6.90 (2.64)
Q1: <5.27	24146 (25.20)	1531 (22.15)
Q2: 5.25-6.30	22970 (23.97)	1569 (22.70)
Q3: 6.30-7.60	23934 (24.97)	1712 (24.76)
Q4: ≥7.60	24786 (25.86)	2101 (30.39)
Inflammation-based Predictive Score (IPS)		
0	94669 (98.78)	6798 (98.34)
1	1167 (1.22)	115 (1.66)

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Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2011 September 01.

Table 2

Hazard Ratio and 95% confidence interval (CI) for categories of CRP, leukocytes, IPS and risk of cancer diagnosis. The models are adjusted for gender, age, SES, and history of circulatory disease.

	Hazard Ratio (95%CI)	Hazard Ratio (95%CI)[1]	Hazard Ratio (95%CI)[2]	Hazard Ratio (95%CI)[3]	Hazard Ratio (95%CI)[4]
CRP (mg/L)					
Categories of CRP					
<10	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
10-15	0.99 (0.92-1.06)	0.94 (0.87-1.03)	0.95 (0.87-1.04)	0.92 (0.83-1.03)	0.96 (0.89-1.04)
15-25	1.28 (1.11-1.47)	1.30 (1.09-1.54)	1.27 (1.04-1.56)	1.15 (0.88-1.51)	1.22 (1.04-1.43)
25-50	1.27 (1.09-1.49)	1.08 (0.88-1.32)	0.95 (0.74-1.23)	0.84 (0.60-1.18)	1.27 (1.07-1.51)
>50	1.22 (1.01-1.48)	0.91 (0.70-1.19)	0.79 (0.57-1.11)	0.79 (0.52-1.21)	1.13 (0.91-1.40)
P-value for trend	<0.001	0.623	0.521	0.142	0.009
Clinical cut-off of CRP (>10)	1.20 (1.10-1.30)	1.10 (0.99-1.22)	1.05 (0.92-1.19)	0.96 (0.81-1.14)	1.05 (1.01-1.08)
Leukocytes (10^9/L)					
Log (leukocytes)	1.48 (1.36-1.61)	1.31 (1.18-1.44)	1.29 (1.15-1.45)	1.42 (1.24-1.63)	1.29 (1.18-1.42)
Quartiles of leukocytes					
<5.27	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
5.27-6.30	1.02 (0.95-1.09)	1.02 (0.94-1.10)	1.06 (0.96-1.16)	1.11 (0.99-1.24)	1.00 (0.93-1.07)
6.30-7.60	1.04 (0.97-1.11)	1.01 (0.93-1.09)	1.02 (0.93-1.12)	1.02 (0.91-1.15)	0.99 (0.92-1.06)
>7.60	1.27 (1.19-1.36)	1.19 (1.10-1.28)	1.21 (1.11-1.32)	1.31 (1.17-1.46)	1.16 (1.08-1.24)
P-value for trend	<0.001	<0.001	<0.001	<0.001	<0.001
Clinical cut-off of leukocytes (>10)	1.47 (1.34-1.61)	1.32 (1.18-1.47)	1.24 (1.08-1.41)	1.35 (1.15-1.58)	1.34 (1.21-1.48)
Inflammation-based Predictive Score (IPS)					
IPS=0	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
IPS=1	1.37 (1.14-1.64)	1.32 (1.05-1.66)	1.23 (0.92-1.63)	1.25 (0.87-1.80)	1.22 (0.99-1.50)

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[1] Sensitivity analysis in which all persons with follow-up <3 years were deleted (n=3,459).

[2] Sensitivity analysis in which all persons with follow-up <5 years were deleted (n=6,173).

[3] Sensitivity analysis in which all persons with follow-up <7 years were deleted (n=20,398).

[4] Sensitivity analysis in which all persons with smoking-related cancer were deleted (n=939).

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Table 3

Hazard Ratio and 95% confidence interval (CI) for categories of CRP and risk of cancer diagnosis, by cancer type in men and women. The models were adjusted for age, SES, and history of circulatory disease.

Men	**Prostate** N_{events}**=1,047**	**Lung** N_{events}**=265**	**Colon** N_{events}**=219**	**Bladder** N_{events}**=210**	**Other skin** N_{events}**=189**
CRP					
Categories of CRP					
<10	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
10-15	0.91 (0.76-1.10)	1.34 (0.96-1.88)	0.63 (0.39-1.02)	1.03 (0.69-1.55)	0.92 (0.60-1.41)
15-25	1.03 (0.70-1.52)	2.48 (1.46-4.19)	1.54 (0.88-3.10)	0.37 (0.09-1.51)	1.20 (0.53-2.72)
25-50	1.18 (0.81-1.73)	2.02 (1.10-3.72)	0.94 (0.39-2.29)	1.25 (0.55-2.81)	0.62 (0.20-1.95)
>50	1.24 (0.80-1.94)	1.38 (0.57-3.36)	0.57 (0.14-2.29)	1.25 (0.46-3.38)	0.33 (0.05-2.33)
P-value for trend	0.461	0.001	0.454	0.922	0.254
Clinical cut-off (>10mg/L)	1.08 (0.87-1.34)	1.83 (1.30-2.58)	0.94 (0.58-1.52)	1.05 (0.66-1.69)	0.81 (0.47-1.40)
Leukocytes					
Log (leukocytes)	0.83 (0.66-1.04)	6.13 (4.33-8.69)	1.86 (1.17-2.96)	1.18 (0.72-1.93)	0.84 (0.50-1.44)
Quartiles of leukocytes					
<5.27	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
5.27-6.30	1.11 (0.93-1.32)	1.67 (0.99-2.80)	1.73 (1.09-2.76)	1.35 (0.90-2.03)	0.90 (0.60-1.36)
6.30-7.60	0.99 (0.83-1.18)	2.53 (1.57-4.09)	1.93 (1.23-3.02)	1.20 (0.79-1.80)	0.81 (0.54-1.22)
>7.60	0.92 (0.77-1.10)	4.69 (2.99-7.38)	2.06 (1.32-3.21)	1.16 (0.78-1.76)	0.84 (0.56-1.27)
P-value for trend	0.173	0.05	0.002	0.152	0.351
Clinical cut-off of CRP (>10 10^9/L)	0.96 (0.73-1.27)	3.11 (2.24-4.33)	1.07 (0.61-1.87)	1.24 (0.72-2.13)	1.13 (0.62-2.08)
Inflammation-based Predictive Score (IPS)					
IPS=0	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
IPS=1	0.88 (0.51-1.53)	2.35 (1.21-4.57)	0.60 (0.15-2.43)	0.98 (0.31-3.08)	1.09 (0.35-3.40)

Women	**Breast** N_{events}**=1,241**	**Colon** N_{events}**=261**	**Cervix** N_{events}**=64**	**Lung** N_{events}**=251**	**Melanoma** N_{events}**=129**
CRP					
Categories of CRP					
<10	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)

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Women	**Breast** N_{events}**=1,241**	**Colon** N_{events}**=261**	**Cervix** N_{events}**=64**	**Lung** N_{events}**=251**	**Melanoma** N_{events}**=129**
10-15	1.00 (0.84-1.18)	0.88 (0.61-1.29)	1.04 (0.49-2.21)	1.10 (0.76-1.60)	0.60 (0.32-1.11)
15-25	1.14 (0.78-1.66)	0.87 (0.36-2.12)	2.43 (0.76-7.78)	1.99 (1.06-3.77)	1.06 (0.33-3.32)
25-50	0.98 (0.62-1.55)	1.30 (0.58-2.93)	NaN	0.76 (0.24-2.38)	NaN
>50	0.76 (0.41-1.43)	1.82 (0.81-4.10)	NaN	1.84 (0.76-4.48)	1.95 (0.62-6.17)
P-value for trend	0.774	0.400	0.749	0.122	0.469
Clinical cut-off (>10mg/L)	0.95 (0.75-1.20)	1.29 (0.84-1.98)	1.02 (0.37-2.82)	1.43 (0.93-2.20)	0.82 (0.38-1.77)
Leukocytes					
Log (leukocytes)	1.05 (0.86-1.28)	1.49 (0.97-2.29)	1.29 (0.55-3.02)	5.13 (3.48-7.55)	1.28 (0.70-2.35)
Quartiles of leukocytes					
<5.27	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
5.27-6.30	0.94 (0.82-1.07)	1.08 (0.75-1.55)	1.06 (0.49-2.29)	1.09 (0.67-1.78)	0.87 (0.53-1.44)
6.30-7.60	0.93 (0.80-1.09)	1.11 (0.78-1.58)	1.31 (0.64-2.69)	2.13 (1.40-3.25)	0.89 (0.54-1.45)
>7.60	1.01 (0.86-1.17)	1.33 (0.95-1.87)	1.49 (0.74-2.97)	3.58 (2.42-5.29)	1.04 (0.65-1.66)
P-value for trend	0.477	0.106	0.206	<0.001	0.857
Clinical cut-off of CRP (>10 10^9/L)	0.97 (0.76-1.25)	1.27 (0.78-2.11)	0.53 (0.13-2.16)	2.62 (1.81-3.80)	1.78 (0.98-3.23)
Inflammation-based Predictive Score (IPS)					
IPS=0	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
IPS=1	0.73 (0.39-1.35)	1.63 (0.67-3.95)	NA	2.82 (1.39-5.71)	0.70 (0.10-4.98)

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2011 September 01.

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Table 4

Descriptive characteristics by cancer status for the cohort with three repeated measurements of CRP and Leukocytes (IPS = Inflammation-based Predictive Score).

	No cancer N=8,398 (90.56%)	Cancer N=875(9.44%)
	n(%)	n(%)
Mean age at third measurement (years) (SD)	59.19 (14.96)	66.48 (11.06)
Gender		
Men	3186 (37.94)	416 (47.54)
Women	5212 (62.06)	459 (52.46)
SES		
White collar	3091 (36.81)	344 (39.31)
Blue collar	3115 (37.09)	256 (29.26)
Not gainfully employed/Missing	2192 (26.10)	275 (31.43)
Circulatory disease prior to CRP measurement		
Yes	2916 (34.72)	372 (42.51)
Mean follow-up time (years) (SD)	7.91 (2.24)	4.36 (2.82)
First Measurement		
CRP (mg/l)		
Mean (SD)	5.39 (10.55)	5.54 (9.09)
>10	529 (6.30)	63 (7.20)
Leukocytes (10^9/l)		
Mean (SD)	0.24 (0.43)	0.23 (0.42)
>10	436 (5.19)	61 (6.97)
Second Measurement		
CRP (mg/l)		
Mean (SD)	5.15 (8.07)	5.46 (10.21)
>10	453 (5.39)	63 (7.20)
Leukocytes (10^9/l)		
Mean (SD)	0.25 (0.43)	0.23 (0.42)
>10	452 (5.38)	61 (6.97)
Third Measurement		
CRP (mg/l)		
Mean (SD)	5.93 (9.26)	6.78 (13.11)
>10	673 (8.01)	94 (10.74)
Leukocytes (10^9/l)		
Mean (SD)	0.24 (0.43)	0.23 (0.42)
>10	429 (5.11)	70 (8.00)
Repeated CRP using clinical cut-off		
0	7056 (84.02)	709 (81.03)
1	1095 (13.04)	123 (14.06)
2	181 (2.16)	32 (3.66)

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	No cancer N=8,398 (90.56%)	Cancer N=875(9.44%)
	n(%)	n(%)
3	66 (0.79)	11 (1.26)
Repeated Leukocytes using clinical cut-off		
0	7488 (98.16)	752 (85.94)
1	613 (7.30)	76 (8.69)
2	187 (2.23)	25 (2.86)
3	110 (1.31)	22 (2.51)
Repeated IPS using clinical cut-off		
0	8165 (97.23)	833 (95.20)
1	200 (2.38)	35 (4.00)
2	28 (0.33)	4 (0.46)
3	5 (0.06)	3 (0.34)

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Table 5

Hazard Ratio and 95% confidence interval (CI) for values of the repeated IPS (Inflammation-based Predictive Score) and risk of cancer diagnosis. The models are adjusted for age, SES, gender and history of circulatory disease.

	Hazard Ratio (95%CI)	Hazard Ratio (95%CI)*	Hazard Ratio (95%CI)^
Repeated CRP with clinical cut-off			
0	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
1	1.15 (0.95-1.39)	1.07 (0.87-1.33)	1.01 (0.81-1.25)
2	1.83 (1.29-2.61)	2.04 (1.41-2.95)	1.82 (1.25-2.66)
3	2.05 (1.13-3.73)	2.44 (1.34-4.34)	1.49 (0.71-3.14)
P-value for trend	<0.001	<0.001	0.025
Repeated Leukocytes with clinical cut-off			
0	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
1	1.42 (1.12-1.79)	1.45 (1.13-1.87)	1.24 (0.95-1.62)
2	1.61 (1.08-2.40)	1.50 (0.96-2.35)	1.48 (0.95-2.31)
3	2.18 (1.43-3.34)	2.38 (1.52-3.71)	1.87 (1.14-3.07)
P-value for trend	<0.001	0.002	0.001
Repeated IPS			
0	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
1	1.87 (1.33-2.63)	2.16 (1.53-3.05)	1.43 (0.94-2.17)
2	1.51 (0.56-4.03)	1.77 (0.66-4.74)	1.31 (0.42-4.06)
3	4.46 (1.43-13.87)	5.29 (1.70-16.50)	3.68 (0.92-14.79)
P-value for trend	<0.001	<0.001	0.03

*Sensitivity analysis in which all persons with follow-up <1 year were deleted (n=219).

^Sensitivity analysis in which all persons with smoking-related cancer were deleted (n=114).