Peer Review File

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Review comments

Although the upper limb is the second most common site of osteosarcoma, investigations into clinical manifestation differences between upper and lower limb patients are still sporadic. In the manuscript "Regional Lymph Node Involvement Is Associated With Poorer Survivorship In Patients With Upper Extremity Osteosarcoma Than With Lower Extremity Osteosarcoma: A SEER analysis", authors investigated the characteristics of these patients to gain a better understanding of the differences between upper and lower limb osteosarcoma patients.

Couple questions are required to be answered before accepted.

(1) There was a similar report **(Clin Orthop Relat Res. 2019 Nov;477(11):2508-2518**) in the PubMed. What is the novel idea in the paper? Please elaborate in the introduction.

Reply 1: Our study is mainly focus on the comparison of clinical manifestation between the upper and lower limb osteosarcoma patients. In previous studies, upper limb lesions are still thought to show similar characteristics to other extremity lesions and thus are rarely reported separately from lower limb patients. Some studies have reported that the upper limb tumor is more proximal and led to more distal metastasis than the lower limb patients and upper limb patients showed poorer survival than lower limb patients. These findings indicate that additional research is needed to determine which characteristics are predictors of survival in upper and lower limb osteosarcoma patients in order to provide a systematic understanding of the differences between upper and lower limb osteosarcoma patients and to avoid inadvertently compromising patient outcomes.

One aspect of the different clinical manifestation between upper and lower limb patients was reflect on the different distal metastasis rate and it was associated with poorer survival in osteosarcoma patients. Osteosarcoma progressed primarily hematogenously and also sometimes lymphogenously. Regional lymph node involvement in upper limb was mainly reported in case report but the prevalence of lymph involvement has not been well defined and their clinical manifestation has not been systematically evaluated and compared with other extremity patients.

Change in the text: We had modified our text as advised (see Page 3-4, line 57-74).

(2) In the introduction, please enrich the progress of the treatment for osteosarcoma.

Reply 2: We had modified our text as advised and add the introduction of treatment for osteosarcoma in introduction. Current treatment for osteosarcoma consists of surgery

and chemotherapy, and the goal of surgery is to complete remove the tumor and preserve as much function as possible.

Change in the text: We had modified our text as advised (see Page 3, line 53-55).

(3) Why to focus on the difference between upper extremity osteosarcoma and lower extremity osteosarcoma in the paper?

Reply 3: The upper limb is the second most common site of osteosarcoma, but these lesions are rarely reported separately from lower limb patients. Some studies have reported that the upper limb tumor is more proximal and led to more distal metastasis than the lower limb patients and upper limb patients showed poorer survival than lower limb patients(8-10). These findings indicate that additional research is needed to determine which characteristics are predictors of survival in upper and lower limb osteosarcoma patients in order to provide a systematic understanding of the differences between upper and lower limb osteosarcoma patients and to avoid inadvertently compromising patient outcomes.

Change in the text: We had modified our text as advised (see Page 3-4, line 58-67).

(4) Are there any causes leading to lymph node involvement?

Reply 4: Previous study had histologically confirmed that osteosarcoma could progress lymphogenously. Lymphatics network on bone are present in the connective tissues overlying the periosteum. It had been demonstrated that lymphatic spread of the tumors could only occur when tumors extend through the periosteum into the adjacent connective tissues. And it was observed in case series of lymph involved osteosarcoma patients.

Change in the text: We had modified our text as advised (see Page 11, line 206-207).

(5) How to identify the lymph node involvement? Please supplement in the methods. How about the lymph node involvement in between upper and lower extremity osteosarcoma in your hospital?

Reply 5: We had modified the method section for clearifying the identify procedure of lymph node involvement in SEER database. SEER provides lymph node involvement data based on a composite of histologically confirmed regional nodes, clinical and radiographic data. The lymph node involvement is based on “Derived AJCC N, 6th ed (2004-2015)”, “Derived AJCC N, 6th ed (2004-2015)”, “Derived SEER Combined N (2016+)”, “Derived SEER Combined N Src (2016+)”, “N value - based on AJCC 3rd (1988-2003)”, “Regional nodes positive (1988+)”, “CS lymph nodes (2004-2015)”, “CS Reg Node Eval (2004-2015)” and the lost message is recode as “NX“. And patients

with localized extend of disease are also enrolled in the no lymph node involvement patients.

In our hospital, diagnostic strategy for lymph node involvement is thorough imaging examinations, including MRI and PET-CT. But our center used to not pay enough attention on lymph node state on osteosarcoma and lack of systemic examination and follow up of these lymph involved patients. And the available cases could not present a clear view of the clinical manifestation of the nature of lymph node involvement in extremity osteosarcoma patients. The rarity and heterogeneity of lymph involvement in osteosarcoma had challenged us to clarify their clinical manifestation and tumor outcome with small sample size in single institution and the heterogeneity of results in previous studies made it difficult to reach a clinical consensus.

Change in the text: We had modified our text as advised (see Page 6, line 107-112).

(6) Are there any risk factors for lymph node involvement?

Reply 6: We had modified the discussion section to present the risk factors for lymph node involvement. Our result identified that tumors located in the upper limb and distal metastasis are risk factors leading to lymph node involvement in extremity osteosarcoma patients ($p<0.05$). It is consistent that some studies have reported that risk location, large tumor size, distal metastasis, high-grade and extraskeletal tumor are important factors to lymph involvement in patients with bone malignance.

Change in the text: We had modified our text as advised (see Page 10-11, line 200-205).

(7) Although there are limitations using SEER database, why not to make the retrospective research based on real world data in your hospital?

Reply 7: Lymph node involvement in osteosarcoma is relatively rare in osteosarcoma patients. For the rarity and heterogeneity of lymph involvement in osteosarcoma, their clinical manifestation and tumor outcome was limited with small sample size in single institution and the heterogeneity of results in previous studies made it difficult to reach a clinical consensus. Currently, population-based databases have become increasingly relevant for studying clinical manifestation and tumor outcome in bone sarcoma research, especially for low incident event like lymph node invovlement. The Surveillance, Epidemiology, and End Results (SEER) database, covering 28% of US population, is one of the most often used large national cancer databases. The main purpose of this study was to systematically evaluated and compared the clinical manifestation and tumor outcome between the upper and lower limbs osteosarcoma patients based on large dataset in SEER database, thereby refining the reginal lymph node involvement prevalence in upper and lower limb patients.

Change in the text: We had modified our text as advised (see Page 4-5, line 75-83).

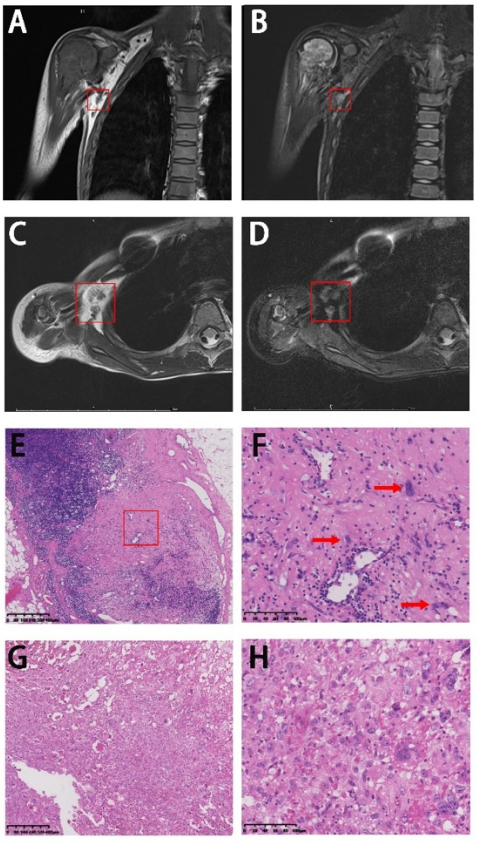
(8) Please supplement the discussion about mechanism in the discussion.

Reply 8: Our studies observed lymph involvement is often occurred with combination of distal metastasis (72.1%). And the result showed the lymph involved patients had no statistical difference in 5-year OS to the metastasis patients (35.8% v 22.9%, p=0.48). It is consistently with previous studies that many case report series observed that lymph node involvement and distal metastasis were occurred simultaneously and had a similarly poor survival. And we postulated that the lymph node involvement presents in a lymphogenous phenotype of distal metastasis and that the difference in lymph node involvement is attributed to the anatomical difference between the reginal lymph node network in upper and lower limbs. It can be interpreted as follows: upper limb lesions are most commonly located in the proximal humerus, close to neighboring axial nodes and vessels, while lower limb lesions are located near the knee joint, close to popliteal vessels but separated by muscle and interosseous membrane from the lymph network.

Change in the text: We had modified our text as advised (see Page 11, line 206-221).

(9) It is better to provide representative PET-CT and bone scan images for lymph node involvement.

Reply 9: We had modified our text as advised and add MRI images and pathological result for axillary node involved humeral osteosarcoma patient.



A & B. for coronal T1-weighted and T2-weighted image for a proximal humeral osteosarcoma patient with axillary lymph node involvement (showed in red box); C & D. for transversal T2-weighted and T2-weighted image (fat suppressed) image for a proximal humeral osteosarcoma patient with axillary lymph node involvement (showed in red box); E for HE staining (x4 scale) of lymph node specimen; F for HE staining (x20 scale) showing the osteosarcoma cell infiltrated in the lymph node specimen (showed with red arrow); G. HE staining (x4 scale) of primary tumor specimen; H HE staining (x20 scale) of primary tumor specimen.

Change in the text: We had modified our text as advised (see Figure 1, page 397-404).