Role of high-sensitivity C-reactive protein in patients with sarcoma

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Abstract. Immunotherapy has shown promising results in lung cancer and melanomas; however, the responses have been poor in patients with sarcoma. Understanding the relationship between the immune system and sarcoma is essential to develop improved immunotherapy approaches. High-sensitivity C-reactive protein (hs-CRP) has been proposed as a prognostic marker in other cancer types; however, to the best of our knowledge, the association between hs-CRP levels and mortality in patients with sarcoma has not been investigated. The present prospective, non-randomised, non-interventional explorative study investigated the prognostic value of hs-CRP in patients with sarcoma. Patients referred to the sarcoma centre of Aarhus University Hospital (Aarhus, Denmark) were included between April 2014 and December 2020. Clinical data were obtained from the national quality sarcoma database and biomarkers other than hs-CRP were obtained from the clinical laboratory information system. The study cohort consisted primarily of patients with localised sarcoma. hs-CRP was significantly higher in patients with bone sarcoma (P=0.022) and soft tissue sarcoma (STS; P<0.001) compared with control patients. For STS, grade III tumours but not metastatic disease were associated with a higher hs-CRP level (P=0.0001). Elevated hs-CRP levels were associated with increased overall mortality [hazard ratio (HR), 1.91; 95% CI, 1.33-2.75; P=0.001]. Furthermore, elevated hs-CRP levels were also associated with decreased progression-free survival (HR, 1.64; 95% CI, 1.17-2.29; P=0.004). Furthermore, for patients with hs-CRP <8 mg/l, higher hs-CRP was associated with an increased risk of recurrent disease and reduced overall survival compared with those of patients with low hs-CRP. In conclusion, the present study demonstrated that hs-CRP was a prognostic factor for overall mortality and progression-free survival in patients with localised sarcoma at the time of diagnosis. Further studies are required to investigate the mechanism behind the association between hs-CRP and sarcoma prognosis and its potential use in clinical practice.

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Introduction

Immunotherapy has transformed cancer treatment in less than a decade. It is used primarily in lung cancer and melanomas, significantly improving overall survival for patients suffering from these cancers (1,2). However, for sarcoma patients, the responses to checkpoint inhibitors have been disappointing, with an objective response rate of only about 4% and progression-free survival of 2.4 months (3). It is proposed that sarcomas possess immune-evading mechanisms, which are not addressed by existing immunotherapeutic drugs. Therefore, understanding the connection between the immune system and sarcomas is crucial in developing improved immunotherapy approaches for sarcoma patients.

One of the earliest and most robust responses to inflammatory conditions is an increased C-reactive protein (CRP) level. CRP is an acute-phase reactant primarily synthesised in the liver and shed into the bloodstream as an early response to acute inflammation and, to a lesser extent, during chronic inflammation (4,5). However, it has been shown that small quantities of CRP are produced by other than liver cells, such as smooth muscle cells (6,7), epithelial cells (8), fat cells (9), and even by immune-modulating macrophages (10). Different cytokines stimulate the production of CRP, such as IL-6, IL-1 and tumour necrosis factor. CRP interacts with the vascular endothelium, contributes to monocyte-endothelium adhesion, increases reactive oxygen species, and triggers platelet aggregation, as shown in rodents (11). Furthermore, CRP binds to lysophosphatidylcholine on the surface of dead or dying eukaryotic cells and bacteria, activating the complement system and an essential player in the innate immune response (12). *In vitro* studies have shown that CRP inhibits the proliferation and activation of T-cells and is, therefore, believed to play a part in both the innate and adaptive immune response (13).

It has been shown that conventional measurement of CRP, where a clinical cut point of 8 mg/l has been used, is a prognostic factor for patients with metastatic soft tissue sarcoma (14,15) and localised soft tissue sarcoma (16,17) and

bone sarcoma (18,19). However, conventional CRP measurements are limited by the sensitivity to detect low values of CRP.

The high-sensitivity C-reactive protein (hs-CRP) quantifies CRP using an assay with a very low detection level. This allows for detecting and graduating low-grade inflammatory states such as obesity, diabetes, and cardiovascular disease (20,21). Hs-CRP is elevated in patients with soft tissue sarcoma (STS) compared to patients without sarcoma, and it has been proposed that it can be a diagnostic marker (22).

Macrophage-related biomarkers (sCD163 and SIRPα) were shown to be prognostic for overall survival and add prognostic values to a model containing known prognostic factors such as grade and age (23). However, the prognostic value and the association of hs-CRP levels and other inflammatory parameters have not been investigated in sarcoma patients.

This study investigates the association between serum hs-CRP and mortality among patients with primarily localised sarcoma.

Materials and methods

Study cohort. This prospective, non-randomised, non-interventional explorative study investigates the prognostic value of hs-CRP in sarcoma patients. Patients referred to the sarcoma centre of Aarhus University Hospital, Denmark, between April 2014 and December 2020 were included in the present study. All patients signed an informed consent form before inclusion. The inclusion criteria were age (18 years or older) and willingness to donate a blood sample. Several patients were diagnosed with conditions other than sarcoma and served as a control group in this study. This control cohort was chosen because they were referred to the sarcoma centre with the suspicion of having a sarcoma. The control groups also included patients with desmoid tumour which is a local aggressive benign tumour and therefore not regarded as a sarcoma. However, these patients include patients with benign tumours such as lipomas or unspecific tissue reactions, to mention a few. After inclusion and following the national guidelines, patients were diagnosed and treated by an experienced multidisciplinary sarcoma team. Most patients were diagnosed with localised grade II and III STS and treated with surgery with or without pre- or postoperative radiation therapy. We have previously published results on immune suppressive macrophages using the same patient cohort (23).

Data sources. Clinical data were obtained from the national quality sarcoma database, which contains comprehensive clinical information on individual sarcoma patients since 2009 in Denmark. The patients' records were reviewed to fill in missing values.

Biomarkers other than hs-sensitive CRP were obtained from the clinical laboratory information system (LABKA) database, which reports all blood tests taken according to the international nomenclature, properties, and Units (NPU) coding system. The values selected for analysis were the biomarkers closest to the day of the sarcoma diagnosis. LABKA results were included from 60 days before sarcoma diagnosis until 60 days after. Monocyte count, C-reactive protein (CRP), albumin, leucocyte count, and neutrophil count were selected for inclusion in this study. Each biomarker was categorised into normal or high/low according to the reference value at Aarhus University Hospital. High monocyte count was defined as $\geq 0.7 \times 10^9$ cells/l; low albumin levels were defined as <36 g/l. Elevated CRP was defined as values ≥8 mg/l; elevated leucocyte count was defined as values $\geq 10 \times 10^9$ cells/l, and high neutrophil count was defined as values $\geq 7 \times 10^9$ cells/l.

Hs-sensitive CRP measurements. Before initiating the treatment, 30 ml of peripheral blood was collected in sodium citrate tubes and centrifuged at 2,000 or 2,500 g for ten minutes. The plasma was isolated and stored at -80˚C until measurement according to the instructions by Danish Cancer Biobank, Bio- and GenomeBank, Denmark. Hs-sensitive CRP was measured with a chemiluminescent immunometric assay using the Atellica CH (Siemens, Germany). The upper limit of normal for hs-CRP is in our institution 3.0 mg/l, which is based on a Danish population. The hs-CRP values were used to allocate patients into high and low hs-CRP groups based on the medium value of hs-CRP.

Prognostic profile. A predictive profile was created as described in our previous publication (23). In short, sCD163 and sSIRPα were divided into low or high groups based on their median serum concentration. CRP was separated into two categories, low or high, based on a threshold of 8 mg/l. Each categorical variable was assigned a score of 1 or 2, while the grade was assigned a score of 1, 2, or 3, depending on grade. The sum of all the assigned scores made up the final profile. The profile was then divided into three risk stratification groups: low-risk (score 4-5), intermediate risk (score 6-7), and high-risk (score 8-9). This profile is named profile 1 (23). In this paper, hs-CRP replaced the normal CRP measurement named profile 2.

Statistical analysis. Clinical data and information on biomarkers were linked by the unique 10-digit civil personal registration (CPR) number, allowing for individual linkage between different reporting systems.

Patient, tumour, and treatment-related variables were reported as frequencies, percentages or continuous variables expressed as medians with interquartile range (IQR). The variables were compared using the chi-squared, Fisher's exact test or Wilcoxon rank-sum test, depending on the variable in question, and stratified by the median value of the hs-CRP in the STS patients. When the median value of multiple groups was compared, the Kruskal Wallis test was used with the Dunn's test for multiple comparisons when significant results were obtained. The median values of hs-CRP were used to categorise patients into a high and low hs-CRP group.

The primary endpoints were recurrent disease and overall survival (OS). Time to recurrent disease was defined as the interval between the primary diagnosis and the first recurrent, local or metastatic. OS was defined as the time from the date of diagnosis until the death of any causes. Kaplan-Meier curves were used to visualise survival and the log rank test was used for comparison of groups. The study period ended on October 15th 2022, and patients alive at this date were censored. Crude (univariate) and adjusted (multivariate) analyses were performed by using the Cox proportional hazard model. A test for proportional hazard was used before including an

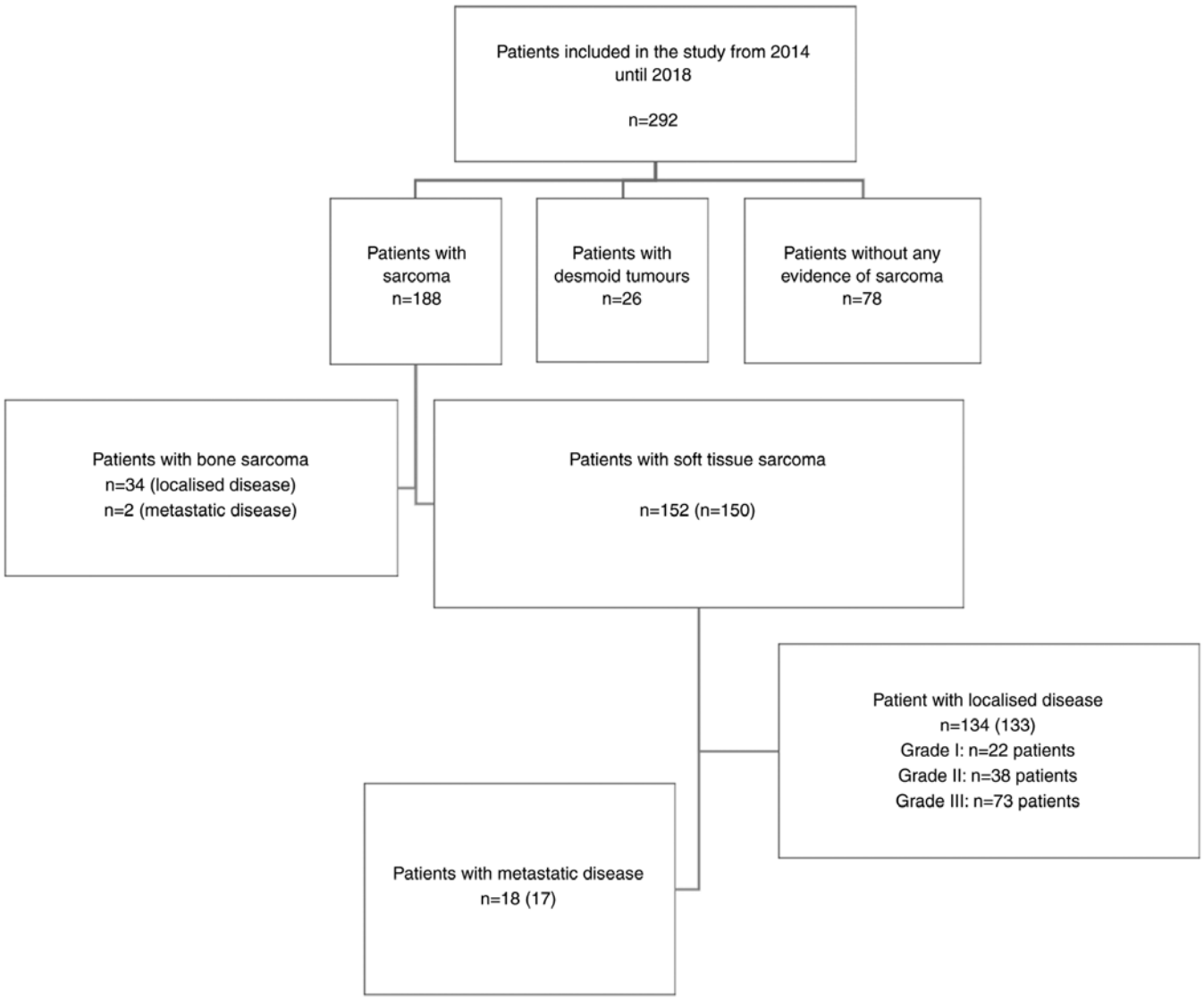

Figure 1. Patient selection according to histological subtype, tumour grade and missing value of hs-CRP. The number indicated in the parentheses is the number of patients with a hs-CRP measurement. hs-CRP, high-sensitivity C-reactive protein.

additional variable in the analysis. Based on the literature, the following variables were included in the adjusted analysis: age, size of the primary tumour, and grade. Tumour size and age were included as continuous variables; all other variables were analysed as categorical. ROC curve for cut point determination using the common criteria with the point on the ROC curve where the sensitivity and specificity of the test are equal (24). Akaike's information criteria (AIC) and Harrell's concordance index determined the model with the minimum AIC values, regarded as the best model. Likelihood-ratio tests were used to evaluate whether the addition of a potential prognostic profile contributed significantly to the models' prognostic value. A Two-sided $P<0.05$ was considered statistically significant. All analyses were performed using Stata (version 17.1) software.

Results

Demographic data. A total of 292 patients were included in this study. The majority of patients were diagnosed with sarcoma (n=188: STS=152, bone sarcoma=36), compared to patients with desmoid fibromatosis (n=26) and patients diagnosed with conditions other than sarcoma or desmoid fibromatosis and therefore defined as control patients (n=78) (Fig. 1).

A significantly higher median hs-CRP was observed in patients with STS or bone sarcoma compared to patients with desmoid tumours and control patients (Table I). For STS, grade III tumours were associated with a higher hs-CRP level ($P=0.0001$), whereas patients with metastatic disease did not have a higher hs-CRP level than those with localised disease. The median hs-CRP values with a 95% confidence interval (CI) are depicted for each subtype of STS, bone sarcoma, desmoid tumours, and the control group in Fig. 2. The median hs-CRP level varies between the different histologic subtypes, with undifferentiated pleomorphic sarcoma having the highest levels. The difference between subtypes is significant compared to the control group and between UPS and the other histological groups.

The diagnostic value of hs-CRP was evaluated by comparing patients with localised sarcoma (bone and soft tissue sarcoma) with patients without sarcoma disease (desmoid fibromatosis

Table I. Median concentration of hs-CRP for different subgroups at diagnosis.

Clinical variables	No.	CRP, mg/l Median	CRP, mg/l Range	Kruskal Wallis test P-value	Dunn's test P-value
Histology				0.0004	
Control	78	1.09	0.20-8.51		
Desmoid tumours	26	0.68	0.20-23.11		0.340[a]
Bone sarcoma	34	2.16	0.23-20.36		0.018[a]
Soft tissue sarcoma (localized only)	133	2.19	0.28-71.69		<0.001[a]
Soft tissue sarcoma				0.0001	
Grade I	22	1.27	0.23-5.42		
Grade II	38	1.34	0.26-19.75		0.220[b]
Grade III	73	3.43	0.46-109.17		<0.001[b]; <0.001[c]
Soft tissue sarcoma				0.9244	
Localised	133	2.23	0.28-71.69		
Metastatic	17	1.53	0.21-38.78		

[a]Compared with the control group; [b]Compared with grade I; [c]Compared with grade II. The range is reported as the 5-95th percentile. The Kruskal-Wallis test was used with Dunn's test for multiple comparisons when significant results were obtained. hs-CRP, high-sensitivity C-reactive protein.

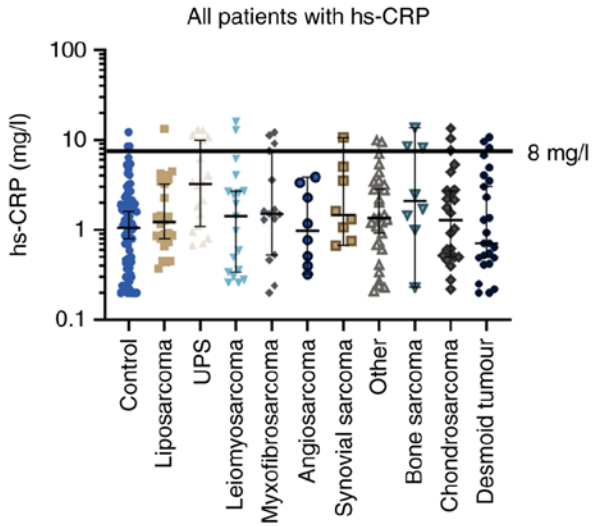

Figure 2. Median hs-CRP value in mg/l according to histological subtype and for control patients. The median value is shown with a 95% confidence interval. The comparisons among different groups were performed using the Kruskal-Wallis test with Dunn's test as post hoc analysis. For patients with liposarcoma (P<0.0001), UPS (P<0.0001), myxofibromsarcoma (P=0.02), other (P=0.01) and bone sarcoma (P=0.02) a significant difference was observed compared with control patients. For patients with UPS compared with the other histological groups, a significant difference was observed for liposarcoma (P=0.003), myxofibrosarcoma (P=0.04), angiosarcoma (P=0.01), others (P=0.007), chondrosarcoma (P=0.005) and desmoid tumours (P<0.001). Hs-CRP, high-sensitivity C-reactive protein; UPS, undifferentiated pleomorphic sarcoma.

and control patients). Fig. 3 shows the ROC curve with the sensitivity and specificity for hs-CRP. The area under the curve is 0.66 (95% CI: 0.59-0.73), and according to the ROC curve, the optimal cut point is 1.41 mg/l for local recurrence and 1.72 mg/l for overall survival.

Prognostic value of hs-CRP. Only patients with localised soft tissue sarcoma (n=133) were included in analysing the effect of hs-CRP.

Clinical and tumour characteristics for patients with localised STS are shown in Table II. Patients were divided into a high and a low hs-CRP group based on the median value (2.19 mg/l). For patients with a high level of hs-CRP, the tumours were more extensive, were of a higher grade, and more patients experienced relapse after curative treatment. Six patients with a high level of hs-CRP also had increased leucocyte and neutrophil cell count; only two patients had increased liver enzymes. For patients with a low hs-CRP, 76% (95% CI: 64-84%) had not experienced a relapse of disease after five years of follow-up compared to 45% (95% CI: 32-57%) of the patient with a high hs-CRP. The 5-year overall survival for patients with a low hs-CRP was 83% (95% CI: 72-90%) compared to 59% (95% CI: 46-70%) for patients with a high hs-CRP.

The univariate analyses are shown in Table III. Patient age, tumour size, tumour grade, CRP level, and neutrophile count are prognostic for recurrent disease, and patient's age, tumour size, tumour grade, CRP level, albumin level, and neutrophile count were prognostic for overall survival. The multivariate analysis showed that a high hs-CRP was an independent prognostic factor for recurrent disease with a hazard ratio of 1.90 (95% CI: 1.03-3.52) and overall survival with a hazard ratio of 2.20 (95% CI: 1.13-4.29).

All patients with localised soft tissue sarcoma treated with curative intent were included in the model selection analysis (n=133). The c statistics showed that adding hs-CRP to known prognostic factors such as grade, tumour size, and age significantly improved the prognostic model. Table IV shows the c-statistics for comparing different models. Replacing normal CRP with hs-CRP lowered the AIC from 414 to 411 when evaluating the risk of disease or recurrent disease. However,

Table II. Clinical, tumour and treatment characteristics of patients with localised soft tissue sarcoma and CRP measurements (n=133).

Characteristics	Total	Low CRP	High CRP	P-value
Sex, n (%)				
Male	59 (44)	29 (43)	30 (45)	0.801
Female	74 (56)	38 (57)	36 (55)	
Median age, years (p5-p95)	67 (27-85)	65 (25-84)	69 (47-85)	0.06
Histological subtype, n (%)				
Liposarcoma	32 (24)	15 (22)	17 (26)	0.792
UPS	23 (17)	8 (12)	15 (23)	
Leiomyosarcoma	17 (13)	9 (13)	8 (12)	
Myxofibrosarcoma	17 (13)	10 (15)	7 (11)	
Angiosarcoma	8 (6)	4 (6)	4 (6)	
Synovial sarcoma	7 (5)	4 (6)	3 (5)	
Others	29 (22)	17 (25)	12 (18)	
Median tumour size, cm (p5-p95)	6 (1-23)	5 (1-16)	8 (1-27)	<0.01
Tumour grade, n (%)				
Low	22 (17)	16 (24)	6 (9)	0.002
Intermediate	38 (29)	23 (34)	15 (23)	
High	73 (55)	28 (42)	45 (68)	
Treatment, n (%)				
Surgery	132 (99)	67 (98)	65 (100)	0.496
Radiation therapy	50 (38)	21 (31)	29 (44)	0.154
Treatment intent, n (%)				
Curative[a]	132 (99)	67 (100)	65 (98)	0.312
Relapse, n (%)				
Yes	51 (39)	17 (25)	34 (52)	0.001
No	82 (61)	50 (75)	32 (48)	

[a]1 patient had local disease but was not treated with curative intent. The χ^2 test and Fisher's exact test were used to compare category variables. Fisher's exact test was used if the expected count in >20% of the cells of the analysed contingency table was 5 or fewer. The Wilcoxon rank sum test was used for continuous variables. CRP, C-reactive protein; p5-p95, 5-95th percentile; UPS, undifferentiated pleomorphic sarcoma.

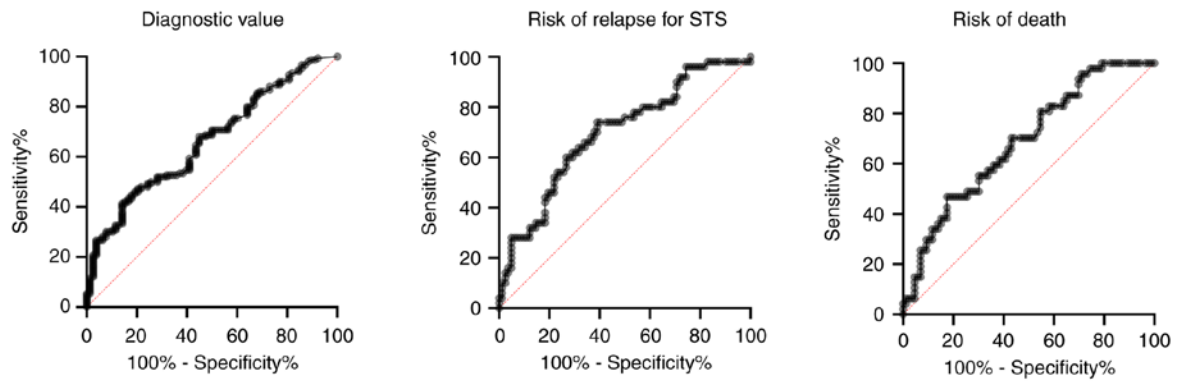

Figure 3. Diagnostic value hs-CRP in all patients with localised sarcoma (n=167) combined with all patients with benign conditions (n=104), including patients without sarcoma and desmoid tumours. All patients with metastatic disease were excluded from the analysis. The risk of relapse includes all patients (n=133) with localised STS at the time of diagnosis. The risk of death included all patients (n=133) with localized STS at the time of diagnosis. hs-CRP, high-sensitivity C-reactive protein; STS, soft tissue sarcoma.

when evaluating the ability to predict overall survival, AIC was unchanged (AIC=322 when CRP was included and AIC=323 when hs-CRP was included). Moreover, a model containing the macrophage markers sCD163 and sSIRPα significantly improved the model, including hs-CRP adjusted for age, tumour size, and grade (P=0.0021).

Table III. Univariate analyses in patients with localised soft tissue sarcoma (n=133) using Cox regression analysis.

	Risk of relapse			Overall survival		
Characteristics	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Age, years	1.03	1.01-1.05	0.007	1.06	1.03-1.09	<0.001
Sex						
Female	1			1		
Male	1.21	0.68-2.16	0.513	1.22	0.67-2.21	0.509
Size, cm	1.04	1.01-1.08	0.018	1.05	1.01-1.08	0.011
Grade						
I	1			1		
II	1.17	0.35-3.90	0.793	2.96	0.64-13.75	0.165
III	4.26	1.52-11.97	0.006	8.22	1.97-34-39	0.004
Serum biomarkers						
Monocytes count, $x10^9$ cells/l	1.09	0.55-2.16	0.796	1.60	0.83-3.10	0.159
hs-CRP, mg/l	2.74	1.52-4.92	0.001	2.62	1.40-4.90	0.002
CRP, mg/l	1.95	1.08-3.51	0.027	2.22	1.19-4.12	0.012
Albumin, g/l	1.60	0.82-3.15	0.165	2.06	1.03-4.09	0.040
Leucocyte count, $x10^9$ cells/l	1.39	0.65-2.98	0.395	2.02	0.99-1.12	0.054
Neutrophil count, $x10^9$ cells/l	2.29	1.10-4.78	0.028	3.06	1.53-6.13	0.002

Monocytes count was categorized into normal ≤$0.7x10^9$ cells/l and high >$0.7x10^9$ cells/l levels. CRP was categorized into normal ≤8 mg/l and high >8 mg/l levels. Albumin was categorized into normal ≥36 g/l and low <36 g/l levels, Leucocyte count was categorized into normal ≤$10x10^9$ cells/l and high >$10x10^9$ cells/l levels. Neutrophil count was categorized into normal ≤$7x10^9$ cells/l and high >$7x10^9$ cells/l levels. hs-CRP, high-sensitivity C-reactive protein.

Table IV. Model fitting.

Prognostic models	Relapse		Survival	
Model	AIC	C-index	AIC	C-index
Grade	456	0.66	397	0.67
Age	466	0.61	392	0.70
Tumour size	468	0.63	409	0.65
hs-CRP	461	0.62	404	0.64
Grade + hs-CRP	451	0.79	394	0.71
Grade + age + hs-CRP	448	0.73	373	0.77
Grade + age + tumour size + hs-CRP	446	0.74	369	0.79
Grade + age + tumour size	448	0.73	373	0.78
Grade + age + tumour size + hs-CRP + sCD163 and sSIRPα	450	0.74	362	0.79

AIC, Akaike's information criteria; hs-CRP, high-sensitivity C-reactive protein; C-index, concordance index; sCD163, soluble CD163; sSIRPα, soluble signal regulatory protein α.

Analyses of patients with a hs hs-CRP <8 mg/l. One hundred patients had a hs hs-CRP <8 mg/l, the reference level defining elevated CRP levels in Denmark. In a subgroup analysis including only patients with hs-CRP <8 mg/l, patients were divided into a high and low group based on the median hs-CRP value (1.32 mg/l). Age and tumour size were associated with high levels of hs-CRP. Again, high hs-CRP was associated with an increased risk of recurrent disease as the 5-year recurrence-free survival for patients with a hs-CRP below the median value was 74% (95% CI: 57-85%) compared to patients with a hs-CRP above the median value 62% (95% CI: 48-73%) see Fig. 4. Furthermore, a higher hs-CRP was associated with a reduced overall survival demonstrated by the 5-year overall survival for patients with a hs-CRP below the median value: of 85% (95% CI: 69-93%) compared to patients with a hs-CRP above the median value: 72% (95% CI: 59-82%) see Fig. 5.

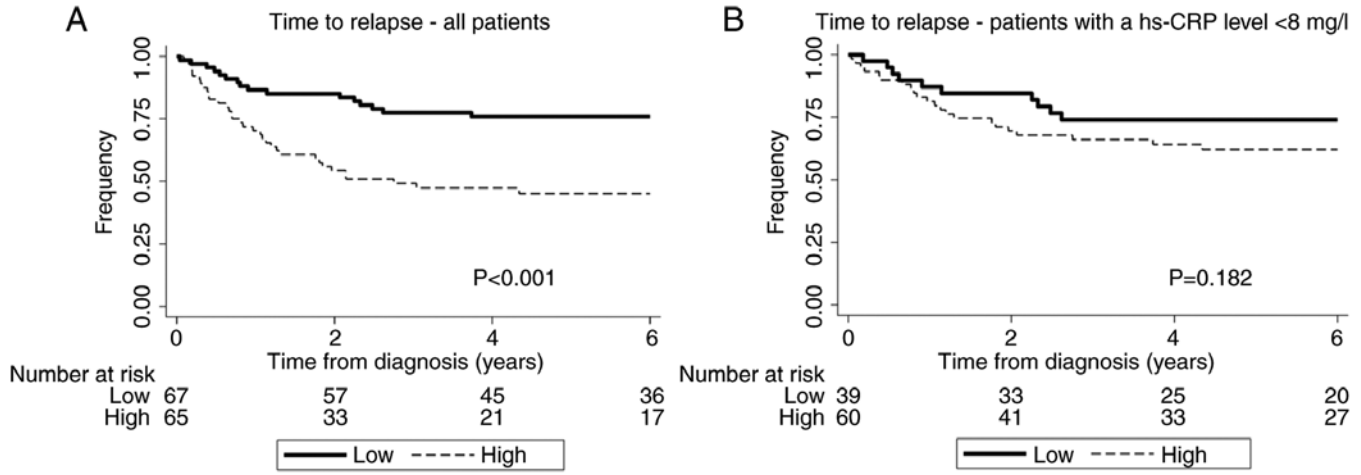

Figure 4. Time to relapse for patients with localised soft tissue sarcoma at the time of diagnosis. (A) All patients with a hs-CRP measurement. (B) Patients with a hs-CRP level <8 mg/l. The log-rank test was used to compare groups. hs-CRP, high-sensitivity C-reactive protein.

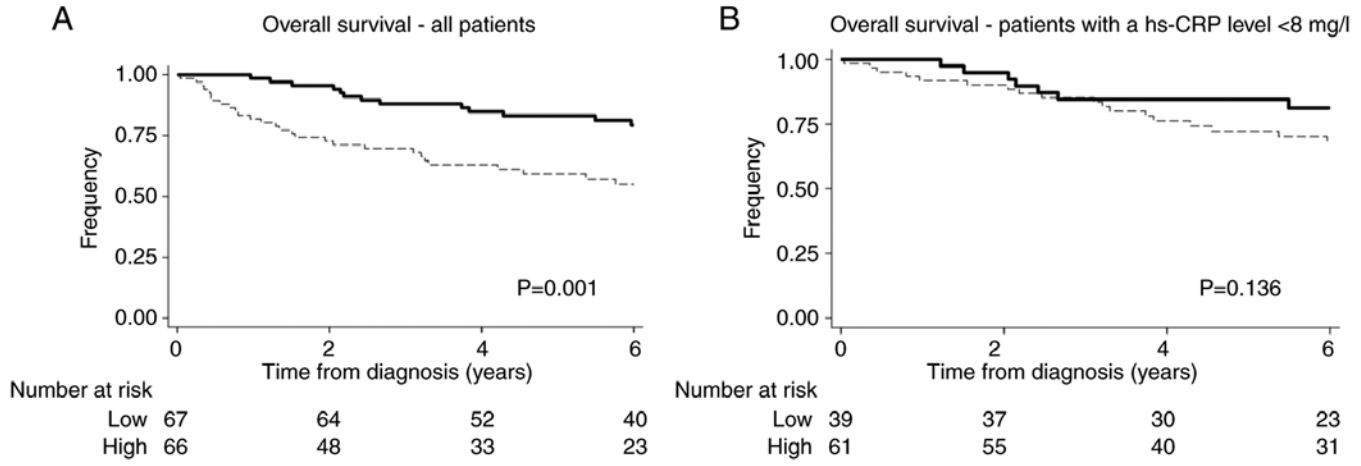

Figure 5. Overall survival for patients with localised soft tissue sarcoma at diagnosis. (A) All patients with hs-CRP measurements. (B) Patients with a hs-CRP level <8 mg/l. The log-rank test was used to compare groups. hs-CRP, high-sensitivity C-reactive protein.

Discussion

This study investigated the association between serum high-sensitivity C-reactive protein (hs-CRP) and progression-free survival in patients with localised sarcoma treated with curative intent. The results showed that hs-CRP is an adverse prognostic factor for progression-free survival in these patients, even after adjusting for known prognostic factors.

Several studies have investigated the relationship between hs-CRP and the presence of cancer. In this article, the optimal cut-off for the prognostic values of hs-CRP was determined as the median values and not the cut-off based on the ROC curve. The ROC curve cut-off is based on the endpoint investigated and, therefore, this can only be applied when using a test and validation cohort. Other studies used the median value.

A study by Lee *et al* (25) found that hs-CRP levels are significantly higher in different cancer patients than in healthy controls, with a median hs-CRP of 0.77 and 0.59 mg/l for men and women, respectively. Nakamura *et al* (22) found a threshold level of 0.95 mg/l for discriminating between sarcoma and healthy control patients. These results are in alliance with our findings. However, the median level in our study was higher than that reported by Lee *et al* (25) and Nakamura *et al* (22). This difference could be due to the body mass index (BMI) being higher in Caucasians than the Asian participants (25). We did not have information about BMI in our study. Nevertheless, this is supported by Cho *et al* (26), who found higher hs-CRP among breast cancer survivors with a larger body mass index than patients with a lower BMI.

Lee *et al* (25) found that a one mg/l increase in hs-CRP was associated with increased mortality after a 17-year follow-up, but the association was found only in women. Our study did not show any difference in the CRP levels between sexes; however, we observed that an increase in CRP by one mg/l led to a 2.69 increased risk of dying (median follow-up time was 6.9 years). Similarly, in the study by Nakamura *et al* (22), hs-CRP levels were associated with poor prognosis and decreased survival in patients with soft tissue sarcoma. All these results suggest a relationship between hs-CRP and sarcoma.

Desmoid tumours included in this study represent locally aggressive neoplasms that do not metastasise but are challenging to treat. As for other cancers, the tumour immune

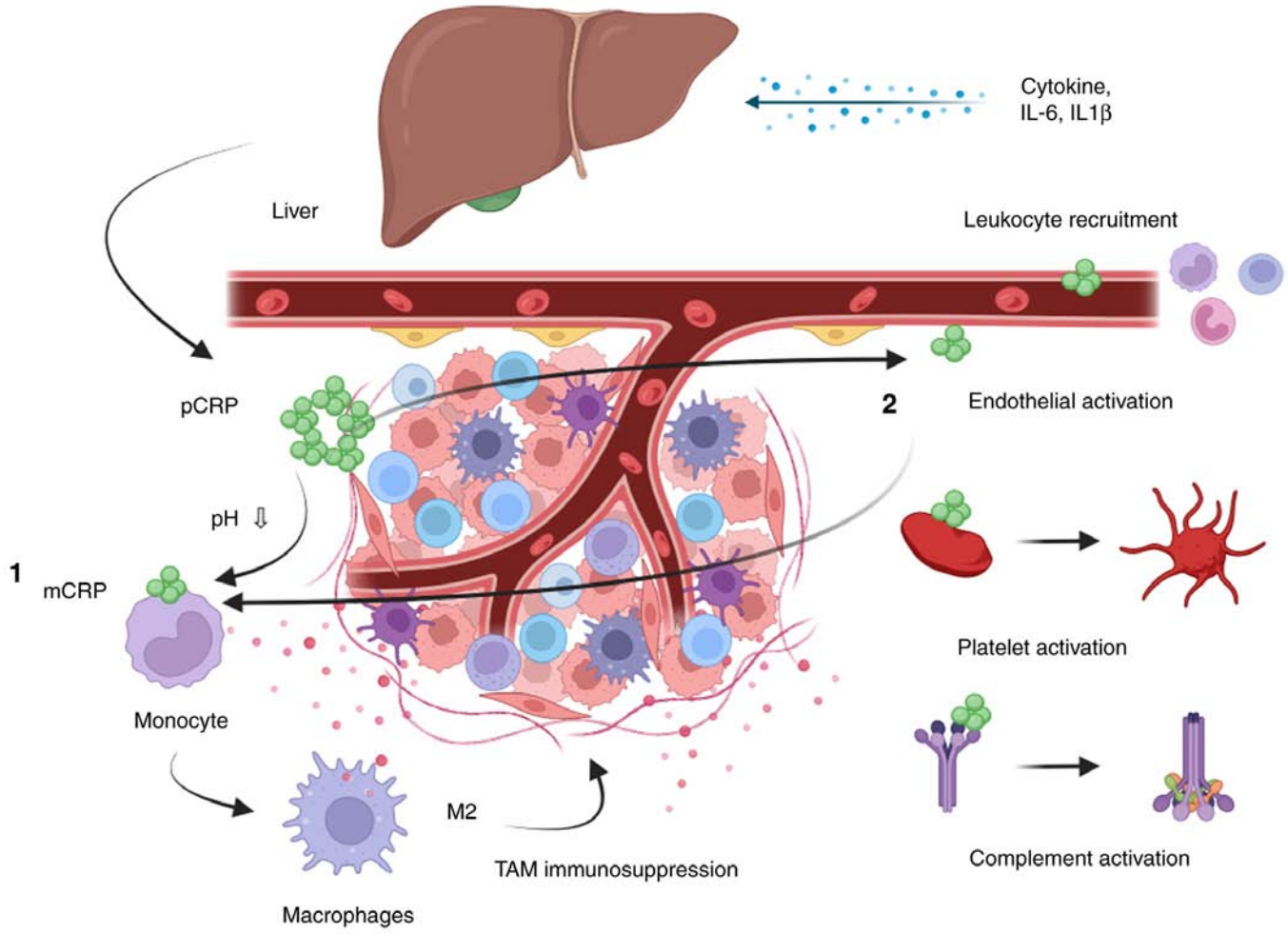

Figure 6. Illustration of the proposed role of CRP and the tumour microenvironment. The liver produces pCRP as a result of inflammatory cytokines, such as IL-6 and IL1β, excreted from cancer cells. pCRP is dissociated into mCRP on the surface of different cells, such as monocytes (1) and endothelial cells (2), through the binding to a ligand called phosphocholine. 1, mCRP together with other inflammatory markers might promote the production of immune-inhibitory TAMs called M2-directed macrophages. 2, The recruitment and activation of M2-directed TAMs could also be due to the endothelial activation by CRP. The endothelial activation might facilitate leukocyte recruitment, including recruitment of monocytes to the tumour. The recruitment of monocytes to the tumour increases and promotes the development into TAMs (M2 directed). Furthermore, platelet and complement activation are facilitated, which are essential in the innate immune response. CRP, C-reactive protein; mCRP, monomeric CRP; pCRP, pentamer of CRP; TAM, tumour-associated macrophage.

microenvironment (TME) is essential when considering immunotherapy. One study investigated the immune expression markers of 33 tissue samples from patients suffering from desmoid tumours. This study concluded that desmoid tumours have a solid immune infiltration in the tumour margins; however, PD-L1 was not present in the tumour cells. PD-L1 is a target for immunotherapy (27). In our study, the level of hs-CRP was not elevated compared to the control group, indicating that the inflammation in these patients may not play a pivotal role.

Several mechanisms have been proposed to explain the link between CRP and cancer. One mechanism involves the role of inflammation in promoting tumour initiation and progression (28,29). CRP is a marker of systemic inflammation, and chronic inflammation has been linked to several types of cancer, including sarcoma (15,17,18). In addition, an *in vivo* experiment has shown that CRP influences the tumour microenvironment by promoting suppressive tumour-associated macrophages (30). These macrophages promote angiogenesis, an essential process for tumour growth and metastases (31). Another proposed mechanism involves the role of CRP as a marker of immune system dysfunction (13).

The strengths of this study include its prospective design, large sample size and the use of a highly sensitive assay to measure hs-CRP, which allowed for the detection of low levels of inflammation. In addition, the comprehensive clinical data and biomarker measurements allowed for the adjustment of potential confounders in the analysis. Patients suspected of having sarcoma but who turned out to have a benign condition comprise the control group in this study. It is a strength in this study as the destining of sarcoma and non-sarcoma patients is essential. However, there are also some limitations to this study. First, the study evaluated soft tissue sarcoma patients as one collective group of patients, which could blur variations between different histological subtypes. Second, the study was not designed to investigate the underlying mechanisms linking hs-CRP and mortality in sarcoma patients, and further studies are needed to elucidate these mechanisms.

However, it is known that tumour-associated macrophages (TAM) are believed to be one of the major immunosuppressive components in cancers. The association between high levels of serum CRP and the number of tumour-associated macrophages of the subtypes CD204 and CD163 has been shown for patients with hepatocellular cancer (32). The activation of monocytes to immunosuppressive macrophages is believed to be facilitated by CRP along with the activation of endothelial cells, platelets and the complement system, which are important in the innate immune response leading to macrophage recruitment and activation (33). Fig. 6 is a proposed association between CRP and the innate immune system.

Additionally, it is essential to note that sarcoma is a rare and complex disease, and more research is needed to understand the underlying mechanisms fully and develop new treatments and therapies for sarcoma patients. Using biomarkers such as hs-CRP and inflammation can help improve the diagnosis, prognosis, and management of sarcoma, but it is crucial to use them in conjunction with other diagnostic tests and imaging studies to make a definitive diagnosis. Furthermore, integrating these biomarkers with other omics technologies can provide a comprehensive understanding of the disease and help identify new diagnostic and therapeutic targets.

In conclusion, High-Sensitive C-reactive protein and inflammation are important biomarkers where high levels of the individual biomarker are linked to more advanced stages of sarcoma and increased risk of mortality. These biomarkers can also influence a patient's response to therapy; therefore, treatment plans for sarcoma must be tailored to account for an individual inflammatory profile. More research is needed to understand the underlying mechanisms and develop new treatments and therapies for sarcoma patients.

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Availability of data and materials

The data that support the findings of this study are available from the Danish Regions but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Danish Ethical commission.

Authors' contributions

NAP, TBH, HJM and BSP conceived the study. NAP and BSP wrote the original draft. NAP, TBH, HJM and BSP wrote, reviewed and edited the manuscript. BSP and NAP confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The regional Ethics Committee of Denmark (approval no. 1-10-72-58-14; Region Midtjylland, Viborg, Denmark) and the Danish Agency of Data Protection (approval no. 1-16-02-112-14; Valby, Denmark) have approved the study. Before obtaining the blood samples, written informed consent was obtained from the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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