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Inflammatory serum markers and risk and severity of prostate cancer: The PROCA-*life* study

Einar Stikbakke[1,2], Elin Richardsen[3,4], Tore Knutsen[1,5], Tom Wilsgaard[6], Edward L. Giovannucci[7,8], Anne McTiernan[9,10], Anne Elise Eggen[6], Hege Sagstuen Haugnes[1,2] and Inger Thune[1,11]

[1]Department of Clinical Medicine, Faculty of Health Sciences, UiT, The Arctic University of Norway, Tromsø, Norway
[2]Department of Oncology, University Hospital of North Norway, Tromsø, Norway
[3]Department of Medical Biology, Faculty of Health Sciences, UiT, The Arctic University of Norway, Tromsø, Norway
[4]Department of Clinical Pathology, University Hospital of North Norway, Tromsø, Norway
[5]Department of Urology, University Hospital of North Norway, Tromsø, Norway
[6]Department of Community Medicine, Faculty of Health Sciences, UiT, The Arctic University of Norway, Tromsø, Norway
[7]Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA
[8]Departments of Nutrition and Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA
[9]Program in Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA
[10]Department of Epidemiology, School of Public Health; and Department of Medicine, School of Medicine, University of Washington, Seattle, WA
[11]Department of Oncology, The Cancer Centre, Ullevaal, Oslo University Hospital, Oslo, Norway

Whether chronic inflammation mirrored by high levels of systemic inflammatory markers such as high sensitive-CRP (hs-CRP) and white blood cell count (WBC) are associated with prostate cancer development remains unclear. In the Prostate Cancer Study throughout Life (PROCA-*life*), a prospective population-based cohort study, 7,356 men were included. Prediagnostic WBC and hs-CRP were assessed from blood collected at study entry; 2,210 participants also had a second CRP measure during follow-up. During a mean 11.8 years follow-up, 509 men developed prostate cancer (mean age at diagnosis 71.7 years). Multivariable Cox proportional hazard regression models were used to study whether individual biomarkers (WBC, hs-CRP), a combined score based on analyte tertiles (score range 2–6), or change in CRP were associated with risk and severity of prostate cancer. We observed a positive dose–response relationship between hs-CRP and prostate cancer risk with a Hazard Ratio (HR) per mg/l of 1.3, 95% CI 1.00–1.07. Men with an increase in hs-CRP between two measurements (Δhs-CRP) of ≥1.00 mg/l had a 36% increased risk of prostate cancer (HR 1.36, 95% CI 1.02–1.82), compared to men with no change or decrease in hs-CRP. Men with a systemic inflammatory score of 5 or 6 had a 68% higher risk of being diagnosed with metastatic disease (HR 1.68, 95% CI, 1.04–2.73) compared to men with lower scores. Our study supports that hs-CRP including repeated measurements alone or in combination with WBC may be a useful inflammation-related biomarker for prostate cancer risk and prognosis.

Additional Supporting Information may be found in the online version of this article.

Key words: prediagnostic inflammatory markers, repeated assessments, prostate cancer, white blood cells, hs-CRP, incidence

Abbreviations: BMI: body mass index; CI: confidence interval; HR: hazard ratio; Hs-CRP: high sensitivity C-reactive protein; ISUP: International Society of Urological Pathology; n: numbers; PSA: prostate-specific antigen; WBC: white blood cell count; Δhs-CRP: change in hs-CRP across two measurements

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Correspondence to: Einar Stikbakke, Department of Clinical Medicine, Faculty of Health Sciences, UiT, The Arctic University of Norway, Breivika, 9037 Tromsø, Norway, Tel: +47-40245123, E-mail: einar.stikbakke@uit.no

What's new?

Although chronic inflammation likely influences prostate cancer development, a clear association is yet to be established. In particular, uncertainties remain regarding the relationship between systemic inflammatory markers and prostate cancer. In this investigation of data for more than 7,350 men, pre-diagnostic levels of C-reactive protein (CRP), measured *via* high-sensitivity CRP (hs-CRP) testing at study entry and at follow-up, were associated with a dose-response increase in prostate cancer risk. Risk and disease severity were further associated with a combined score incorporating both hs-CRP and white blood cell count, highlighting the relevance of inflammation in prostate cancer development and prognosis.

Introduction

Chronic inflammation, one hallmark of cancer development[1] has been questioned as playing a key role in prostate cancer development. The suggested hypothesis is partly based on observations of inflammatory cells in the prostate microenvironment of adult men, and on inflammation being associated with precursor lesions in the prostate gland, termed proliferative inflammatory atrophy. However, a causal relationship between inflammation and prostate cancer development—one of the most common invasive cancers among men globally—has yet to be established.[2–4]

Currently, prostate-specific antigen (PSA) is the only non-invasive biomarker in clinical use to detect and evaluate efficacy of prostate cancer treatment, but has a low sensitivity in prostate cancer diagnosis.[5] However, PSA testing has led to a dramatic increase in incidence of prostate cancer, and the majority of prostate cancer cases have been localized disease.[6] Key challenges in diagnostics of prostate cancer are to develop better tools to identify individuals at high risk for prostate cancer, and to distinguish between tumors with a low malignant potential that are unlikely to require therapeutic intervention compared to tumors that should be treated.

Blood levels of two commonly available measures—C-reactive protein (CRP) and white blood cell count (WBC) are indicators of systemic inflammation. Interesting observations suggest that these biomarkers could predict risk for prostate cancer development and progression.[7–10] CRP is an acute phase protein that reflects tissue injury and has become a widely used systemic biomarker of acute infection or inflammation in clinical practice. CRP is relatively stable in serial measurements in healthy individuals.[11,12] Furthermore, local inflammation has been observed in 35–100% of prostate cancer biopsies.[2,13–15]

Previous studies investigating the association between CRP and risk for prostate cancer development have shown conflicting results, as some studies found positive associations between level of inflammation-related biomarkers and risk of prostate cancer[7,16,17]; others have not.[16–24] However, most studies have included only one single measurement of CRP, with a limited number of prostate cancer cases and short follow-up time.[7,16–24]

The aim of the present study was to investigate associations between the inflammation-related biomarkers CRP and WBC and risk of prostate cancer development and severity. A second aim is to determine whether markers of inflammation (WBC and high sensitivity-CRP, hs-CRP) independently or in combination were associated with risk and severity of prostate cancer, and to look at change in CRP and risk of prostate cancer development and severity. The Prostate Cancer Study throughout life (PROCA-*life*) study includes a subset of men included in the population-based Tromsø Study, who had available measures of CRP and WBC.

Methods

Study population

The PROCA-*life* study includes all men, age > 25 years who enrolled in the population-based, prospective cohort Tromsø study between 1994 and 2008 (Tromsø 4, 1994–1995, Tromsø 5, 2001, Tromsø 6, 2007–2008).[25,26] The procedures were almost identical and assessments were done by trained research technicians. All age-eligible men in the Tromsø geographic area were invited to participate *via* a personal written invitation, and non-respondents were given one reminder. Once enrolled, all participants were invited to participate in the regular next follow-up survey (second measurement). The attendance rate for men was on average 67% in the three health surveys.[26] For the present study, only men who attended the second visit in Tromsø 4 or Tromsø 5, and all men in Tromsø 6, were eligible (n = 7,720). Measurements of prediagnostic hs-CRP > 20 mg/l and/or prediagnostic WBC > 15 x 10^9cells/l, which may mirror other acute or chronic diseases, were excluded (high hs-CRP: n = 285, high WBC: n = 44). Participants with prevalent or previous cancer (n = 334), or who developed cancer within the first year after the enrollment in the study (n = 58) were excluded to account for the possibility that undiagnosed cancer or severe illness could influence the results (Fig. 1). All men completed questionnaires, blood draws and basic clinical measurements. The PROCA-*life* study has been approved by the Regional Committee for Medical and Health Research Ethics North (REK) (2015/1059). All participants have signed consent declarations when enrolled in the Tromsø Study.

Questionnaires and clinical assessments

Information about medical history, lifestyle factors, dietary factors, medication, smoking history, and level of physical activity were obtained from the questionnaires. We defined being physical active as: more than 1 hour/week of strenuous exercise, or any leisure time exercise more than two to three times/week.

Height and weight were measured on an electronic scale with the participants wearing light clothing and no shoes. Height was

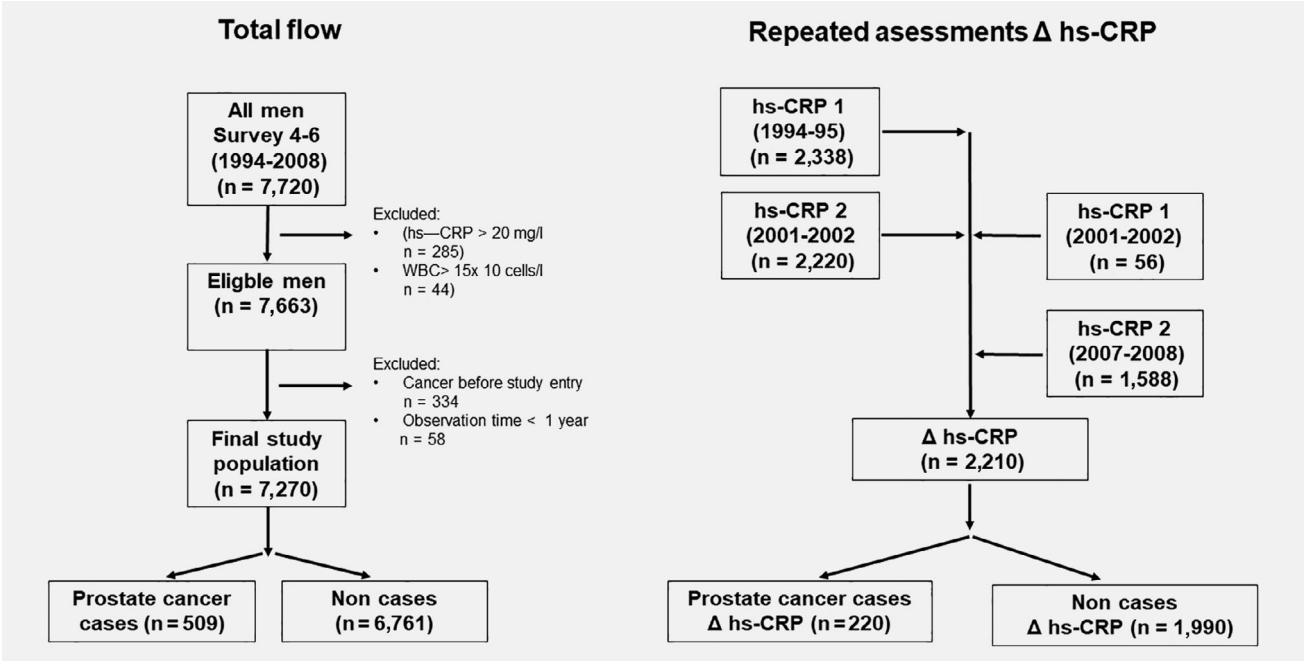

Figure 1. Flow chart for the population included in the PROCA-life study between 1994 and 2008.

measured to the nearest centimeter (cm) and weight to the nearest kilogram. BMI was calculated using the formula weight/height^2 (kg/m^2).[25] Blood pressure was measured three times in a resting position, and the mean of the two last measurements were used.

Assessment of serum inflammation-related biomarkers and other serum markers

Blood samples were drawn by trained research assistants on attendance at each survey, and were nonfasting. Analyses of serum samples were done at the Department of Laboratory Medicine, University Hospital of Northern Norway (UNN), Tromsø, Norway.[25] Serum samples from men who attended the first two surveys (Tromsø 4 or 5: 1994–95 and/or 2001) were kept frozen up to 12 years at −70°C and later analyzed, while hs-CRP was assessed in fresh samples from men who attended the final survey (Tromsø 6: 2007–08). Hs-CRP was analyzed by a particle-enhanced immune turbid metric assay on a Modular P auto-analyzer (Roche Diagnostics, Mannheim, Germany) with reagents from the manufacturer with a detection limit of 0.12 mg/l. For WBC counts, 5 ml of blood was collected into Vacutainer tubes containing EDTA as an anticoagulant (K3-EDTA 40 ll, 0.37 mol/l per tube), and analyzed within 12 hr by an automated blood cell counter (Coulter CounterÒ, Coulter Electronics, Luton, UK and Coulter LH750, Nerliens Meszansky). Total cholesterol was analyzed by enzymatic colorimetric methods with commercially available kits (CHOD-PAP for cholesterol). PSA measurements were done for cancer cases only, as part of clinical routine in diagnosis and follow-up (1990–1994 Stratus® PSA Fluorometric Enzyme Immunoassay, 1994–2001 AxSYM Psa Reagent Pack, Abbot®, 2001 Bayer® PSA Reagens Pack Immuno I (Prod. Nr.T01-3450-51), Technicon Immuno I). For prostate cancer cases diagnosed or treated in other institutions (n = 21), PSA values from their local laboratories were recorded.

Identification of prostate cancer cases during follow-up

Prostate cancer cases during follow-up (until December 31, 2016) were identified by using the unique national 11-digit identification number through linkage with the Cancer Registry of Norway. Among 7,270 men that were included in our study, 509 men were diagnosed with verified invasive prostate cancer during follow-up, and there was no ongoing screening programs for prostate cancer in Tromsø during the study period. Follow-up time was calculated from date of entry into the study, to the date of censoring (prostate cancer diagnosis, emigration, death, or end of follow-up [December 31, 2016]).

Detailed clinical information for the prostate cancer cases was obtained from the medical records (e.g., disease stage, treatments, recurrence) by trained physicians (TK and ES). All histopathological specimens were reexamined by the same uropathologist (ER) and classified according to the latest International Society of Urological Pathology (ISUP) guidelines on Gleason score and ISUP grade group.[27]

Prostate cancer cases were divided into four risk groups based on PSA level at diagnosis, highest ISUP grade group and clinical T-stage, according to the EAU guidelines.[28] Risk group 1 (low) was defined as: PSA < 10 μg/l, clinical T-stage (cT-) 1, and ISUP grade group 1. Risk group 2 (intermediate) was defined as: PSA: 10–20 μg/l, cT-stage 2, or ISUP grade

group 2–3. Risk group 3 (high) was defined as: PSA: > 20–100 μg/l, cT-stage 3, or ISUP grade group 4–5. Risk group 4 (metastatic) was defined as: PSA > 100 μg/l, or with radiological evidence of metastatic disease. ISUP grade group were reported after reclassification when available. PSA values above 100 were not included in calculation of mean or median PSA.

Statistical methods

Descriptive characteristics of the study population were presented as means (standard deviation) or percent (numbers). Differences in the distribution of characteristics at study entry between nonprostate cancer cases and prostate cancer cases were assessed using *t*-tests for continuous variables and Chi-square tests for categorical data. No large differences was observed

Table 1. Distribution of selected characteristics for men with prostate cancer (cases) and without prostate cancer (noncases) in the PROCA-*life* Study (1994–2008)

Characteristics	Overall (*n* = 7,270)	Noncases (*n* = 6,761)	Prostate cancer cases (*n* = 509)
Age at first attendance (years)	56.9 (10.5)	56.5 (10.6)	66.8 (7.6.)
Observation time (years)	11.8 (6.0)	11.9 (6.0)	9.9 (5.9)
Observation time ≤5 years (%)	5.8	4.3	24.8
Observation time 5.1–10 years (%)	59.9	61.9	33.2
Observation time › 10 years (%)	34.3	33.8	42.0
Systolic blood pressure (mm Hg)	137.7 (19.8)	137.2 (19.6)	143.3 (20.5)
Body mass index (kg/m^2)	26.8 (3.67)	26.8 (3.7)	26.6 (3.6)
Serum samples at study entry			
Total cholesterol (mmol/l)	5.98 (1.21)	5.96 (1.21)	6.19 (1.24)
Hs-CRP (mg/l)	2.10 (2.46)	2.10 (2.46)	2.17 (2.47)
Hs-CRP (mg/l) median (interquartile range)	1.28 (0.70–2.45)	1.27 (0.69–2.44)	1.36 (0.78–2.61)
White blood cells (x10^9/l)	6.62 (1.79)	6.61 (1.80)	6.75 (1.68)
White blood cells (x10^9/l) median (interqu. range)	6.3 (5.3–7.6)	6.3 (5.3–7.6)	6.4 (5.6–7.7)
Lifestyle factors at study entry			
Lipid-lowering drugs, current use (%)	8.5	8.5	8.3
Current smokers (%)	26.7	26.6	28.5
Physically active (%)	41.0	41.1	39.2
Characteristics among prostate cancer cases			
Age at diagnosis (years)			71.7 (7.5)
Cancer specific mortality (%)			8.8
PSA at diagnosis (μg/l)[1]			14.3 (14.3)
PSA at diagnosis, median (μg/l)[1]			9.9
Time from last blood sample to diagnosis (years)			5.4 (3.2)
Tumor characteristics			
T-stage			
T1 + T2 (%)			74.1
T3 + T4 (%)			22.0
Tx (%)			3.9
ISUP grade group			
1–3 (Gleason score 6–7) (%)			72.7
4–5 (Gleason score 8–10) (%)			18.3
ISUP missing (%)			9.0
Risk group			
Low (%)			16.1
Intermediate (%)			41.9
High (%)			24.2
Metastatic (%)			12.1
Unknown (%)			5.5

Numbers may vary due to missing information. Values are mean (standard deviation) unless otherwise specified.
Abbreviations: Hs-CRP, high sensitivity C-reactive protein; ISUP, International Society of Urological Pathology; PSA, prostate-specific antigen.
[1]PSA values above 100 are excluded from calculation of mean and median.

between cases and noncases and therefore not shown in text or tables. Multivariable Cox proportional hazard models were used to investigate whether inflammation biomarkers (hs-CRP and WBC) or repeated assessments of hs-CRP (Fig. 1) were associated with prostate cancer risk and severity, presented with hazard ratio (HR) and 95% confidence interval (CI). The inflammatory markers (hs-CRP and WBC) were not normally distributed, and log-transformation was tested, but did not influence results.

To study the importance of the variation in inflammation-related biomarkers in more detail, we used hs-CRP and WBC both as continuous and categorical variables, with tertile cut-points based on the distribution in the overall data set. Continuous variables are presented as HR per unit increase. We defined the systemic inflammatory score as the sum of tertile ranking for hs-CRP and WBC: tertile 1. hs-CRP: ≥0.01 – ≤0.91 mg/l, WBC: ≥1.1 – ≤5.6 × 10^9/l/l, tertile 2. hs-CRP: ≥0.92 – ≤2.03 mg/l, WBC: ≥5.7 – ≤7.0 × 10^9/l and tertile 3. hs-CRP: ≥2.04 – ≤20 mg/l, WBC: ≥7.1 – ≤15 × 10^9/l. The systemic inflammatory score ranged from 2 to 6 points; 5–6 were defined as a high score. The endpoints in the study were prostate cancer overall (Table 2), or prostate cancer split into risk groups as separate endpoints (Table 3). When using prostate cancer of a specific risk group as endpoint, prostate cancer cases in other or unknown risk group were excluded from the analysis.

Participating men with more than one measurement of hs-CRP during follow-up (n = 2,210) were included in the data set by using the "reshape" command in STATA, thus updating the measured levels of inflammation-related biomarkers for the next period at risk. We then calculated Δhs-CRP: the difference in hs-CRP between the first and the second measurement. In separate models, Δhs-CRP was included as a continuous variable or dichotomized as Δhs-CRP ≥1.00 mg/l (yes/no).

Based on suggested biological mechanisms influencing our inflammation-related biomarkers, and/or prostate cancer risk, several variables were assessed as potential confounders. Age at entry (continuous) and BMI (continuous), were included as covariates in the final models. Lipid-lowering drugs (categorical), alcohol habits (categorical), and physical activity (categorical) did not influence our results and were not included. The analyses with Δhs-CRP as an explanatory variable were also adjusted for hs-CRP at baseline. We performed stratified analyses by age at study entry (<60 years *vs.* ≥60 years), systolic BP (<140 mm Hg *vs.* ≥140 mm Hg), BMI (<25 kg/m^2 *vs.* ≤25 kg/m^2).

The proportional hazard assumption was verified by visual inspection of log minus log survival curves in tertiles of hs-CRP and WBC and in groups according to Δhs-CRP or systemic inflammation score. All statistical tests were two-sided using a significance level of $p < 0.05$, and conducted with STATA/MP version 15.1 (StataCorp LLC, College station, TX).

Data availability

The data set used in our study is available upon request, pending permission from the Tromsø Study (www.tromsoundersokelsen.no).

Results

The cohort of 7,270 participating men had the following means: age at entry 56.9 years, hs-CRP 2.10 mg/l, and WBC 6.62 (×10^9cells/l) (Table 1). A total of 509 men developed prostate cancer during 11.8 years of follow-up. Men with one measurement of inflammatory markers compared to men with two measurements had a mean follow up and incidence rate of 8.4 years and 60.9/1000 men, and 18.3 years and 124/1000 men, respectively (not presented in tables). The prostate cancer cases with a mean age at diagnosis of 71.7 years had a mean PSA at diagnosis of 14.3 μg/l. Among prostate cancer cases, 16.1% were in the low-risk group, 41.9% were in the intermediate-risk group,

Table 2. Hazard ratios (HR) for risk of prostate cancer by prediagnostic hs-CRP, WBC or by a combination of hs-CRP and WBC (systemic inflammatory score). The PROCA-*life* study (1994–2008)

Inflammatory markers	Cases *N*	Age-adjusted (*n* = 7,270) HR (95% CI)	Multivariable[1] (*n* = 7,270) HR (95% CI)
Hs-CRP			
Continuous, mg/l	509	**1.04 (1.01–1.07)**	**1.03 (1.00–1.07)**
Continuous, 1 SD	509	**1.09 (1.01–1.17)**	**1.09 (1.01–1.17)**
Tertiles			
‹0.91 mg/l	131	1.00 (reference)	1.00 (reference)
0.92–2.03 mg/l	174	1.16 (0.93–1.46)	1.16 (0.92–1.46)
›2.04 mg/l	204	**1.31 (1.05–1.63)**	**1.30 (1.04–1.63)**
WBC			
Continuous, x 10^9/l	490	1.04 (0.98–1.09)	1.04 (0.98–1.09)
Continuous, 1 SD	490	1.06 (0.97–1.17)	1.06 (0.97–1.17)
Tertiles			
≤5.6 x 10^9/l	147	1.00 (reference)	1.00 (reference)
5.7– ≤7.0 x 10^9/l	196	**1.46 (1.18–1.80)**	**1.46 (1.17–1.80)**
≥7.1 x 10^9/l	147	1.23 (0.98–1.55)	1.23 (0.98–1.55)
Systemic inflammatory score (SIS)			
Continuous per 1 point	490	**1.09 (1.02–1.17)**	**1.09 (1.02–1.17)**
SIS low[2–4]	285	1.00 (reference)	1.00 (reference)
SIS high[5,6]	205	**1.28 (1.07–1.53)**	**1.28 (1.06–1.53)**
Δhs-CRP[2]			
Continuous, mg/l	220	1.04 (0.99–1.09)	**1.05 (1.01–1.10)**
Positive change			
‹1.00 mg/l	155	1.00 (reference)	1.00 (reference)
≥1.00 mg/l	65	**1.35 (1.01–1.80)**	**1.36 (1.02–1.82)**

Statistically significant (p value ‹0.05) hazard ratios are marked in bold letters. The systemic inflammatory score ranged from two to six points; high systemic inflammatory score: 5–6 were defined as a high systemic inflammatory score-score. Low systemic inflammatory score: Systemic inflammatory score = 2, 3 and 4.

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; Hs-CRP, high sensitivity C-reactive protein; *n*, numbers; WBC, white blood cell count.

[1]Adjusted for age at entry and BMI. Analyses with Δhs-CRP also adjusted for hs-CRP at baseline.

[2]Δhs-CRP: Change in hs-CRP across two measurements. Analyzed in subgroup with repeated measurements available.

Table 3. Age-adjusted hazard ratios (HR) for different risk groups of prostate cancer, by prediagnostic hs-CRP, WBC or by a combination of hs-CRP and WBC (systemic inflammatory score). The PROCA-*life* study (1994–2008)

	Low-risk prostate cancer (N_{total}: 7,023 [1])		Intermediate-risk prostate cancer (N_{total}: 7,112 [1])		High-risk prostate cancer (N_{total}:: 7,064 [1])		Metastatic prostate cancer (N_{total}: 7,031 [1])	
Inflammatory markers	**Cases**	**HR (95% CI)**	**Cases**	**HR (95% CI)**	**Cases**	**HR (95% CI)**	**Cases**	**HR (95% CI)**
Hs-CRP								
Continuous, mg/l	80	1.00 (0.92–1.09)	195	**1.07 (1.03–1.22)**	132	1.01 (0.95–1.08)	72	1.00 (0.92–1.09)
Continuous, 1 SD	80	1.00 (0.81–1.25)	195	**1.20 (1.08–1.33)**	132	1.04 (0.89–1.21)	72	1.00 (0.81–1.24)
Tertiles								
‹0.91 mg/l	24	1.00 (ref.)	44	1.00 (ref.)	39	1.00 (ref.)	16	1.00 (ref.)
0.92–2.03 mg/l	30	1.12 (0.66–1.92)	65	1.31 (0.90–1.93)	44	0.99 (0.64–1.53)	25	1.30 (0.69–2.43)
›2.04 mg/l	26	0.99 (0.56–1.73)	86	**1.72 (1.19–2.48)**	49	1.04 (0.68–1.60)	31	1.44 (0.79–2.65)
WBC								
Continuous, x 10^9/l	77	1.01 (0.89–1.15)	192	1.03 (0.94–1.11)	126	1.00 (0.90–1.11)	66	1.11 (0.97–1.28)
Continuous, 1 SD	77	1.02 (0.80–1.29)	192	1.05 (0.90–1.21)	126	1.00 (0.83–1.21)	66	1.21 (0.95–1.55)
Tertiles								
≤5.6 x 10^9/l	28	1.00 (ref.)	59	1.00 (ref.)	38	1.00 (ref.)	17	1.00 (ref.)
5.7– ≤7.0 x 10^9/l	30	1.22 (0.73–2.04)	76	**1.44 (1.03–2.03)**	54	**1.54 (1.02–2.33)**	22	1.41 (0.75–2.66)
≥7.1 x 10^9/l	19	0.88 (0.49–1.58)	57	1.23 (0.85–1.77)	34	1.07 (0.67–1.70)	27	**1.91 (1.03–3.52)**
Systemic inflammatory score								
Continuous per 1 point	77	0.96 (0.80–1.14)	192	**1.16 (1.04–1.30)**	126	1.06 (0.88–1.17)	66	**1.25 (1.02–1.51)**
Low[2–4]	51	1.00 (ref.)	108	1.00 (ref.)	77	1.00 (ref.)	33	1.00 (ref.)
High[5,6]	26	0.96 (0.60–1.54)	84	**1.43 (1.07–1.90)**	49	1.11 (0.78–1.60)	33	**1.68 (1.04–2.73)**

Statistically significant (p value ‹0.05) hazard ratios are marked in bold letters. Values given are hazard ratios with 95% confidence interval. Numbers may vary due to missing information. The systemic inflammatory score ranged from 2 to 6 points; High Systemic inflammatory score: 5–6 were defined as a high systemic inflammatory score. Low systemic inflammatory score: Systemic inflammatory Score = 2, 3 and 4.
Abbreviations: CI, confidence interval; HR, hazard ratio; Hs-CRP, high sensitivity C-reactive protein; *n*, numbers; WBC, white blood cell count.
[1]Prostate cancer cases in other risk groups or unknown risk group were excluded from the analysis.

24.2% in the high-risk group, and 12.1% had metastatic disease at the time at diagnosis.

Inflammation-related biomarkers and prostate cancer risk

We observed a positive dose–response relationship between hs-CRP and prostate cancer risk (HR per unit 1.03, 95% CI 1.00–1.07) after adjustments for potential confounding factors (Table 2). Men in the upper tertile of hs-CRP (>2.04 mg/l) had a 30% increased prostate cancer risk (HR 1.30, 95% CI 1.04–1.63) compared to men in the lower tertile of hs-CRP (<0.91 mg/l). We also observed that an increase in hs-CRP between two measurements (Δhs-CRP) of more than 1.00 mg/l increased the risk of prostate cancer by 36% (HR 1.36, 95% CI 1.02–1.82), compared to those men who had a small increase or a decrease in hs-CRP level between two measurements (Table 2). Time between two measurements did not influence these observed results, and mean time between measurements was 6.7 years (range 5.7–14.1 years) (results not presented in table). A similar dose–response relationship was observed between WBC and prostate cancer risk, but the results were not statistically significant.

When the levels of hs-CRP and WBC were combined in a systemic inflammatory score (range 2–6), a positive dose–response association was observed between systemic inflammatory score and prostate cancer risk (HR per unit 1.09, 95% CI 1.02–1.17). Men with a high systemic inflammatory score[5,6] had a 28% increased risk of prostate cancer (HR 1.28, 95% CI 1.07–1.53) when compared to men with a lower systemic inflammatory score.[2–4] When stratified by age at study entry (<60 years *vs.* ≥60 years at study entry), we observed a positive dose–response relationship between systemic inflammation score and prostate cancer risk (HR 1.08, 95% CI 1.00–1.17) only among men who were ≥60 years at study entry, but interaction terms between groups were not significant (Supporting Information Table S1). Among those with a prediagnostic BMI ≥25 kg/m^2 we observed a 1.27 times increased risk (95% CI 1.03–1.58) of prostate cancer for men with an high systemic inflammatory score when compared to men with a low score (Supporting Information Table S2). When stratified by systolic blood pressure (<140 mm Hg *vs.* ≥140 mm Hg), we observed a positive dose–response relationship between hs-CRP and prostate cancer risk (HR 1.06, 95% CI 1.02–1.11) among men who had a systolic blood pressure < 140 mm Hg. Interaction terms between groups were not significant (Supporting Information Table S3).

Inflammation-related biomarkers and severity of prostate cancer

Men with a WBC count in the upper tertile (≥7.1 x 10^9cells/l), had a 1.91 (95% CI 1.03–3.52) times increased risk of metastatic

prostate cancer when compared to men with the lowest tertile of WBC ($\leq 5.6 \times 10^9$/l).

We observed a dose–response association between systemic inflammatory score and both being diagnosed within an intermediate prostate cancer risk group (HR 1.16, 95% CI 1.04–1.30) and being diagnosed with metastatic disease, (HR 1.25, 95% CI 1.02–1.51). Men with a high systemic inflammatory score[5,6] had a 43% increased risk for intermediate risk prostate cancer (HR 1.43, 95% CI 1.07–1.90), and a 68% increased risk of metastatic prostate cancer (HR 1.68, 95% CI 1.04–2.73) when compared to men having a systemic inflammatory score between 2 and 4 (Table 3).

Discussion

In this population-based prospective study with repeated measurements of prediagnostic inflammatory markers, we observed that hs-CRP measured at one and two time points was associated with prostate cancer risk in a positive dose–response manner; among men with an increase in hs-CRP between two measurements (≥1.00 mg/l), we observed a 36% higher prostate cancer risk compared to those who had small increase or a decrease in hs-CRP level. Men with a high systemic inflammatory score (hs-CRP and WBC in combination) had a 28% higher prostate cancer risk, and were more likely to be diagnosed with metastatic prostate cancer compared to men having a low systemic inflammatory score (2–4).

Results from previous studies of the association between hs-CRP or WBC and prostate cancer risk have been inconsistent. Our findings that hs-CRP measured at one time point were associated with prostate cancer risk are supported by some studies,[7,17,24] but our results are also in contrast to others.[16,18,19,21–23] In a nested case–control study including 622 prostate cancer cases, a positive association was observed between prediagnostic CRP and prostate cancer risk among men with BMI <25 kg/m^2, even when CRP was measured several years before the diagnosis.[21] In the present study, we did not observe any clear pattern of variation in the associations studied between inflammatory markers and prostate cancer when stratified by BMI (BMI <25 kg/m^2 *vs*. BMI ≥25 kg/m^2). However, among those with a prediagnostic BMI ≥25 kg/m^2 we observed a 1.27 times increased risk (95% CI 1.03–1.58) of prostate cancer for men with a high systemic inflammatory score when compared to men with a low score. These findings support that excess weight may mirror a low grade inflammation by resulting in a higher systemic inflammatory score not observed among the leaner men (BMI <25 kg/m^2).

Our findings suggesting that both hs-CRP and an increase in hs-CRP during follow-up were associated with risk of metastatic prostate cancer are partly in line with the Swedish AMORIS study.[17] In the AMORIS study,[17] CRP was dichotomized into low (<10 mg/ml) and high (≥10 mg/ml) and it was observed that CRP levels assessed on average 14 years before being diagnosed with prostate cancer predicted worse outcome (high-risk prostate cancer and metastatic prostate cancer). A positive association between hs-CRP and advanced prostate cancer is also supported by others.[29–31]

However, to our knowledge, this is the first study to assess the combination of hs-CRP and WBC creating a systemic inflammatory score in relation to both prostate cancer risk and severity. Interestingly, our findings suggest that compared to using either WBC or hs-CRP alone, a combination of these markers may be more useful. The score was strongly associated with both risk for prostate cancer and for severity of prostate cancer. Thus, an inflammatory score might be a useful way of combining two or more inflammatory markers that could be used for risk classification.[24] In a large population-based study by Morrison *et al*., CRP and WBC were combined into a Z-score.[32] They found an association between the inflammation Z-score and risk for overall cancer, including prostate cancer. In contrast, in another study, a high score based on three inflammatory biomarkers (CRP, WBC and fibrinogen) was not associated with prostate cancer risk.[33] Additionally, several studies have questioned whether a systemic inflammatory score could be a valuable predictive tool for worse outcome in several types of cancers including prostate cancer,[34,35] and our results support the hypothesis that it might be valuable for prostate cancer severity.

Published studies suggest a dual effect of obesity: an increased risk for advanced prostate cancer,[36] low-grade systemic inflammation,[37] severity of prostate cancer and a decreased risk of localized prostate cancer.[38] In our study, we did not find any variation by measured BMI (kg/m^2), in contrast to others,[39] but we included only one BMI measurement. Wang *et al*. found that men with an increase in BMI from normal to an overweight or obese condition experienced increased risk of prostate cancer compared to men with persistently normal BMI, and that this was most pronounced for men with ISUP grade group ≥7. The biological explanation is not fully understood, but there is evidence suggesting that substantial crosstalk occurs between molecular pathways involved in inflammation and obesity. Studies have investigated the association between inflammatory markers and hypertension,[40,41] where low-grade systemic inflammation might be a common cause. We stratified our results by systolic BP ($\lessgtr$140 mm Hg), but did not observe any significant association between the systemic inflammatory score and risk of prostate cancer (Supporting Information Table S3).

Inflammation is one of the hallmarks of cancer development,[33] and CRP is found in blood plasma, with rising levels in response to factors released by inflammatory associated cells as macrophages and fat cells.[42] Chronic inflammation is evident in the adult prostate and probably has a role in formation of lesions such as proliferative inflammatory atrophy, which is proliferative glandular epithelium with morphological appearance of simple atrophy that occurs in association with inflammation.[2,43,44] These lesions are thought to be possible precursors for prostate cancer.[2,45] Further, there is evidence that regenerative epithelium in response to environmental insults may precede development of prostate intraepithelial neoplasia and early carcinoma.[44,45] The origin of prostate inflammation is multifactorial

and in many cases without symptoms. The inflammation could be either acute or chronic.[46]

The strengths of our study include its prospective and population-based design and the high attendance rate (65.7–78.5%[25]), which lessens the chance of biased observations. In addition, a high completeness rate of identification of prostate cancer cases (Cancer Registry of Norway) at 98.8% is another strength.[47] Furthermore, the rather long follow-up time, broad information about baseline characteristics and repeated measurements of hs-CRP strengthen the results observed. All medical records for the prostate patients were carefully reviewed by trained physicians with systematic abstraction of histopathology and clinical characteristics. The study was able to control for several potential confounding factors, and to address effect modification, such as age, body mass index, smoking habits, and physical activity.

However, our study also has some limitations. The population in Tromsø is mainly Caucasian, and the results may therefore not be relevant for populations including other ethnicities. Repeated assessments of inflammation-related biomarkers were only assessed among a subgroup of men, thus limiting the sample size. A limitation of our study is the long time between exposure measurement and diagnosis. Thus, changes in various clinical variables over time may have occurred, and two measurements of variables may only in part account for the cumulative effect of the markers on risk of prostate cancer. The levels of these exposures may be affected by various factors over the life-course and may tend to fluctuate. However, measurements of BMI made earlier in life have been found to be strongly related to measurements later in life.[48,49] Moreover, adjustment for time between measurement and diagnosis did not change our results. Information regarding family history of prostate cancer was not available and could therefore not be included in the analysis.

Conclusion

Our study supports a positive association between hs-CRP, hs-CRP and WBC in combination and risk for both prostate cancer and for metastatic prostate cancer. Importantly, hs-CRP and WBC are often used in routine clinical practice, and thus easily accessible. Our findings contribute to understanding the relationship between inflammation and prostate cancer development, and may be useful in future research on prostate cancer etiology and possibly prevention. However, our results are based on a relatively small sample size and should be interpreted with caution.

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References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–74.
2. De Marzo AM, Platz EA, Sutcliffe S, et al. Inflammation in prostate carcinogenesis. *Nat Rev Cancer* 2007;7:256–69.
3. Luo JL, Tan W, Ricono JM, et al. Nuclear cytokine-activated IKKalpha controls prostate cancer metastasis by repressing Maspin. *Nature* 2007;446:690–4.
4. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. *Nat Rev Cancer* 2006;6:24–37.
5. Cooperberg MR, Broering JM, Carroll PR. Time trends and local variation in primary treatment of localized prostate cancer. *J Clin Oncol* 2010;28:1117–23.
6. Cancer in Norway. In: Larsen IK, ed. *Cancer incidence, mortality, survival and prevalence in Norway*. Oslo: Cancer Registry of Norway, 2016. 2017.
7. Touvier M, Fezeu L, Ahluwalia N, et al. Association between prediagnostic biomarkers of inflammation and endothelial function and cancer risk: a nested case-control study. *Am J Epidemiol* 2013;177:3–13.
8. Beer TM, Lalani AS, Lee S, et al. C-reactive protein as a prognostic marker for men with androgen-independent prostate cancer: results from the ASCENT trial. *Cancer* 2008;112:2377–83.
9. Prins RC, Rademacher BL, Mongoue-Tchokote S, et al. C-reactive protein as an adverse prognostic marker for men with castration-resistant prostate cancer (CRPC): confirmatory results. *Urol Oncol* 2012;30:33–7.
10. Elsberger B, Lankston L, McMillan DC, et al. Presence of tumoural C-reactive protein correlates with progressive prostate cancer. *Prostate Cancer Prostatic Dis* 2011;14:122–8.
11. Buckley DI, Fu R, Freeman M, et al. Helfand M. C-reactive protein as a risk factor for coronary heart disease: a systematic review and meta-analyses for the U.S. preventive services task force. *Ann Intern Med* 2009;151:483–95.
12. Ockene IS, Matthews CE, Rifai N, et al. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. *Clin Chem* 2001;47:444–50.
13. De Nunzio C, Kramer G, Marberger M, et al. The controversial relationship between benign prostatic hyperplasia and prostate cancer: the role of inflammation. *Eur Urol* 2011;60:106–17.
14. Sharma S, Zapatero-Rodriguez J, O'Kennedy R. Prostate cancer diagnostics: clinical challenges and the ongoing need for disruptive and effective diagnostic tools. *Biotechnol Adv* 2017;35:135–49.
15. Maia MC, Hansen AR. A comprehensive review of immunotherapies in prostate cancer. *Crit Rev Oncol Hematol* 2017;113:292–303.
16. St Hill CA, Lutfiyya MN. An epidemiological analysis of potential associations between C-reactive protein, inflammation, and prostate cancer in the male US population using the 2009-2010 National Health and Nutrition Examination Survey (NHANES) data. *Front Chem* 2015;3:55.
17. Arthur R, Williams R, Garmo H, et al. Serum inflammatory markers in relation to prostate cancer severity and death in the Swedish AMORIS study. *Int J Cancer* 2018;142:2254–62.
18. Platz EA, De Marzo AM, Erlinger TP, et al. No association between pre-diagnostic plasma C-reactive protein concentration and subsequent prostate cancer. *Prostate* 2004;59:393–400.
19. Van Hemelrijck M, Jungner I, Walldius G, et al. Risk of prostate cancer is not associated with levels of C-reactive protein and other commonly used markers of inflammation. *Int J Cancer* 2011;129:1485–92.
20. Pierce BL, Biggs ML, DeCambre M, et al. C-reactive protein, interleukin-6, and prostate cancer risk in men aged 65 years and older. *Cancer Causes Control* 2009;20:1193–203.
21. Stark JR, Li H, Kraft P, et al. Circulating prediagnostic interleukin-6 and C-reactive protein and prostate cancer incidence and mortality. *Int J Cancer* 2009;124:2683–9.
22. Siemes C, Visser LE, Coebergh JW, et al. C-reactive protein levels, variation in the C-reactive protein gene, and cancer risk: the Rotterdam study. *J Clin Oncol* 2006;24:5216–22.
23. Heikkila K, Harris R, Lowe G, et al. Associations of circulating C-reactive protein and interleukin-6 with cancer risk: findings from two prospective cohorts and a meta-analysis. *Cancer Causes Control* 2009;20:15–26.
24. Toriola AT, Laukkanen JA, Kurl S, et al. Prediagnostic circulating markers of inflammation

and risk of prostate cancer. *Int J Cancer* 2013;133: 2961–7.
25. Eggen AE, Mathiesen EB, Wilsgaard T, et al. The sixth survey of the Tromso Study (Tromso 6) in 2007-08: collaborative research in the interface between clinical medicine and epidemiology: study objectives, design, data collection procedures, and attendance in a multipurpose population-based health survey. *Scand J Public Health* 2013;41:65–80.
26. Jacobsen BK, Eggen AE, Mathiesen EB, et al. Cohort profile: the Tromso Study. *Int J Epidemiol* 2012;41:961–7.
27. Epstein JI, Allsbrook WC Jr, Amin MB, et al. The 2005 International Society of Urological Pathology (ISUP) consensus conference on Gleason grading of prostatic carcinoma. *Am J Surg Pathol* 2005;29: 1228–42.
28. Heidenreich A, Bastian PJ, Bellmunt J, et al. EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and local treatment with curative intent-update 2013. *Eur Urol* 2014;65:124–37.
29. Aldemir M, Ener K, Dehni D, et al. KayÄ±gil Ã. evaluation of the relationship between prostate cancer and serum inflammation markers. *Nephrourol Mon* 2010;02:244–50.
30. Lehrer S, Diamond EJ, Mamkine B, et al. C-reactive protein is significantly associated with prostate-specific antigen and metastatic disease in prostate cancer. *BJU Int* 2005;95:961–2.
31. Chang C-C, Lin ATL, Chen K-K, et al. The significance of plasma C-reactive protein in patients with elevated serum prostate-specific antigen levels. *Urolog Sci* 2010;21:88–92.
32. Morrison L, Laukkanen JA, Ronkainen K, et al. Inflammatory biomarker score and cancer: a population-based prospective cohort study. *BMC Cancer* 2016;16:80.
33. Allin KH, Bojesen SE, Nordestgaard BG. Inflammatory biomarkers and risk of cancer in 84,000 individuals from the general population. *Int J Cancer* 2016;139:1493–500.
34. Shibutani M, Maeda K, Nagahara H, et al. The prognostic value of the systemic inflammatory score in patients with unresectable metastatic colorectal cancer. *Oncol Lett* 2018;16:666–72.
35. McMillan DC. The systemic inflammation-based Glasgow prognostic score: a decade of experience in patients with cancer. *Cancer Treat Rev* 2013;39: 534–40.
36. Giovannucci EL, Liu Y, Leitzmann MF, et al. A prospective study of physical activity and incident and fatal prostate cancer. *Arch Intern Med* 2005; 165:1005–10.
37. Choi J, Joseph L, Pilote L. Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. *Obes Rev* 2013;14: 232–44.
38. Discacciati A, Orsini N, Wolk A. Body mass index and incidence of localized and advanced prostate cancer–a dose-response meta-analysis of prospective studies. *Ann Oncol* 2012;23:1665–71.
39. Wang K, Chen X, Gerke TA, et al. BMI trajectories and risk of overall and grade-specific prostate cancer: an observational cohort study among men seen for prostatic conditions. *Cancer Med* 2018;7: 5272–80.
40. Dauphinot V, Roche F, Kossovsky MP, et al. C-reactive protein implications in new-onset hypertension in a healthy population initially aged 65 years: the proof study. *J Hypertens* 2009; 27:736–43.
41. Fukutomi M, Hoshide S, Eguchi K, et al. Low-grade inflammation and ambulatory blood pressure response to antihypertensive treatment: the ALPHABET study. *Am J Hypertens* 2013;26: 784–92.
42. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure* 1999; 7:169–77.
43. Nakai Y, Nonomura N. Inflammation and prostate carcinogenesis. *Int J Urol* 2013;20:150–60.
44. Davidsson S, Fiorentino M, Andren O, et al. Inflammation, focal atrophic lesions, and prostatic intraepithelial neoplasia with respect to risk of lethal prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2011;20:2280–7.
45. Bostwick DG, de la Roza G, Dundore P, et al. Intraepithelial and stromal lymphocytes in the normal human prostate. *Prostate* 2003; 55:187–93.
46. Moreira DM, Nickel JC, Gerber L, et al. Baseline prostate inflammation is associated with a reduced risk of prostate cancer in men undergoing repeat prostate biopsy: results from the REDUCE study. *Cancer* 2014;120:190–6.
47. Larsen IK. Data quality at the Cancer Registry of Norway. *Eur J Cancer* 2009;45:1218–31.
48. Evensen E, Wilsgaard T, Furberg AS, et al. Tracking of overweight and obesity from early childhood to adolescence in a population-based cohort - the Tromso Study, fit futures. *BMC Pediatr* 2016;16:64.
49. Wilsgaard T, Jacobsen BK, Schirmer H, et al. Tracking of cardiovascular risk factors: the Tromso study, 1979-1995. *Am J Epidemiol* 2001; 154:418–26.