

Pretreatment C-reactive protein and neutrophil counts as a predictor of metastasis in patients with osteosarcoma

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Abstract

Background: Systemic inflammation responses have been associated with cancer development, progression and metastasis. However, little is known about the risk of metastasis based on inflammatory-based scores in patients with osteosarcoma before treatment. We therefore estimated the predictive value of these parameters for metastasis in osteosarcoma.

Methods: A total of 54 osteosarcoma patients were enrolled in this retrospective study. All had been diagnosed histologically, and their laboratory data at the first visit were collected from medical records. The lymphocyte-monocyte ratio (LMR), neutrophil-lymphocyte ratio (NLR), monocyte-neutrophil ratio (MNR), platelet-lymphocyte ratio (PLR), Glasgow prognostic score (GPS), neutrophil-platelet score (NPS), neutrophil counts (NC), lymphocyte counts (LC), monocyte counts (MC), and C-reactive protein (CRP) were evaluated.

Results: High values of CRP, PLR, MNR, and NPS and a low NC were significantly associated with metastasis of osteosarcoma patients in the univariate analysis. A multivariate Cox regression analysis revealed that a high CRP level ($>0.6\text{mg/dL}$) (hazard ratio=9.7, 95% confidence interval=3.0-31 ; $p=0.00010$) and low NC ($<4087/\mu\text{L}$) (hazard ratio=0.13, 95% confidence interval =0.040-0.42 ; $p=0.00080$) were risk factors significantly associated with metastasis of osteosarcoma patients.

Conclusions: Our study demonstrated that the combination of a high CRP level and low NC before treatment was a useful inflammatory-based prognostic indicator for metastasis in patients with osteosarcoma.

Background

Osteosarcoma is the most common primary bone tumor, predominantly affecting adolescents and young adults[1]. The standard treatment is the combination of chemotherapy and surgery[2]. With the assistance of radiologists and medical oncologists, multi-disciplinary treatment had led to better clinical outcomes and prognoses ; however, some patients still have a poor outcome due to distant metastases[3,4].

Some types of cancer have a specific marker revealing the grade or prognosis ; however, osteosarcoma has no oncological marker reflecting its prognosis. Recently, miR-138-5p, miR-1285-3p, miR-199a-3p, and ERp29 were reported as prognostic factors for osteosarcoma[5-9], but these markers are costly and inconvenient to measure, and cannot be used in the standard management of patients yet. The simple measurement of prognostic factors is extremely useful and has been widely accepted for guiding the treatment course of patients. Identifying such novel and simple prognostic factors will help distinguish high risk patients who need specific therapy.

Smoldering inflammation in the tumor microenvironment is reported to have many tumor-promoting effects[10]. The inflammatory reaction leads to the proliferation and survival of malignant cells, and

promotes angiogenesis and metastasis[9]. The addition of anti-inflammatory drugs during treatment has been suggested to be a new effective treatment for increasing the patient survival[12,13].

In various types of cancer, the prognostic significance of several inflammation-related biomarkers or hematological indexes, including the C-reactive protein (CRP), Glasgow prognostic score (GPS), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and neutrophil-platelet score (NPS), have been associated with cancer development[14-18]. A high NPS has been commonly associated with a poor survival in a variety of cancers[17]. In addition, a low LMR was linked to a decreased survival[15,17]. In osteosarcoma, there are a few reports that high values of CRP, and NLR and a low LMR were significantly associated with a poor prognosis[19-23]. However, the prognostic role of inflammation biomarkers in metastasis of osteosarcoma patients remains unclear.

In this retrospective study, we evaluated the clinical significance of pretreatment inflammation-based scores and determined the independent prognostic factors for metastasis in patients with osteosarcoma.

Methods

Patients.

Between January 1999 and December 2019, we treated 138 patients with a histological diagnosis of primary osteosarcoma. The patients who had received any previous treatment, such as anti-cancer treatment or administration of non-steroidal inflammatory drugs (NSAIDs) for painkiller or a cancer fever, and those with incomplete laboratory data before any procedures were all excluded from this study for the precise evaluation of the inflammatory-based scores. The lack of any treatment with NSAIDs was all confirmed by a questionnaire at the first visit. Patients with evidence of infection or autoimmune disease were also excluded. A total of 84 patients (61%) were excluded, leaving 54 for the retrospective analysis.

Data collection.

The laboratory data at the first visit were assessed from the medical records. The lymphocyte-monocyte ratio (LMR), NLR, PLR, monocyte-neutrophil ratio (MNR), GPS, NPS, neutrophil count (NC), lymphocyte count (LC), monocyte count (MC) and CRP level were evaluated from the peripheral blood cells before a biopsy or any treatment. GPS 0 is defined at $CRP \leq 1.0$ mg/dL and albumin ≥ 3.5 mg/dL, and GPS 2 is defined as $CRP \geq 1.0$ mg/dL and albumin ≤ 3.5 mg/dL. GPS 1 is defined as neither GPS 0 nor 2. Also, NPS 0 is defined when the NC is $\leq 7.5 \times 10^9$ /L and the PC is $\leq 400 \times 10^9$ /L. NPS 2 is defined as an NC $\geq 7.5 \times 10^9$ /L and PC $\geq 400 \times 10^9$ /L. NPS 1 is defined as neither NPS 0 nor 2. Using receiver operating characteristic (ROC) curves, the optimal threshold of the inflammation-based score of LMR, NLR, MNR, PLR, NC, LC, MC, and CRP was obtained when the Youden index was maximal. Patients were divided into two groups based on the cut-off values, with either a low or high value.

Patient follow-up.

Patients were followed up every 4 months during the first 5 years after treatment completion and every 6-12 months thereafter. The routine assessment for distant metastasis includes systemic computed tomography (CT) and chest X-ray for the first five years. Positron emission tomography (PET) is performed annually or when necessary. The metastasis-free survival was the period from the time of no metastasis at the completion of treatment to the time of the detection of distant metastasis. In case of detecting metastasis at first visit, the metastasis-free period was defined as 0 months. In case of metastasis being detected in the course of treatment, the period from the first visit to the time of the detection of metastasis was defined as the metastasis-free period.

Statistical analysis.

All statistical analyses were performed using the EZR software program (Saitama Medical Center, Jichi Medical University, Saitama, Japan) ²². The metastasis-free survival was plotted using the Kaplan-Meier method in each group and compared by the log-rank test and Bonferroni test. A P-value ≤ 0.1 was defined as a significant difference in the univariate analysis. Cox regression models were employed to determine the independent prognostic factors of metastasis for the multivariate analysis. P-value < 0.01 was defined as a significant difference.

Results

Patient characteristics.

This study consisted of 54 osteosarcoma patients with complete clinical data, including 30 men and 24 women. The mean age was 28 [range, 8-93] years old, and tumors were located at the appendicular skeleton in 41 patients, axial skeleton in 10 patients, and other sites in 3 patients. All of the patients were diagnosed with primary osteosarcoma pathologically by a biopsy at our department, and 93% of the patients (50/54) received chemotherapy. All but seven patients underwent surgery. Radiotherapy, including carbon-ion radiotherapy, was performed in seven patients because of their unresectable lesions. The mean follow-up period was 72 (Interquartile range [IQR]: 48-147) months. The number of patients suffering from distant metastasis during the follow-up periods was 27 (50%), including 6 (11%) at the first visit.

The optimal cut off value for inflammatory-based scores.

We compared the significance of CRP, LMR, NLR, PLR, NC, LC, and MC for discriminating between metastasis and non-metastasis using ROC curves. The optimal cut-off points of CRP, LMR, NLR, PLR, NC, LC, and MC were 0.60, 5.68, 2.82, 116, 4087, 1973, and 360, respectively. The areas under the curve (AUCs) of CRP, LMR, NLR, PLR, NC, LC, and MC were 0.59, 0.58, 0.52, 0.69, 0.66, 0.59, and 0.62, respectively (Table 1). Of the 54 patients, 36 had a GPS of 0, while 17 and 1 showed a GPS of 1 and 2, respectively. The patient numbers with an NPS of 0, 1, and 2 were 49, 3, and 2, respectively. The patient numbers in the high groups of CRP, NLR, MNR, LMR, and PLR were 13, 15, 19, and 25, respectively, and the patient numbers in the high groups of NC, LC, and MC were 23, 25, and 23, respectively.

Survival analysis and multivariate analysis for prognostic factors of metastasis.

High values of CRP, PLR, MNR, NPS, and a low NC were significantly associated with distant metastasis in the univariate analysis. Among them, a multivariate Cox regression analysis revealed that a high level of CRP ($>0.6\text{mg/dL}$) (hazard ratio [HR]=9.7, 95% confidence interval [CI]=3.0-30.9; $p=0.0001$) and low NC ($<4087/\mu\text{L}$) (HR=0.13, 95%CI=0.04-0.42 ; $p=0.0008$) were risk factors significantly associated with metastasis of osteosarcoma patients (Table 2). The survival curve revealed that the 5-year distant metastasis-free survival of patients with a high level of CRP was 29%, while the 5-year distant metastasis-free survival of patients with a low level of CRP was 61% ($p=0.02$) (Fig.1a). In addition, the 5-year distant metastasis-free survival of patients with a low NC was 42%, while the 5-year distant metastasis-free survival of patients with a high NC was 73% ($p=0.04$) (Fig.1b). Furthermore, among patients who had both a low level of CRP and a high NC ($n=13$), the 5-year distant metastasis-free survival was 100% until metastasis of only one patient in the group was first detected at 114 months ($p=0.004$) (Fig.1c).

Patients characteristics in each group according to the values of CRP and NC

The patient characteristics in each group according to the values of CRP and NC are shown in Table 3. There were no significant differences in the groups with higher and lower CRP (Table 3a). In the group with a low NC, radiation therapy including carbon ion radiotherapy, was performed more often than in the group with a high NC, instead of surgery (Table 3b). However, the rate of chemotherapy did not differ significantly between the groups with low and high NC.

Discussion

Several prognostic factors for osteosarcoma have been established, including alkaline phosphatase, lactate dehydrogenase, tumor site, tumor size and Enneking surgical criteria[25-27]. However, these factors were not associated with specific therapy, which has proven effective in improving clinical outcomes directly. Recently, miR-138-5p, miR-1285-3p, miR-199a-3p, and ERp29 were reported as prognostic factors for osteosarcoma[5-9] : however, these markers are costly and inconvenient to measure, and cannot be used in the standard management of patients yet.

Elevated CRP levels reflected an increased systemic inflammatory response, which is involved in tumor development, progression, and metastasis[12]. In patients with nasopharyngeal carcinoma or colorectal cancer, elevated CRP levels were significantly associated with metastasis, and a high level of CRP can predict an unfavorable prognosis[29,30]. Systemic inflammation also affected the prognosis of osteosarcoma, and increased CRP levels were reported to be useful as a key indicator of the prognosis of osteosarcoma[19,28] ; however, very little is known about the association between high levels of CRP and the metastasis of osteosarcoma. Our results revealed that elevated CRP levels were significantly associated with metastasis of osteosarcoma using the cut-off values of 0.6 mg/dL, as calculated by ROC curves. In a few reports of in vitro and in vivo studies, the growth and metastasis of osteosarcoma was suppressed by aspirin[31]. Blocking interleukin-6 (IL-6) signaling with a humanized monoclonal antibody reduced the rate of lung metastasis and prolonged the survival of xenografted mice[32]. However, anti-

inflammatory drugs such as aspirin or IL-6 antibody were not used for the treatment of osteosarcoma patients. Our results suggested that anti-inflammatory drugs, such as NSAIDs, anti-IL-6 antibody and steroids, might prevent the metastasis of osteosarcoma by decreasing the high levels of CRP. In addition, the cut-off value of CRP in our study was not very high, but the early detection of signs of metastasis and the regulation of early inflammation by tumor cells might lead to the prevention of metastasis with an unfavorable prognosis.

The role of neutrophils in cancer development remains controversial[34,35]. There have been few reports on the correlation between the NC and metastasis in osteosarcoma patients[36-38]. A pretreatment NC $\geq 6400/\mu\text{L}$ in osteosarcoma patients was reported to be associated with the 5-year lung metastasis-free survival (65% vs. 44%, in comparison with an NC $< 6400/\mu\text{L}$). However, in their report, whether the blood test had been performed prior to the biopsy or not was unclear, as was how to decide the cut-off value of NC. Granulocyte-macrophage colony stimulating factor (GM-CSF) showed no detectable effect in recurrent pulmonary metastasis in osteosarcoma[36]. However, a meta-analysis of the influence of neutropenia on cancer patients revealed that neutropenia in patients without Granulocyte colony stimulating factor (G-CSF) administration was independently associated with a poor outcome, compared with patients receiving G-CSF administration during chemotherapy [38]. Various stimuli in the tumor microenvironment have been reported to result in the differentiation of neutrophils into either an anti-tumor or pro-tumor phenotype[39]. As with tumor-associated macrophages, neutrophils are classified into two polarization states, N1 (anti-tumor neutrophils) and N2 (pro-tumor neutrophils)[34,35,40]. Our results revealed that decreased NCs were significantly associated with metastasis of osteosarcoma patients. These data suggested that the osteosarcoma cells might suppress the differentiation of anti-tumor neutrophils in the first stage, subsequently causing the number of neutrophils to decrease and metastasis to advance. Inducing the differentiation of neutrophils or reviving the exhausted neutrophils might encourage the anti-tumor effect and possibly become a novel treatment for inhibiting the progression of osteosarcoma. Further investigations will be necessary to confirm the role of neutrophils in the treatment of osteosarcoma patients in the near future.

In our study, the patients with a higher NC underwent radiotherapy more often than those with a lower NC, instead of surgery, although the rate of chemotherapy in the patients with a lower NC was the same as in those with a higher NC. Recently, radiotherapy, especially carbon ion radiotherapy has been reported to be as effective as surgical treatment[41-43]. Therefore, treatment methods do not markedly influence the occurrence of metastasis.

The present study is the first attempt to evaluate the correlation between metastasis and inflammation-based scores in patients with osteosarcoma. Inflammation-based scores are simple and can be calculated solely using a blood test at a low cost. Currently available anti-inflammation drugs, such as NSAIDs, anti-IL-6 antibody, and steroids, can decrease high CRP levels, while the administration of G-CSF can increase the NC. The further development of these agents combined with standard chemotherapy may help prevent metastasis of osteosarcoma.

Several limitations associated with the present study warrant mention. First, this was a retrospective, small sample-size, and single-institution study, providing a lower level of confidence than randomized controlled trials. Second, the peripheral blood findings were not compared with the histological findings of peritumoral inflammation in the primary osteosarcoma tissue. Third, the changes in the inflammatory-based scores over the treatment course were not evaluated. That was because the timing of the blood test was not the same among all patients due to the fact that the efficacy of the treatment differed from patient to patient.

Conclusions

In conclusion, our study is the first attempt to evaluate the correlation between metastasis and inflammation-based scores in patients with osteosarcoma. Inflammation-based scores are simple and can be calculated solely using a blood test at a low cost. The present study demonstrated that the combination of a high pretreatment CRP level and low NC was a useful inflammatory-based prognostic indicator for metastasis in patients with osteosarcoma. The further studies for the treatment of these high-risk osteosarcoma patients will be necessary.

Abbreviations

GPS : Glasgow prognostic score; CRP : C-reactive protein; LMR : Lymphocyte-monocyte ratio; MNR : Monocyte-neutrophil ratio; NLR : Neutrophil-lymphocyte ratio; PLR : Platelet-lymphocyte ratio; NPS : Neutrophil-platelet score; NC : Neutrophil counts; LC : Lymphocyte counts; MC : Monocyte counts ; NSAIDs : Non-steroidal inflammatory drugs; IL-6 : Interleukin-6 ; GM-CSF : Granulocyte-macrophage colony stimulating factor; G-CSF : Granulocyte colony stimulating factor; HR : Hazard ratio ; CI : Confidence interval; IQR : Interquartile range ; CT : Computed tomography ; PET : Positron emission tomography; ROC : Receiver operating characteristic ; AUC : Area under the curve

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Institutional Review Board of the Kanazawa University Hospital (2019-061 (3094)), and written informed consent was obtained from all study participants.

Consent for publication

The consent for publication of the manuscript and the related images from the patients and/or their relatives was obtained by the Kanazawa University Hospital.

Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Competing interests

The authors declare no conflicts of interest in association with the present study.

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Authors' contributions

T.H., Y.N., and A.Yos. conceived and designed the study. A.Yos. carried out data acquisition. H.T., A.K., T.Y., Y.H., M.S., and A.Yoh. provided assistance for data acquisition. T.H., Y.N., H.K., T.A., M.S. and I.K. managed the patients for the appropriate treatment and observed them at the follow-up outpatient clinic after treatment completion. T.H. and Y.N. and A.Yos. contributed to the analysis and interpretation of laboratory data and critical appraisal. A.Yos. analyzed all the patient's data and wrote the manuscript. All authors read and approved the final manuscript.

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Figures

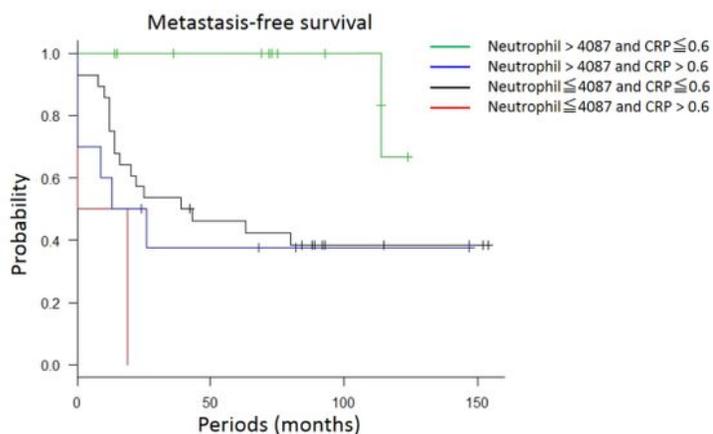
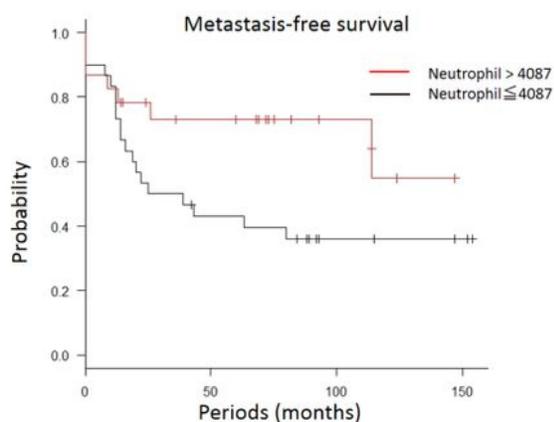
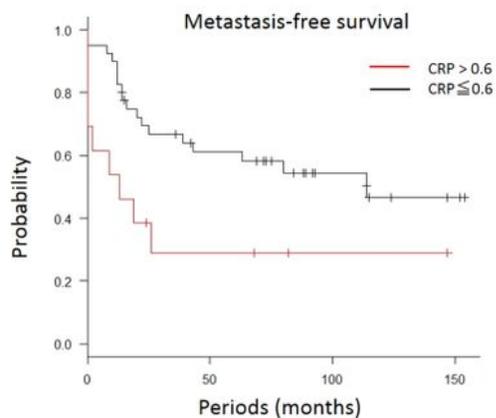


Figure 1

Figure 1(a). Kaplan-Meier curve showing the distant metastasis-free survival for patients with low and high levels of C-reactive protein (CRP). The lower CRP group was significantly associated with a longer rate of distant metastasis-free survival ($p = 0.02$). Figure 1(b). Kaplan-Meier curve showing the distant metastasis-free survival for patients with low and high neutrophil counts (NCs). The higher NC group was significantly associated with longer distant metastasis-free survival ($p = 0.04$). Figure 1(c). Kaplan-Meier

curve showing the distant metastasis-free survival for patients according to the values of CRP and NC. The group with a lower CRP level and higher NC showed a 0% distant metastasis rate until distant metastasis of only one patient was first detected at 114 months of follow-up ($p = 0.004$).