

# Serum C-reactive protein and overall survival of patients with osteosarcoma

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Received: 1 July 2014 / Accepted: 9 February 2015 / Published online: 19 May 2015  
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**Abstract** Increased level of serum C-reactive protein (CRP) has been identified as an important prognostic factor in several types of cancers. However, the prognostic significance of serum CRP levels in patients with osteosarcoma was still unclear. A retrospective cohort study of 85 patients was performed to assess the prognostic significance of serum CRP level in osteosarcoma. Both log-rank test and multivariable analysis by Cox regression model were used to assess the impact of serum CRP levels on the overall survival in patients with osteosarcoma. Among those 85 patients, 28 (32.9 %) had high serum CRP level ( $>1$  mg/dL), while the other 57 (67.1 %) patients had normal serum CRP level ( $\leq 1$  mg/dL). There was no obvious difference in the baseline characteristics between high CRP group and normal CRP group. Kaplan-Meier product-limit method showed that patients with high serum CRP levels had significantly poorer overall survival than those patients with normal serum CRP levels (log-rank test  $P=0.0008$ ). Multivariable analysis by Cox regression model further showed that high serum CRP level was an independent predictor of poor overall survival (hazard ratio [HR]=2.39; 95 % confidence interval [95 % CI] 1.22–4.67,  $P=0.01$ ). Thus, serum CRP level has an important prognostic significance in patients with osteosarcoma, and high CRP level is associated with worse overall survival.

**Keyword** Osteosarcoma · C-reactive protein · Prognosis

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## Introduction

Osteosarcoma is the most common primary malignant bone tumor [1, 2]. Though there are many advances in the surgical resection and adjuvant chemotherapy for osteosarcoma in recent years, the prognosis for osteosarcoma patients has not been obviously improved [3]. A study from Norway showed that there was no improvement in the overall survival for osteosarcoma since the 1990s, and the survival rates were still poor for elderly people or patients with metastatic diseases [2]. Previous studies have suggested that patients with poor histological response to chemotherapy or advanced stages usually have worse prognosis [4–6]. It is no doubt that identification of prognostic factors to choose starting and reasonable therapy is very helpful to improve patients' survival. C-reactive protein (CRP) is an important acute phase protein, and it is produced mainly by the hepatocytes [7, 8]. Currently, there is a strong evidence for important roles of CRP in the development of chronic infections and autoimmune diseases [7, 8]. Previous studies suggest that patients with cancer often have increased levels of circulating CRP, and increased level of serum CRP has been proven to be an important prognostic factor in several types of cancers including colorectal cancer and breast cancer [9–11]. However, the prognostic significance of serum CRP level in patients with osteosarcoma was still unclear. The aim of this retrospective cohort study was to investigate whether serum CRP level was a predictor of overall survival in patients with osteosarcoma.

## Methods

### Study design and patients

A retrospective cohort study of 85 patients (average age, 20 years) was designed and performed to assess the prognostic

significance of serum CRP levels in patients with osteosarcoma. From July 2004 to July 2006, a total of 136 osteosarcoma patients were initially enrolled in the retrospective study. Fifty-one patients were excluded because their pretreatment CRP levels were not available. Information on patient and tumor characteristics, such as age, gender, stage, histological grade, and CRP levels, were obtained from the databases of our hospital. Follow-up information, including the cause of mortality, was obtained through a review of clinical notes or contact with patients' family. The Ethics Committee of our hospital approved the study. Written informed consent was obtained from all of the patients according to the guidelines approved by the Institutional Research Board of the hospital. The stage classification was defined by the Enneking Staging System [12]. For response to adjuvant chemotherapy, a "good response" was classified as a tumor showing 90 % or more necrosis, while "poor response" was classified as less than 90 % tumor necrosis in response to chemotherapy.

### Serum CRP

The blood of osteosarcoma patients was obtained before tumor resection through venous puncture. Using standardized phlebotomy procedures, up to 30 ml of peripheral blood was drawn from each of the patients. The blood samples were temporarily stored at 4 °C. Immediately after the blood was centrifuged, serum samples or the supernatant were frozen and stored at -80 °C until use. CRP measurements were not expressly ascertained for study purposes but performed as part of a preoperative clinical routine by a latex-enhanced immunoturbidimetric test according to the manufacturer's instructions. A serum CRP level less than 1 mg/dL was regarded normal corresponding to our clinical routine practice. When investigating the correlation between the levels of serum CRP with the prognosis of osteosarcoma patients, the pretreatment serum CRP values were classified into CRP  $\geq 1$  and  $< 1$  mg/dL groups.

### Statistical analysis

The primary endpoint in present study was overall survival. Overall survival was calculated as the period from diagnosis to death or last follow-up, and non-tumor-related death was also included into censored. Analyses were performed to assess the relationships between serum CRP levels, baseline clinical factors, and overall survival of osteosarcoma patients. The  $\chi^2$  or Fisher's exact test was used to compare relationships between serum CRP levels and baseline factors. Kaplan-Meier product-limit methods and the log-rank test were used to estimate overall survival distribution and test the difference. Multivariate survival analysis of the group variables was performed using the Cox proportional hazard model to validate the prognostic role of serum CRP levels on overall survival by

adjusting for baseline factors, and the corresponding *P* value had to be more than 0.10 when removing a variable from the model. A two-tailed *P* value less than 0.05 was considered statistically significant and was determined using STATA 12.0 software.

## Results

### Patient characteristics

Of the 85 patients, 43 patients (50.6 %) were male and 42 patients (49.4 %) were female. Of those 85 patients, 44 (51.8 %) had tumor size more than 10 cm and 41 (48.2 %) had tumor size less than 10 cm. As for Enneking stage, 24 patients were stage I, 24 patients were stage II, and 37 patients were stage III. In addition, 37 patients had metastatic diseases (43.5 %). Among those 85 patients, 28 (32.9 %) had high serum CRP level ( $> 1$  mg/dL), while the other 57 patients had normal serum CRP level ( $< 1$  mg/dL). In those patients included into the study, only 60 patients provided information on whether they used or did not use chemotherapy (Table 1). Osteosarcoma affected regions around the knee in 55 patients, 9 patients around the hip, 10 patients at the shoulder, and 11 patients in the other bones. Table 1 showed the associations

**Table 1** Association between pretreatment CRP levels and baseline characteristics of osteosarcoma patients

Variables	Groups		<i>P</i> value
	Group 1 (CRP $\geq 1$ mg/dL) (n=28)	Group 2 (CRP $< 1$ mg/dL) (n=57)	
Age, years			
<20	10	26	0.32
$\geq 20$	18	29	
Gender			
Male	13	30	0.59
Female	15	27	
Tumor size			
<10 cm	13	31	0.49
$\geq 10$ cm	15	26	
Enneking stage			
I	5	19	0.07
II	6	18	
III	17	20	
Response to chemotherapy			
Good responders	7	24	0.19
Poor responders	13	16	
Unclear	8	17	
Metastatic diseases			
No	11	37	0.03
Yes	17	20	

CRP C-reactive protein

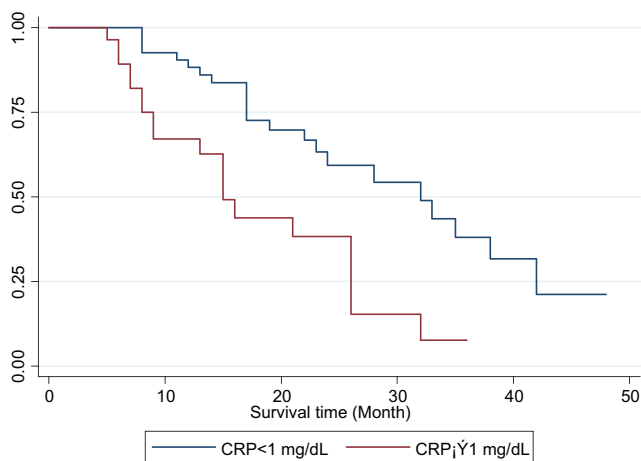
between pretreatment CRP levels and baseline characteristics of osteosarcoma patients. For the 28 patients with high serum CRP level ( $>1$  mg/dL), 60 % of those patients had metastatic diseases ( $P=0.03$ , Table 1).

#### CRP levels and osteosarcoma prognosis

Kaplan-Meier product-limit method and log-rank test showed that patients with high serum CRP level had significantly worse overall survival than those patients with normal serum CRP level ( $P=0.0008$ , Fig. 1). The 5-year overall survival rates in patients with or without elevated CRP levels were 36 and 15 %, respectively. Multivariable analysis by Cox regression model further showed that high serum CRP level was a significantly independent predictor of overall survival (hazard ratio [HR]=2.39; 95 % confidence interval [95 % CI] 1.22–4.67,  $P=0.01$ ) (Table 2). In addition, tumor size ( $\geq 10$  cm) and poor response to chemotherapy were also independent predictors of overall survival in patients with osteosarcoma (Table 2).

#### Discussion

In the present retrospective cohort, a total of 85 patients with osteosarcoma were included, 28 (32.9 %) had high serum CRP level ( $>1$  mg/dL), while the other 57 (67.1 %) patients had normal serum CRP level ( $\leq 1$  mg/dL). The prognostic significance of serum CRP level in patients with osteosarcoma was identified in both log-rank test and multivariable analysis by Cox regression model. In the multivariable analysis by Cox regression model, high serum CRP level was a significantly independent predictor of overall survival (HR=2.39; 95 % CI 1.22–4.67,  $P=0.01$ ). Thus, the results in the study show that high serum CRP level is a significantly independent predictor of worse overall survival in osteosarcoma patients.



**Fig. 1** Kaplan-Meier curve for overall survival of 85 patients with osteosarcoma according to serum C-reactive protein (CRP) levels

**Table 2** Multivariable analysis by Cox regression model in the retrospective cohort study

Factors	Adjusted estimates <sup>a</sup>		P values
	HR	95%CI	
Age ( $\geq 20$ years)	1.64	0.82–3.30	0.16
Gender (male)	0.69	0.35–1.36	0.28
Tumor size ( $\geq 10$ cm)	2.48	1.07–5.74	0.03
Enneking stage	1.25	0.74–2.12	0.40
CRP levels ( $\geq 1$ mg/dL)	2.39	1.22–4.67	0.01
Poor response to chemotherapy	1.56	1.04–2.34	0.03
Metastatic diseases	2.18	0.98–4.82	0.06

HR hazard ratio, 95 % CI 95 % confidence interval

<sup>a</sup> Variables included age, gender (male), tumor size ( $\geq 10$  cm), Enneking stage, grade, CRP levels, and metastatic diseases

Osteosarcoma is the most frequent and primary malignant bone tumor [1, 13]. It is no doubt that identification of prognostic factors to choose starting and reasonable therapy can predict and improve patients' survival [3, 14]. In the present retrospective cohort, the prognostic significance of serum CRP level in patients with osteosarcoma was successfully identified. For those osteosarcoma patients with high serum CRP level, more adjuvant treatments may be necessary and can be added to improve the survival [4, 5, 14].

CRP is an important acute-phase protein, and it is produced mainly by the hepatocytes, which is a response to body's inflammation [7, 8]. Currently, there is a strong evidence for the elevated levels of CRP in patients with chronic infections and autoimmune diseases [7–9]. In addition, elevated levels of CRP had been found in patients with cancers [9–12, 15]. Previous studies also showed that elevated levels of CRP were associated with decreased survival and increased risk of mortality in various cancers [16–22]. However, there are few studies performed to assess the association between CRP levels and prognosis of osteosarcoma patients. Nakamura et al. assessed the prognostic value of the serum level of C-reactive protein for the survival of patients with a primary sarcoma of bone [23]. A study published in 2011 showed that preoperative serum CRP was an independent prognostic factor for survival in patients with high-grade osteosarcoma, which was consistent with the findings in our study [24]. Our study was the first study in Asians designed to assess the association between CRP levels and overall survival of osteosarcoma patients. The results in our study show that high serum CRP level is a significantly independent predictor of worse overall survival in osteosarcoma patients. However, our study did not assess the influence of serum CRP level on alive with disease (AWD) and a completely disease-free (CDF) in osteosarcoma patients. Further studies in the future are needed to assess the influence of serum CRP level on AWD and CDF in osteosarcoma patients.

Identifying prognostic factors including serum biomarkers may offer the opportunity to improve the prognosis by adding more effective therapy. Conventional factors, such as pathological clinical stages and metastasis, are important predictors of poor clinical outcome of osteosarcoma in clinical practice. Furthermore, a number of less common molecular markers have also shown prognostic roles, such as Mediator of RNA polymerase II transcription subunit 19 (Med19), microRNA-9 (miR-9), and human leukocyte antigen (HLA) class I antigen expressions [25–27]. However, these markers are only available following surgery or their measurement is complex, and therefore, they are often not used in clinical practice. By contrast, measurement of serum CRP levels is easy and relatively inexpensive in daily clinical practice. Thus, CRP levels may be measured routinely in patients with osteosarcoma as a further prognostic indicator of overall survival.

In conclusion, serum CRP level has an important prognostic significance in patients with osteosarcoma, and high CRP level is associated with worse overall survival. CRP levels may be measured routinely in patients with osteosarcoma as a further prognostic indicator of overall survival. In addition, more studies with large number of participants are necessary to validate the prognostic role of serum CRP levels in patients with osteosarcoma.

**Conflicts of interest** None

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