EUROPEAN JOURNAL OF CANCER 46 (2010) 3425–3433

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journal homepage: www.ejconline.com

C-reactive protein; a potential marker of second cancer and cardiovascular disease in testicular cancer survivors?

T. Wethal [a,*,g], H.S. Haugnes [b,h], J. Kjekshus [a,g], M.C. Småstuen [c,f], T. Ueland [d,g], P. Aukrust [d,e,g], S.D. Fosså [f,i]

[a] Department of Cardiology, Faculty Division Rikshospitalet, University of Oslo, Norway
[b] Department of Oncology, Institute of Clinical Medicine, University of Tromsø, Norway
[c] The Cancer Registry of Norway, Norway
[d] Research Institute for Internal Medicine, Faculty Division Rikshospitalet, University of Oslo, Norway
[e] Section of Clinical Immunology and Infectious Diseases, Rikshospitalet, Oslo University Hospital, Norway
[f] Department of Clinical Cancer Research, The Norwegian Radium Hospital, Oslo, Norway

ARTICLE INFO

Article history:
Received 2 June 2010
Received in revised form 28 July 2010
Accepted 9 August 2010
Available online 9 September 2010

Keywords:
Testicular cancer survivors
C-reactive protein
Second cancer
Cardiovascular disease

ABSTRACT

Introduction: C-reactive protein (CRP) is a marker of cardiovascular disease (CVD). There is conflicting evidence regarding CRP as a marker of future cancer. We studied whether CRP predicts CVD and consecutive cancer in testicular cancer survivors (TCSs).

Patients and methods: During 1998–2001, 586 TCSs with a high sensitivity CRP ⩽10 mg/L were identified median 11 (4–21) years after treatment (FU-1). A second follow-up survey (FU-2) was conducted median 8 (6–9) years after FU-1. At FU-2 we obtained information about post-FU-1 CVD (cardiovascular death, nonfatal myocardial infarction, stroke, revascularisation or heart failure). Information about post-FU-1 non-germ cell cancer and cardiovascular death in all patients were retrieved from the Cancer Registry of Norway.

Results: After FU-1 31 (5.3%) of 586 patients developed non-germ cell cancer (excluding localised prostate cancer), while 28 (4.9%) developed CVD. Cox regression analyses showed that patients with CRP ⩾1.5 mg/L had 2.21 (95% CI 1.04–4.70) times higher risk of developing non-germ cell cancer and 2.79 (95% CI 1.22–6.34) times higher risk for CVD compared to patients with CRP <1.5 mg/L at FU-1.

Conclusion: In long-term TCSs, CRP may serve as a potential marker of cardiovascular events and a second cancer.

1. Introduction

C-reactive protein (CRP) is established as a predictor of cardiovascular events in patients with known cardiovascular disease (CVD) as well as in asymptomatic individuals with risk factors for CVD.[1,2] CRP is an acute phase protein produced by the liver in response to inflammatory stimuli.[3] Chronic subclinical inflammation may also be associated with cancer, for example as a pathogenic mediator in colon cancer, cervical cancer or gastric carcinoma.[4] This hypothesis is supported

* *Corresponding author. Address*: Department of Cardiology, Faculty Division Rikshospitalet, University of Oslo, Sognsvannsveien 20, 0027 Oslo, Norway. Tel.: +47 23 07 00 00; fax: +47 23 07 39 17.
E-mail address: torgeir.wethal@medisin.uio.no (T. Wethal).
[g] Address: Sognsvannsveien 20, 0027 Oslo, Norway.
[h] Address: Breivika, 9037 Tromsø, Norway.
[i] Address: Ullernschausséen 70, 0310 Oslo, Norway.
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2010.08.004

by the observation of elevated CRP in patients with prevalent cancer as well as in patients who develop cancer several years after the registration of an elevated CRP.[4–7] Recently, a CRP level ⩾3 mg/L was identified as a marker of incident lung cancer in the general population in comparison to individuals with CRP <1 mg/L.[5] However, the association between CRP and cancer is still controversial.[7–9]

Long-term testicular cancer survivors (TCSs) treated with radiotherapy and/or chemotherapy have an increased risk of second malignancies and cardiovascular morbidity.[10–13] Elevated levels of CRP and other markers of inflammation have been observed in TCSs several years after treatment with radiotherapy and chemotherapy.[14,15] We have previously shown that TCSs treated with radiotherapy had elevated levels of CRP median 11 years after treatment compared with TCSs treated only with surgery or chemotherapy.[15] However, it is still unknown whether elevated CRP levels seen in TCSs identify individuals at risk for developing a second cancer or CVD.

The aim of this exploratory study was primarily to explore the hypothesis that elevated high sensitivity CRP levels determined at one occasion during long-term follow-up of TCSs may predict subsequent CVD (i.e. cardiovascular death, non-fatal myocardial infarction, stroke, revascularisation or heart failure) or non-germ cell cancer in TCSs. Secondly, we wanted to study if treatment with radiotherapy shown to be associated with raised CRP levels was associated with an increased risk of CVD or a consecutive cancer.

2. Patients and methods

2.1. Patients

Surviving TCSs treated for unilateral testicular cancer at the Norwegian Radium Hospital (NRH) from 1980 to 1994 participated in a questionnaire-based survey conducted during 1998–2001 (FU-1).[16] They also underwent an out-patient clinical examination at the hospital with determination of several biomarkers, among them CRP. Post-orchiectomy treatment consisted of retroperitoneal lymph node dissection alone or surveillance (n = 140), cisplatin-based chemotherapy with or without additional surgery (n = 240) or abdominal radiotherapy alone (n = 231, median dose 35 Gy). Only one patient received mediastinal radiotherapy. Twenty-eight patients received combined chemotherapy and radiotherapy. The chemotherapeutic agents used were primarily cisplatin in the combination with bleomycin and vinblastin (before 1985) or etoposid (after 1985). Detailed treatment strategies of the surveyed men have been reported previously.[15,16] At FU-1 patients were categorised as smokers versus never or previous smokers and as physically active or inactive.

A second questionnaire-based survey (FU-2) was conducted in 2007–2008 median 8 (range 6–9) years after FU-1. Surviving TCSs were asked to have a clinical examination and blood sampling performed at their family doctor's office. The blood samples were immediately mailed to the NRH where all biochemical analyses were performed.

Eligibility criteria for the present study were aged ⩽60 years at FU-1, no known second cancer and no apparent acute or chronic infections at blood sampling thus excluding men with CRP >10 mg/L (n = 17) and those in whom CRP was missing (n = 36) due to logistic reasons (Fig. 1).

At the time of FU-2, 19 men had died after FU-1; four died from CVD and 10 from cancer. The post-FU-1 development of a non-germ cell cancer or a first-time cardiovascular event represented the end-point of our analyses.

2.2. Post-FU-1 non-germ cell cancer and CVD

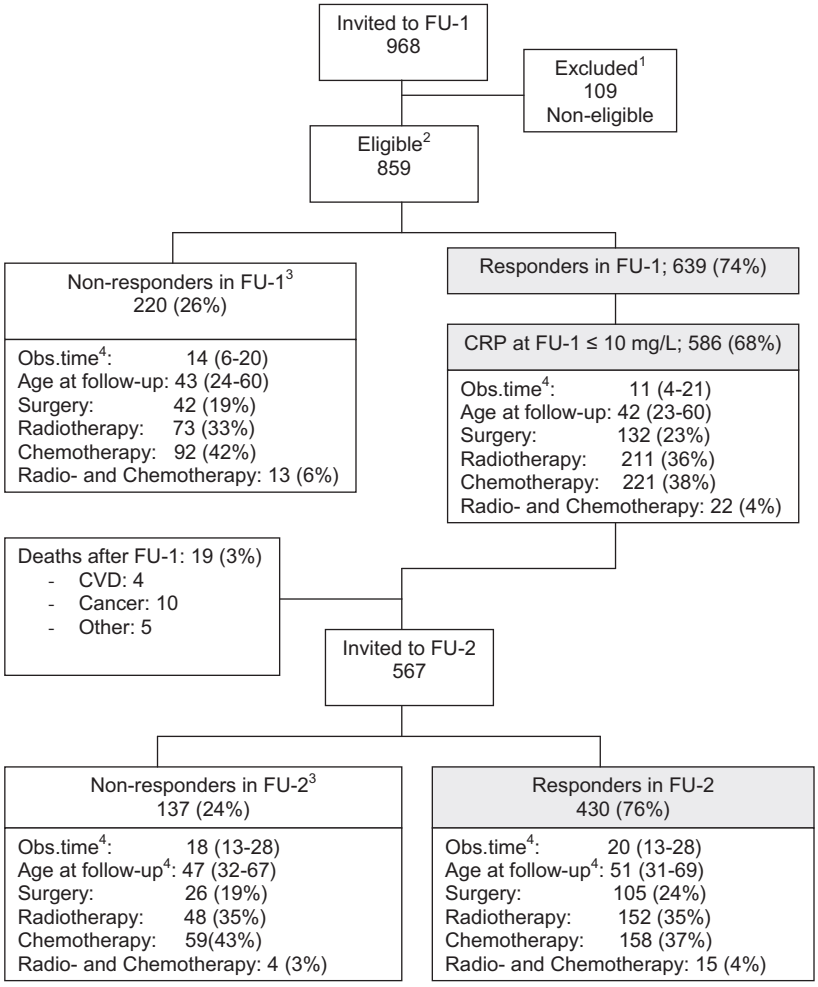
From the Cancer Registry of Norway we received data (type of cancer, date of diagnosis) about non-germ cell cancer diagnosed after FU-1 among our eligible TCSs as registered by December 2008. The diagnosis of localised prostate cancer (n = 5) was not considered to be a valid event as this malignancy is increasingly detected by PSA screening. From the questionnaire at FU-2 we extracted information about first-time post-FU-1 cardiovascular events (myocardial infarction, stroke, revascularisation and hospitalisation for heart failure) after exclusion of 15 men who had reported such an event prior to FU-1. All self-reported cardiovascular events were validated by the patient's hospital medical record. The study was approved by the Institutional and Regional Ethical Committees.

2.3. Biochemical analyses of serum and plasma

At FU-1 non-fasting blood was sampled between 9 am and noon into pyrogen-free, pre-cooled vials without additives (serum) or with EDTA as anticoagulant (plasma). The tubes were centrifuged at 1000g for 10 min within 30 min (plasma) or allowed to clot before centrifugation (serum). High sensitivity CRP in plasma was determined by a high-sensitive particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Basel Switzerland). Plasma levels of total cholesterol, low- and high density lipoprotein (LDL and HDL) cholesterol and triglycerides were measured enzymatically with a Roche/Hitachi 917 analyser (Roche Diagnostics). Serum levels of luteinising hormone (LH) and testosterone were analysed by immunoassays.[16] Patients were classified as having hypogonadism when any of the following three conditions were met: serum testosterone <8 nmol/L, serum LH >12 U/L, or regular use of exogenous testosterone.[16] The intra- and inter-assay coefficients of variation were less than 10% for all assays.

2.4. Statistical analyses

Continuous variables were described with median (range or interquartile range; IQR) and categorical variables with proportions (percentages). Spearman's correlation coefficient was computed to assess the correlation between CRP at FU-1 and -2. Mann-Whitney, Wilcoxon and Kruskal-Wallis tests were used to compare continuous data with skewed distributions. Chi-square test or Fisher's exact test was applied to compare categorical data. To determine the optimally discriminating CRP, we depicted the area under the curve (AUC) for the composite end-point of cardiovascular event and a second cancer and calculated that a cut-off level of CRP at 1.5 mg/L reached a sensitivity of 65% and a specificity of 58% (Fig. 2). This cut-off level was used to construct groups of patients with CRP <1.5 and ⩾1.5 mg/L. We also constructed



Abbreviations: FU-1: first follow-up survey; FU-2: second follow-up survey; CVD: cardiovascular disease
[1]Age at survey >60 years; [2]Invited patients eligible for FU-1; [3]Without out-patient visit, questionnaire or lack of blood sampling; [4] p:<0.01, all other comparisons p:>0.05; Observation time and age at follow-up are presented as median and range. Other values are number (%).

Fig. 1 – Flow-diagram of the recruited patients.

groups of patients with CRP <1.0 and ⩾1.0 mg/L and with CRP <3.0 and ⩾3.0 mg/L to reveal if alternative cut-off levels were associated with increased risks of second cancer or CVD.

The cumulative incidences of CVD and a second cancer as separate end-points were computed for the groups using Kaplan–Meier plots. The time to event was calculated from FU-1 to the date of the end-point, the date of FU-2 (for cardiovascular events) or 31st December 2008 (for second cancer), whatever occurred first. Differences in time to event between groups were assessed using the log-rank test. Additional Kaplan–Meier plots were drawn to illustrate the impact of treatment modalities for which the log-rank test could not be performed.

Univariate and multiple Cox-regression models were fitted to explore the relation between clinically important predictors of second cancer or CVD. The limited number of events for each end-point allowed only three variables to be included in the multiple Cox-regression model. Based on the univariate analyses and the main purpose of the study CRP, treatment (surgery ± chemotherapy versus radiotherapy ± chemotherapy) and age were entered into the multiple model. To fulfil the proportional hazard assumption patients treated with only surgery and patients treated with chemotherapy were considered as one group in the Cox-regression models. The proportional hazard assumption was tested by visual inspection of log–log plots.

The categorical variables current smoking, physical active, education, hypogonadism and diabetes mellitus were not included in the multiple analyses because there were no associations between these variables and the end-points in univariate analyses except from an association between diabetes mellitus and a second cancer ($p < 0.001$). Additionally these variables did not fulfil the proportional hazards assumption. There were significant correlations between

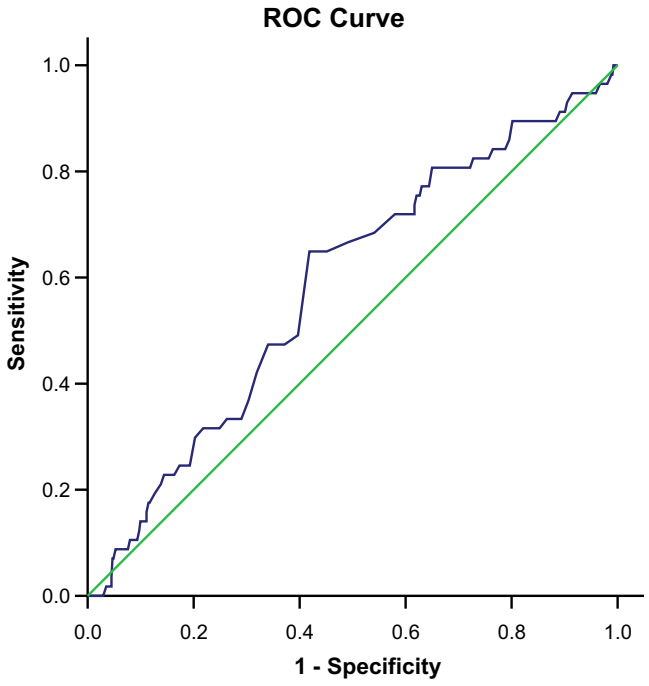


Fig. 2 – Receiver operating characteristic curve for CRP and the presence of either second cancer or cardiovascular disease. The area under the curve was 0.58 (95% CI 0.51–0.66), $p = 0.04$. Inspection of the curve revealed that the optimal discriminating CRP value had a sensitivity of 65% and specificity at 58% in predicting cardiovascular disease or a second cancer. This corresponded to a CRP value of 1.5 mg/L.

systolic and diastolic blood pressure as well as between the total-, LDL- and HDL-cholesterol. To avoid multi-colinearity, only systolic blood pressure and LDL-cholesterol were selected for the multiple Cox-regression. A two-sided p-value <0.05 was considered statistically significant. Data were analysed using SPSS 14.0 (SPSS Inc.).

3. Results

Overall, 586 patients were included in FU-1 at a median of 11 (range 4–21) years after treatment. All were eligible for analyses of a second cancer while 571 patients were eligible for analyses of CVD between FU-1 and FU-2. Patients who had been treated with radiotherapy (alone or with additional chemotherapy) were older both at diagnosis and at FU-1 and had a higher level of CRP at FU-1 compared to the other two treatment groups (Table 1). There was a strong relationship between CRP-levels at FU-1 and FU-2 (Spearman $r = 0.59$, $p < 0.001$).

3.1. *Non-germ cell cancer*

In total, 31 (5.3%) of 586 patients developed non-germ cell cancer after FU-1, excluding five patients who were diagnosed with localised prostate cancer (Table 2). Gastrointestinal cancer, kidney cancer, bladder cancer and skin cancer were the most frequent consecutive cancer types seen in TCSs. The cumulative incidence of non-germ cell cancer was significantly increased among TCSs with a CRP ⩾1.5 mg/L as compared to those with CRP <1.5 mg/L (Fig. 3a; $p = 0.01$). The multiple Cox-regression analysis revealed that patients with a CRP level ⩾1.5 mg/L at FU-1 had a 2.21-fold (95% CI 1.04–4.70) higher risk of developing non-germ cell cancer after adjustment for age and treatment (Table 3). Systolic blood pressure was not associated with non-germ cell cancer after adjustment for age. Four patients developed non-germ cell cancer within 2 years after the measurement of CRP. The estimated risk of a second cancer was 2.16 (95% CI 0.97–4.82) after excluding these patients.

Patients treated with radiotherapy as compared to patients treated with only surgery or chemotherapy had a higher cumulative incidence of non-germ cell cancer and in particular gastrointestinal cancer (Fig. 3b). Cox-regression analyses demonstrated that patients treated with infra-diaphragmatic radiotherapy alone or in combination with chemotherapy had 2.56-fold (95% CI 1.19–5.51) higher risk of developing a second cancer as compared to patients treated with surgery with or without chemotherapy (Table 3). Additionally, after the exclusion of the 22 patients who were registered to be treated with both radiotherapy and chemotherapy, treatment with only infra-diaphragmatic radiotherapy was associated with increased risk of a second cancer (HR 2.29 (95% CI 1.03–5.07)).

3.2. *CVD*

Twenty-eight (4.9%) TCSs were diagnosed with a post-FU-1 cardiovascular event (Table 2). The most frequent cardiovascular events were myocardial infarction and revascularisation. At FU-1 CRP was generally higher among patients who eventually developed CVD compared to those without events (median 1.9 (IQR 1.2–3.6) versus median 1.2 (IQR 0.7–2.1) mg/L, $p = 0.005$). The cumulative incidence of CVD was increased among TCSs with a CRP ⩾1.5 mg/L as compared to those with CRP <1.5 mg/L (Fig. 3c). Cox regression analyses demonstrated that patients with a CRP ⩾1.5 mg/L at FU-1 had 2.79-fold (95% CI 1.22–6.34) times higher risk of developing cardiovascular events after adjustment for age (Table 3). Systolic blood pressure and LDL-cholesterol were not associated with non-germ cell cancer after adjustment for age. The cumulative incidence of CVD was no different in the three treatment groups (Fig. 3d). Cox-regression analyses did not reveal any influence of treatment modality on cardiovascular outcome. The use of a cut-off of 3 mg/L showed that patients with a CRP ⩾3 mg/L had 2.27 (95% CI 1.03–5.03) times higher risk of CVD after adjustment for age compared to TCSs with CRP <3 mg/L. Additionally, patients with a CRP ⩾1 mg/L had 2.61 (95% CI 0.99–6.89) times higher risk of developing CVD after adjustment for age compared to those with CRP <1 mg/L.

4. Discussion

TCSs included in our study with a CRP level ⩾1.5 mg/L had more than twice as large risk for developing non-germ cell cancer and almost three times as high risk of CVD compared to survivors with CRP <1.5 mg/L. Prior radiotherapy represented an additional risk factor for cancer development.

Table 1 – Demographic and clinical characteristics among the 586 patients at FU-1 (median 11 years after diagnosis).

Number of patients (n = 586)	Surgery only 132	Chemotherapy 221	Radiotherapy ± chemotherapy 233
Age (years)[a]			
at diagnosis	29 (24–35)	28 (23–33)	34 (29–38)
at follow up	40 (36–47)	40 (34–45)	45 (40–51)
Current smoker, *n* (%)	42 (32)	83 (38)	82 (35)
Physical active, *n* (%)	107 (81)	186 (84)	191 (82)
Education at university level, *n* (%)	44 (33)	90 (41)	94 (40)
Gonadal function			
Hypogonadism, *n* (%)	7 (5)	31 (14)	21 (9)
Lipid-related markers			
Total cholesterol (mmol/L)	5.6 (4.8–6.4)	5.6 (4.9–6.3)	5.6 (5.0–6.3)
LDL cholesterol (mmol/L)	3.7 (3.1–4.3)	3.6 (3.0–4.2)	3.6 (3.1–4.2)
HDL cholesterol (mmol/L)	1.2 (1.0–1.4)	1.1 (1.0–1.3)	1.1 (1.0–1.3)
Triglycerides (mmol/L)	1.3 (0.9–2.0)	1.6 (1.0–2.5)	1.6 (1.0–2.4)
Clinical markers			
Body mass index (kg/m^2)	26.3 (24.4–28.6)	25.7 (23.7–27.9)	26.2 (24.1–28.4)
Systolic blood pressure (mm Hg)	120 (115–130)	125 (120–140)	130 (120–140)
Diastolic blood pressure (mm Hg)	80 (70–85)	80 (70–90)	80 (75–85)
Diabetes mellitus I or II, *n* (%)	2	6	8
Inflammatory marker			
hsCRP (mg/L)	1.2 (0.6–2.0)	1.2 (0.7–2.4)	1.5 (0.9–2.6)

Abbreviation: FU-1, first follow-up; hsCRP, high sensitivity C-reactive protein.
[a] Values are median (interquartile range) unless otherwise specified.

Table 2 – Clinical end points registered after FU-1 according to treatment.

	Surgery	Chemotherapy	Radiotherapy ± chemotherapy	Total	*p*-Value
A: Second cancer					
Eligible patients (FU-1)	132	221	233	586	
Gastrointestinal cancer	2 (1.5)	1 (0.5)	7 (3.0)	10 (1.7)	
Kidney- and bladder cancer	0	4 (1.8)	3 (1.3)	7 (1.2)	
Prostate cancer	1 (0.8)	1 (0.5)	4 (1.7)	6 (1.0)	
Melanoma	1 (0.8)	0	2 (0.9)	3 (0.5)	
Other[a]	0	2 (0.9)	8 (3.4)	10 (1.7)	
All second cancer	4 (3.0)	8 (3.6)	24 (10.3)	36 (6.1)	0.003
All, except localised prostate cancer	3 (2.3)	7 (3.2)	21 (9.0)	31 (5.3)	0.004
B: Cardiovascular events[b]					
Eligible patients (FU-2)	130	216	225	571	
Nonfatal myocardial infarction	2 (1.5)	5 (2.3)	6 (2.6)	13 (2.3)	
Nonfatal stroke	2 (1.5)	2 (0.9)	2 (0.9)	6 (1.1)	
Revascularisation	3 (2.3)	6 (2.8)	6 (2.6)	15 (2.6)	
Heart failure	0	2 (0.9)	1 (0.4)	3 (0.5)	
Death from cardiovascular disease	0	1 (0.5)	3 (1.3)	4 (0.7)	
Any	5 (3.8)	10 (4.6)	13 (5.8)	28 (4.9)	0.70

Abbreviations: FU-1 and FU-2, first and second follow-up.
Data are given as numbers of patients (%).
[a] Pulmonary cancer (1), brain cancer (1), peripheral nerve system, pelvis (1), pancreatic cancer (1), laryngeal cancer (2), cancer in the lymphatic system (1), peritoneal cancer (1), squamous cell carcinoma (2).
[b] Fifteen patients with CVD before FU-1 were excluded. Cardiovascular events registered after FU-1 included death due to CVD, nonfatal myocardial infarction, nonfatal stroke, coronary revascularisation and heart failure.

The increased risk of non-germ cell cancer or CVD in TCSs is documented in several studies.[11–13,17,18] Radiotherapy has been shown to be the most important etiological factor for a second malignancy but it also contributes to CVD even if only administered infra-diaphragmatically.[11,12] Second cancer is only partly explained by radiation-induced genetic mutations. Long-term subclinical inflammation caused by infra-diaphragmatic radiotherapy in TCSs with release of cytokines may also be of importance in mediating second cancer.[15] The role of cisplatin-based chemotherapy in second solid cancer development is suggested but is less obvious; probably due to short observation times.[18] On the other hand, cisplatin,

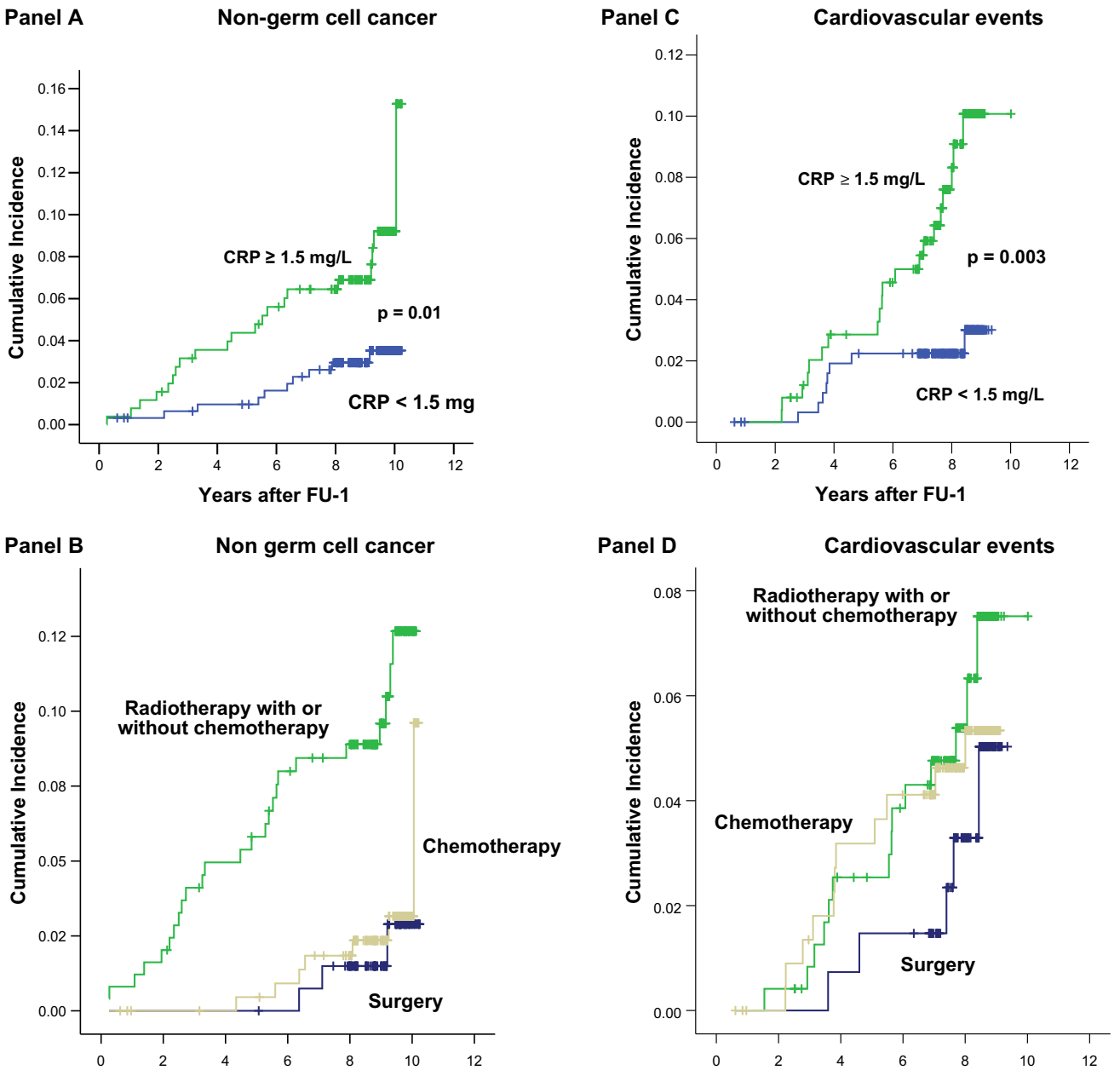


Fig. 3 – Cumulative Incidence of non-germ cell cancer according to CRP-level ⩾ or below 1.5 mg/L (Panel A) and according to treatment group (Panel B). Cumulative Incidence of cardiovascular disease according to CRP-level ⩾ or below 1.5 mg/L (Panel C) and according to treatment group (Panel D).

which may be an etiological factor for both second cancer and CVD, can be demonstrated in serum or urine of TCSs more than 10 years after their chemotherapy.[19,20] Oncologists have increasingly discussed the need of life-long monitoring of TCSs, at least of high-risk TCSs. However, no biomarker is accepted for the identification of TCSs at particular high risk of a second solid cancer or a CVD event. With this background we suggest that CRP might represent such a predictive factor.

In patients with manifest cancer-elevated CRP indicates a poor prognosis.[21–23] However, CRP may also predict a malignancy as cancer may etiologically be related to chronic infections by viral or bacterial agents.[4–7] The majority of evidence is related to patients with colorectal cancer and lung cancer as demonstrated by recent studies and meta-analyses, although not all studies have supported this view.[5–8] The results from the abovementioned studies may be confounded by patients who already had occult cancer at the time of CRP measurement or incidental elevation of CRP. Allin and colleagues demonstrated that the association between CRP and cancer was attenuated after excluding patients diagnosed within 2 years after CRP measurement.[5] In our study only four patients were diagnosed with non-germ

Table 3 – Cox regression with non-germ cell cancer (left panel) and cardiovascular disease (right panel) as the dependent variable.

	Univariate analyses			Multiple results[a]			Univariate analyses			Multiple results[b]		
	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age at FU-1	1.08	1.03–1.13	0.001	1.07	1.02–1.12	0.01	1.12	1.06–1.18	<0.001	1.11	1.06–1.17	<0.001
Lipid-related markers												
Total cholesterol	1.26	0.94–1.68	0.13				1.36	1.00–1.84	0.05			
LDL cholesterol	1.08	0.75–1.55	0.67				1.52	1.06–2.18	0.02			
HDL cholesterol	1.00	0.28–3.59	0.99				0.51	0.12–2.08	0.35			
Clinical markers												
Body mass index	1.01	0.92–1.11	0.85				1.04	0.95–1.15	0.42			
Systolic blood pressure	1.02	1.00–1.04	0.02				1.03	1.01–1.04	0.004			
Diastolic blood pressure	1.03	1.01–1.06	0.04				1.03	1.00–1.06	0.10			
Inflammatory markers												
hsCRP	1.08	0.91–1.29	0.39				1.17	0.99–1.38	0.07			
CRP $\geqslant$1.5 mg/L	2.60	1.22–5.51	0.01	2.21	1.04–4.70	0.04	3.27	1.44–7.42	0.005	2.79	1.22–6.34	0.02
Treatment												
Surgery ± chemotherapy	1.00	Reference		1.00	Reference		1.00	Reference				
Radiotherapy ± chemotherapy	3.46	1.62–7.35	<0.001	2.56	1.19–5.51	0.02	1.40	0.67–2.95	0.37			

Due to multi co-linearity we could possibly choose systolic or diastolic blood pressure and one of the lipid-related markers for the multiple Cox-regressions. For the analysis of second cancer systolic blood pressure was regarded as possible confounder. For the analysis of cardiovascular events LDL-cholesterol and systolic blood pressure were possible confounders. After adjustment for age no significant associations between these markers and a second cancer or cardiovascular disease were revealed.

Abbreviations: FU-1, first follow-up; hsCRP, high sensitivity C-reactive protein.

Hazard ratios (HR) and 95% confidence interval (CI) for different predictors of non-germ cell cancer and cardiovascular disease. HR for the continuous variables refers to the increased risk of non-germ cell cancer and cardiovascular disease for every unit increase in the continuous variable.

HR = 1 (reference) for the following categorical variables; a CRP level below 1.5 mg/L and treatment with surgery ± chemotherapy.

[a] For the analysis of second cancer age at FU-1, CRP $\geqslant$1.5 mg/L and treatment were included.

[b] For the analysis of cardiovascular events age at FU-1 and CRP $\geqslant$1.5 mg/L were included.

cell cancer within 2 years from CRP measurement. After the exclusion of these patients, CRP $\geqslant$1.5 mg/L was still associated with future cancer, even though this association was attenuated, possibly due to the limited number of events among our TCSs.

Cisplatin-based chemotherapy is today considered to be a possible factor in the development of premature atherosclerosis and CVD.[11,12,24] Hypertension, hypercholesterolaemia and the metabolic syndrome are frequently observed among TCSs and may contribute to the increased risk of CVD.[24,25] Cisplatin-based chemotherapy may lead to an increase in inflammatory markers and endothelial dysfunction as demonstrated by reduced flow-mediated dilatation and increased intima-media thickness.[14,26] Large-field radiotherapy has similar effects predominantly on the vessels within the target field.[27] The acute effects of radiotherapy include small vessel thrombosis due to endothelial cell swelling and endothelial inflammation.[28] Previously we demonstrated elevated levels of CRP and von Willebrand factor in irradiated TCSs, most of them included in the present study.[15] Other groups have observed similar alterations of inflammatory serum markers after chemotherapy.[14,26] It is tempting to hypothesise that persistent subclinical endothelial inflammation after radiotherapy and/or chemotherapy, with on-going production of cytokines, may represent a common link between local or systemic cytotoxic treatment and the development of cardiovascular events in TCSs. Large studies with long follow-up have to confirm this hypothesis.

After the demonstration that a CRP level above 2 mg/L identified individuals with increased risk of coronary heart disease, CRP has gained much interest as a potentially important tool in primary prevention of CVD.[1,29] This is reflected in the recent JUPITER trial where cardiovascular morbidity and mortality were reduced by 44% in presumably healthy individuals with normal LDL-cholesterol but a CRP-level above 2 mg/L.[2] There is an ongoing debate whether measurements of individual CRP values will give additional information beyond what is provided by the traditional risk scores. In the present study, a CRP level $\geqslant$1.5 mg/L was better in predicting CVD than traditional risk factors. Recently, it has been demonstrated that biomarkers such as CRP and brain natriuretic peptide (BNP) were not better than traditional risk factors.[30] In contrast; our study suggests that CRP may assist in identifying TCSs at increased risk of developing CVD. If used together with other markers of future CVD it would be possible to compose a test battery to identify individuals at risk of premature CVD who might benefit from primary prophylaxis with statins.

The strength of our study is the prospective design, with the use of high sensitivity analyses of CRP applied for the first time in the follow-up of TCSs. Furthermore, only 23% were lost during follow-up, making selection bias unlikely. Nevertheless, some limitations have to be pointed out: the use of a cut-off at 1.5 mg/L optimised the results for our data set, and the results have to be validated in an independent data set. Even though, previous studies and guidelines have demonstrated increased risk of CVD or cancer with the use of a cut-off at 1, 2 or 3 mg/L which makes our cut-off at 1.5 mg/L reasonable.[1,2,29] Furthermore, the use of a cut-off at either 1.0 or 3.0 mg/L was able to identify TCSs at an increased risk of CVD. The total number of our patients under observation and the number of TCSs who experienced an end-point were limited which gave some restrictions to our Cox-regression analyses. The cardiovascular events in those alive are self-reported suggesting that the incidence of cardiovascular events might be underestimated, but all events reported by the patients were validated by medical records. We did not have an age-matched male control group available to investigate whether determination of elevated CRP is of greater value in TCSs than in the general population.

From this exploratory study, we conclude that high sensitivity CRP may be a useful marker of cardiovascular events and non-germ cell cancer among TCS irrespective of cancer treatment modality. Future large-scaled studies should evaluate if CRP can be a useful marker in the screening of TCSs for the prevention of a second cancer and CVD.

Conflict of interest statement

We have no conflict of interest to declare.

Acknowledgement

This work was supported by the University of Oslo, Norway.

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