



# Expression of *HER-2* in surgical specimen and biopsy as a biomarker of metastasis in patients with osteosarcoma: a meta-analysis

Jian Zhou<sup>1,2</sup>, Wanchun Wang<sup>1</sup>, Qian Yan<sup>3</sup>, Yingquan Luo<sup>3</sup>

<sup>1</sup>Department of Orthopedics, The Second Xiangya Hospital, Central South University, Changsha 410011, China; <sup>2</sup>Department of Sports Medicine Research Center, Central South University, Changsha 410008, China; <sup>3</sup>Department of Geriatrics, The Second Xiangya Hospital, Central South University, Changsha 410011, China

**Contributions:** (I) Conception and design: J Zhou, W Wang; (II) Administrative support: J Zhou, W Wang; (III) Provision of study materials or patients: J Zhou; (IV) Collection and assembly of data: J Zhou, W Wang; (V) Data analysis and interpretation: J Zhou, Q Yan, Y Luo; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

**Correspondence to:** Wanchun Wang. Department of Orthopedics, The Second Xiangya Hospital, Central South University, 139 Renmin Middle Road, Changsha 410011, China. Email: wanchun.wang@csu.edu.cn; Yingquan Luo. Department of Geriatrics, The Second Xiangya Hospital, Central South University, Changsha 410011, China. Email: luoyingquan@csu.edu.cn.

**Background:** Previous studies have evaluated the effect of *human epidermal growth factor receptor 2 (HER-2)* expression on the metastasis of patients with osteosarcoma (OS) while the results remain conflicting. Here we performed a systematic review and meta-analysis to determine the value of *HER-2* in prognosis of OS.

**Methods:** A comprehensive search using NCBI PubMed, the Cochrane library, Embase, ISI Web of Knowledge, Springer, China National Knowledge Internet database (CNKI), Wanfang database, Chinese VIP database and Chinese Biological Medical Database (CBM) from inception through Aug 28, 2018 was conducted to investigate *HER-2* expression and OS metastasis. We evaluated the quantity of the studies using Newcastle-Ottawa quality assessment scale (NOS).

**Results:** There were 15 studies with 652 OS patients involved. The results of meta-analysis showed that positive expression of *HER-2* was associated with OS metastasis (OR =4.42; 95% CI, 2.91–6.71; P<0.0001). No significant heterogeneity or publication bias was observed among all studies.

**Conclusions:** The results of this study suggest that *HER-2* positive expression indicates OS metastasis, while it's needed to perform more prospective studies to confirm the prognostic value of *HER-2* in patients with OS.

**Keywords:** *Human epidermal growth factor receptor 2 (HER-2)*; osteosarcoma (OS); metastasis; meta-analysis

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## Introduction

Osteosarcoma (OS) is the most common malignancy in bones and is limited to the metaphysis of long bones (1,2). In adolescents and young adults, OS is one of the most common primary malignant bone tumors. The survival rate of patients with OS increased with the introduction of advanced surgery and combination chemotherapy. However, a large proportion of patients are still involved in fatal metastasis, which significantly reduces the survival rate

at present. Because the mechanism of oncogenes has not yet been fully elucidated, the ability to predict the metastasis of OS is currently very limited. Therefore, it's urgently needed to understand the prognostic biomarkers of metastatic OS. These markers can identify patients at higher risk, so the patients can use more intensive and aggressive treatment at the time of initial diagnosis. So far, *human epidermal growth factor receptor 2 (HER-2)* expression has been identified as one of the potential prognostic biomarkers of OS (3).

Over-expression of *HER-2/neu/C-erbB2/ErbB-2* is closely related to the degree of malignancy of many epithelial cell cancers. *HER-2* high-expression tumors show strong migratory and invasive properties, poor sensitivity of chemotherapeutic drugs, poor post-hepatology, and recurrence. *HER-2* belongs to the human epidermal growth factor receptor family, which consists of 4 members: *EGFR*, *HER-2*, *HER-3*, and *HER-4* (4). These receptors are located on the surface of the cell membrane and have similar structures. *HER-2* expression is extremely low in normal cells, but it is expressed at high levels during embryonic development. *HER-2* can spontaneously form homodimeric receptors or ligands to induce the formation of heterodimeric receptors, triggering a signal transduction network (5,6), which plays an important regulatory role in cell proliferation, differentiation, development, adhesion, and migration. The prognostic effect of *HER-2* was first described about 20 years ago. Data from many studies indicate that *HER-2* over-expression is linked to risk of tumor metastasis in OS patients, while some other studies have shown controversial results. In order to investigate its relationship with metastasis, we conducted a meta-analysis of all available studies on *HER-2* expression and OS patients. In the current work, we confirmed that *HER-2* expression is increased in metastatic OS. Therefore, our study shows that *HER-2* can be used as a tool to judge the prognosis and metastasis of OS.

## Methods

### *Search strategy and study selection*

A systematic search using NCBI PubMed, the Cochrane library, Embase, ISI Web of Knowledge, Springer, CNKI, Wanfang database, Chinese VIP database and CBM was performed to investigate *HER-2* expression and OS metastasis. We performed the last search on Aug 28, 2018. The following terms: (*HER-2* or *neu* or *ErbB-2* or *C-erbB-2*) and (osteogenic tumor or osteosarcoma) were included in the search strategy by 2 investigators (JZ and QY) independently, which was checked repeatedly and no language limitations were imposed.

### *Inclusion and exclusion criteria*

Inclusion criteria: (I) publications were written in Chinese or English; (II) sufficient information was provided to construct the 2×2 contingency table; (III) pathological diagnosis (gold standard) was used to diagnose OS; (IV) *HER-2* in OS was measured using commercial reagents.

Exclusion criteria: (I) studies absence of survival outcome were excluded; (II) studies of non-dichotomous *HER-2* expression levels were excluded; (III) reviews, cell and animal experiments, case reports, correspondences, talks, expert opinions, letters, and editorials without original data were excluded; (IV) there was no cut-off value in the paper; (V) OS was diagnosed without a biopsy; (VI) multiple duplicate data were published in the different works, excluding earlier and smaller sample data; (VII) similar studies were published by the same author.

### *Data extraction*

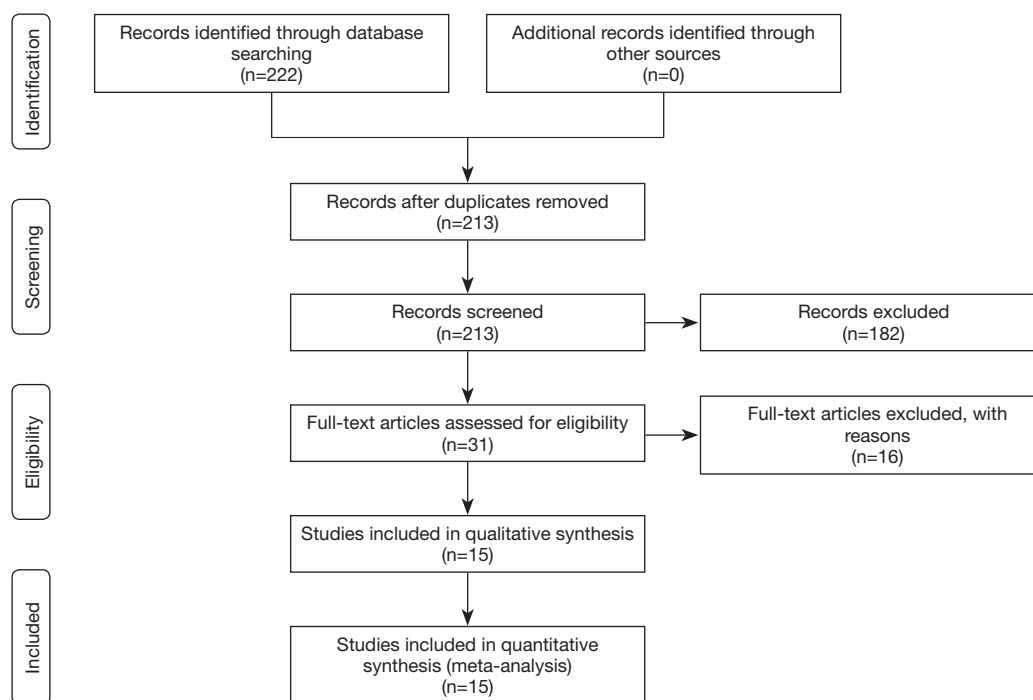
The eligibility of all retrieved studies was evaluated by two investigators (JZ and QY). Two investigators (JZ and QY) extracted the relevant data independently. Extracted databases were then crosschecked between the two authors to rule out any discrepancy. Data regarding the following for each included study were extracted independently: publication year, first authors' surname, *HER-2* assessment methods, and the cut-off definition. Corresponding authors were contacted if further information was needed. The study was excluded if no response was received after sending a reminder.

### *Assessment of included studies*

We assessed the quality of included studies using NOS (7) with three categories (selection, comparability, and exposure) and eight items. The quality assessment values ranged from 0 to 9 stars. Studies with score >6 stars were included for our analysis.

### *Statistical analysis*

The pooled odds ratio (OR) with corresponding 95% confidence interval (95% CI) was calculated to evaluate the effect of *HER-2* positive expression on metastasis of OS. The heterogeneity between the included studies was assessed by  $I^2$  statistics, which quantified the proportion of the total variation in meta-analysis assessment from 0 to 100% (8). When there was no significant heterogeneity ( $I^2 \leq 50\%$ ), the fixed effects model was used (9); otherwise, a random effects model was used for the analysis (10). Moreover, sensitivity analysis was performed by sequentially omitting individual studies to assess the stability of the results. The possibility of publication bias was assessed via visually assessing the symmetry of Egger's test and Begg's funnel plots (11). All the analyses were conducted using STATA version 12 software



**Figure 1** Schematic representation of the study selection.

(StataCorp LP, College Station, TX, USA). A two-tailed  $P < 0.05$  was considered statistically significant.

## Results

### Eligible studies

We retrieved 222 potentially relevant articles from our initial search in NCBI PubMed and so on. After removing duplicate data, 213 articles were remained. After reviewing the title and abstract, 182 manuscripts were excluded because they were either commentary or case reports, either duplicated or not relevant to the current analysis. Next, we further evaluated the remaining 31 studies. Among these, 16 studies were excluded for the following reasons: 3 items were not provided in full, 7 items were restricted to the study of molecular biology mechanisms, and 6 items did not provide enough data to calculate OR and 95% CI. A total of 652 patients participated in the current analysis in 15 studies (12-26) (Figure 1, Table 1), with 14 to 80 patients in each study (median of 47). The main features of the included studies are summarized in Table 1. In general, all studies used immunohistochemistry (IHC) to determine *HER-2* expression. We judged the results by two methods

of cut-off: positive cell percentage and staining intensity.

### Qualitative assessment

The study quality was evaluated using NOS, generating scores ranging from 7 to 8 (with a mean of 7.40). A higher value [0–9] indicates better methodology. The results of the quality assessment are shown in Table 1 and detailed information are shown in Table S1.

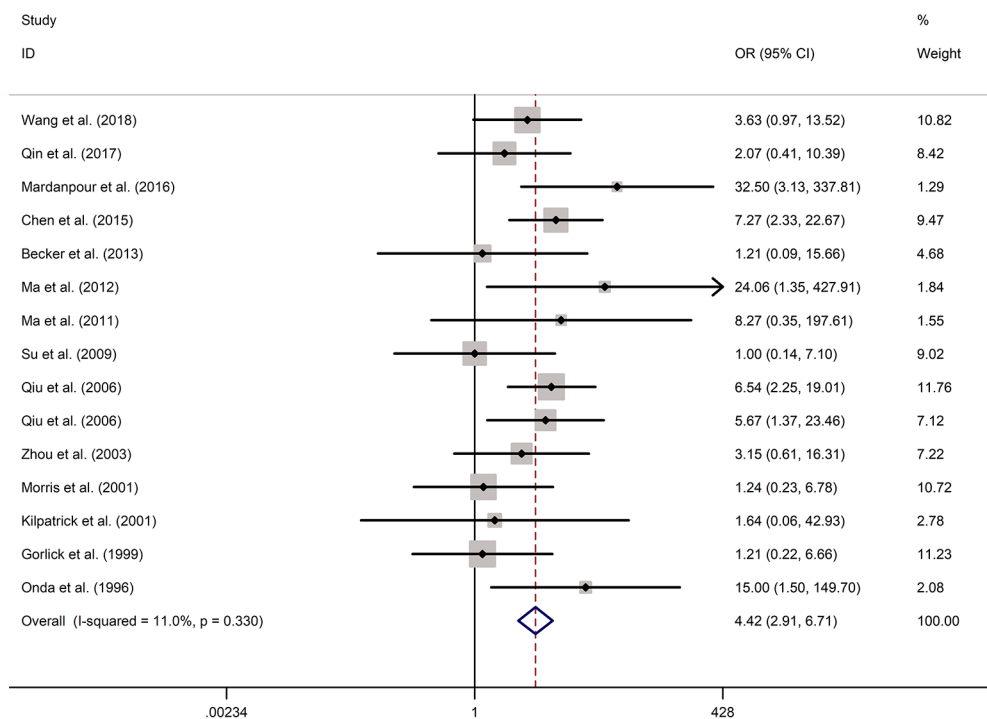
### Meta-analysis

In order to evaluate the heterogeneity of the included studies ( $I^2$ ), a I-squared test was used, where  $I^2$  evaluated the number of inconsistencies throughout the study ( $P < 0.05$  was considered significant). The value of  $I^2$  close to zero represents homogeneity, while the following ranges of  $I^2$  are used to explain heterogeneity: low heterogeneity if  $I^2 = 25\text{--}50\%$ , moderate heterogeneity if  $I^2 = 51\text{--}75\%$  and significant heterogeneity if  $I^2 > 75\%$ . In this study, OR was used to evaluate the relationship between metastasis rate and *HER-2* positive expression in patients with OS. As shown in Figure 2, the OR that assessed the *HER-2* positive expression on OS metastasis was 4.42 (95% CI, 2.91–6.71;  $P < 0.0001$ ;

**Table 1** Characteristics of studies included in the meta-analysis

Ref	Study	Year	No. of patients	Age (median)	Method	Assay kit	HER-2 cut-off	HER-2 positive		HER-2 negative		NOS score
								Metastasis	Non metastasis	Metastasis	Non metastasis	
(12)	Wang <i>et al.</i>	2018	60	24	IHC	OriGene	A1*B1>2	9	4	18	29	8
(13)	Qin <i>et al.</i>	2017	45	28	IHC	MXB	A2*B1≥3	3	6	7	29	7
(14)	Mardanpour <i>et al.</i>	2016	28	26.44	IHC	DAKO	A3≥2	13	4	1	10	8
(15)	Chen <i>et al.</i>	2015	80	31.2	IHC	MXB	-	47	14	6	13	7
(16)	Becker <i>et al.</i>	2013	27	13	IHC	DAKO	A4≥2	1	2	7	17	7
(17)	Ma <i>et al.</i>	2012	63	16	IHC	Santa cruz	B2	12	26	0	25	7
(18)	Ma <i>et al.</i>	2011	14	16	IHC	Santa cruz	B2	3	0	5	6	8
(19)	Su <i>et al.</i>	2009	30	18	IHC	MXB	A5>1	3	15	2	10	7
(20)	Qiu <i>et al.</i>	2006	69	17.4	IHC	MXB	A5+B1≥4	19	9	10	31	8
(21)	Qiu <i>et al.</i>	2006	38	17.7	IHC	MXB	-	10	5	6	17	7
(22)	Zhou <i>et al.</i>	2003	25	14	IHC	Ventana	A1≥2	7	4	5	9	8
(23)	Morris <i>et al.</i>	2001	53	16.9	IHC	DAKO	A1≥2	3	21	3	26	7
(24)	Kilpatrick <i>et al.</i>	2001	41	29	IHC	DAKO	B1≥2	14	26	0	1	7
(25)	Gorlick <i>et al.</i>	1999	53	16.9	IHC	DAKO	A2≥2	8	33	2	10	8
(26)	Onda <i>et al.</i>	1996	26	19.9	IHC	Nichirei Inc	-	10	1	6	9	7

A, positive cell percentage; A1, scored 1 (<25%), 2 (25–75%), 3 (>75%); A2, scored 0 (<5%), 1 (6–25%), 2 (26–50%), 3 (>50%); A3, scored 0–3+; A4, scored 0–1 (<30%), 2–3 (>30%); A5, scored 1 (>10%); B, staining intensity; B1, scored 0 (absence of staining), 1 (weak staining), 2 (moderate staining), 3 (strong staining); B2, scored by a pathologist.



**Figure 2** *HER-2* expression and metastasis of osteosarcoma patients.

$I^2=11.0\%$ ), which suggests a low heterogeneity among the 15 studies analyzed ( $I^2=11.0\%$ ) in the meta-analysis of the effect of *HER-2* expression on the metastasis rate of OS. Therefore, a fixed effect model was used to calculate the combined OR and the corresponding 95% CI.

**Sensitivity analysis**

We performed a sensitivity analysis to assess the stability of the results, indicating that the combined OR was stable and there was no significant change in heterogeneity when removing a single study. We evaluated the robustness of the results by canceling one study at a time and recalculating the overall OR. A one-time sensitivity analysis was conducted to show that our analysis was not too dependent on a study and the conclusion was stable (Figure 3). These results indicate that *HER-2* positive expression may be an indicator of metastasis in patients with OS.

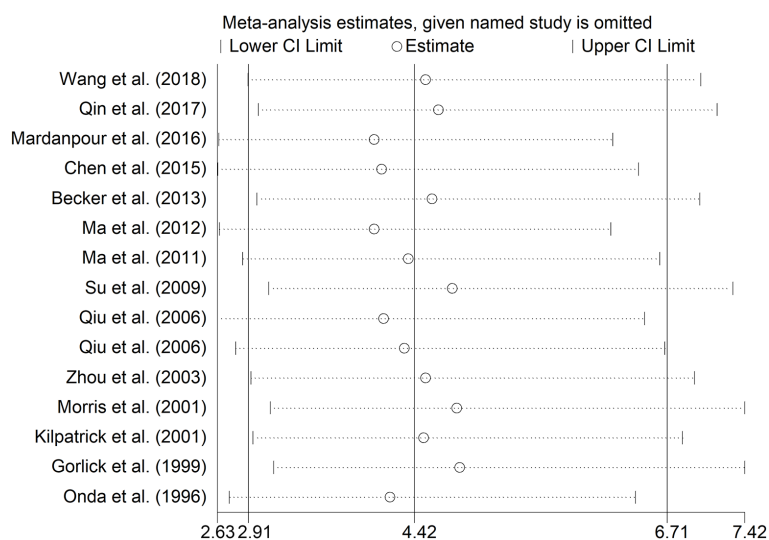
**Publication bias**

In order to test whether there is obvious evidence of asymmetry and the publication bias of the quantitative analysis literature, we used a Begg’s funnel plot with STATA. Begg’s

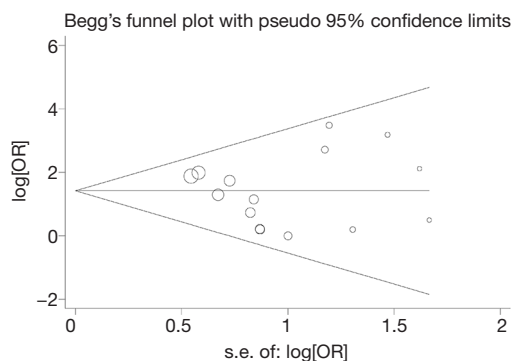
funnel plot does not show obvious asymmetric evidence in metastasis, with a P value of 0.838 ( $P>0.05$ ) (Figure 4).

**Discussion**

OS is the most common malignant bone tumors in adolescents and young adults. The OS prognosis is poor with low sensitivity to chemotherapy and radiotherapy. It also has the characteristics of easy recurrence and metastasis. Therapy for OS has hit the bottleneck. The 10-year survival rate of amputation therapy for primary OS patients is only 15% (2) and the combination chemotherapy does not improve OS (27). Recently, many studies about the prognosis of OS have been reported. CRP and ESR levels of pre-operation may have value in building a prognostic model for OS (28). *Hypoxia-inducible factor-2 alpha (HIF2α)* may be a biomarker of OS (29). Controlled and predictive marker studies of primary bone tumors was allowed by the newly developed and validated preclinical model (30). All that reports would contribute to the development of prognostic biomarker for OS. Additionally, the impact of Managed Clinical Network for Sarcoma on patient outcomes was also assessed. Previous study indicated that Scotland have similar outcomes to the UK following diagnosis of a primary malignant bone tumor (31). Although



**Figure 3** Forest plot for the sensitivity analysis in the meta-analysis.



**Figure 4** Funnel plot in the meta-analysis of the effect of *HER-2* expression on osteosarcoma (OS) metastasis.

many efforts on OS have been done, the survival of patients with metastatic OS remains extremely poor. Because OS has a high degree of malignancy, early metastasis is the main factor affecting whether the patient's cure rate can be improved. Therefore, it is urgently needed to update the early prognostic biomarkers to adapt to the appropriate treatment of malignant tumors. Many researchers report that high expression of certain cell surface markers suggests poor clinical characteristics and prognosis. *HER-2* is one of the potential prognostic indicators of OS. It has been reported that the *HER-2* gene is of irreplaceable significance in predicting the survival and recurrence time of many cancer patients. In terms of hormone receptor status, it has higher prognostic value than many currently used prognostic factors. *HER-2* has been proved to have a role in the transfer of promoters

via acting PI3K/Akt pathway to increase the proliferation of cancer cells. *HER-2* gene and protein expression levels in some tumors, especially breast cancer metastases (32,33), are significantly higher than those in primary tumors. In addition, high expression of *HER-2* protein was also found in the pseudopodia of invasive cancer cells, indicating that this gene is closely related to cancer metastasis. Moreover, *HER-2*, like other members of other tyrosine kinase receptor families, not only regulates the growth and differentiation of cells, but also influences the abilities of cells to move and adhere, and is involved in the process of metastasis of tumor cells in many ways. The high expression of *HER-2* increases the proliferation, survival and anti-apoptotic capacity of tumor cells and increases the ability to migrate and infiltrate, which often indicates a poor prognosis (34). Therefore, selective *HER-2* inhibitors have potential application value in the prevention of OS, enhancing the sensitivity of chemotherapy and radiotherapy, and reducing their toxic and side effects. However, whether it can be used as a new drug for the treatment of OS in clinical applications requires further research. In conclusion, *HER-2* molecule is an important molecule involved in tumor growth, invasion and metastasis, and it may be a valuable prognostic biomarker in OS.

Meta-analysis is a quantitative approach combing information from different studies on related topics to facilitate the assessment of cancer-related prognostic indicators (35). In order to conduct a precise assessment about the prognostic role of *HER-2* positive expression in OS, a meta-analysis was conducted and fifteen published studies was included.

The results showed that *HER-2* positive expression indicates higher rates of OS metastasis 4.42 (95% CI, 2.91–6.71;  $P < 0.0001$ ). Additionally, a sensitivity analysis was performed to determine the stability of the results. When any single study was removed, the pooled OR was stable with no significant changes. In summary, meta-analysis shows that *HER-2* is a valuable biomarker to guide the clinical therapy for OS.

However, there are still several limitations in this study and following questions should be considered. Firstly, there is no publication bias in the selection of documents, but there may still be potential publication bias. Because these studies with desired results are more easily released, which may lead to bias in the overall accuracy. Secondly, the inclusion of the literature is limited to English and Chinese publications, which also have an impact on the results. Thirdly, because of the scarcity of OS, the total sample size included in this Meta-analysis was 652, of which 332 were *HER-2* positive and 320 were *HER-2* negative. Fewer samples will have unavoidable random errors and sample biases in the meta-analysis process. This requires us to conduct a larger sample size study to better assess the correlation between *HER-2* positive expression and OS metastasis. Fourthly, tumor metastasis is used as the main outcome, but it still lacks sufficient data. We can't stratify data according to patient age, tumor size, tumor stage, and site of metastasis. This requires a larger sample size and more detailed research program designed to more comprehensively evaluate the link of connection between the two.

In conclusion, this meta-analysis was performed to evaluate the association between *HER-2* positive expression and metastasis of patients with OS. Results of this meta-analysis indicated that *HER-2* was an effective biomarker that correlates with OS metastasis. More well-designed studies with larger sample sizes are still needed to obtain a more comprehensive evaluation about the prognostic role of *HER-2* positive expression in OS patients.

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### Footnote

**Conflicts of Interest:** All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2019.06.26>). The authors have no conflicts of interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Table S1 Qualitative assessment of included study for osteosarcoma (OS) metastasis

Column	Entries	Study													
		Wang et al., 2018	Qin et al., 2017	Mardanpour et al., 2016	Chen et al., 2015	Becker et al., 2013	Ma et al., 2012	Ma et al., 2011	Su et al., 2009	Qiu et al., 2006	Zhou et al., 2003	Morris et al., 2001	Kilpatrick et al., 2001	Gorlick et al., 1999	Onda et al., 1996
Section	Is the definition adequate	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆
	Representativeness of the cases	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆
	Selection of controls														
	Definition of controls	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆
Comparability	Comparability of cases and controls on the basis of the design and analysis	☆☆	☆	☆☆	☆	☆	☆☆	☆	☆☆	☆☆	☆☆	☆	☆☆	☆☆	☆
Exposure	Ascertainment of exposure	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆
	Same method of ascertainment for cases and controls	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆
	Non-Response rate	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆
Total scores		8	7	8	7	7	8	7	8	7	8	7	8	7	7