

Review Article

Her-2/ErbB-2 expression is not a prognostic factor in high-grade osteosarcoma: a systematic review and meta-analysis

Jinping Mao¹, Tao Xie², Hongliang Gao², Hailin Yan², Lingling Sun², Peijian Tong¹

¹Department of Orthopedics, The First Affiliated Hospital of Zhejiang Chinese Medicine University, Hangzhou 310009, P. R. China; ²Department of Orthopedics, The Second Affiliated Hospital of Zhejiang University, School of Medicine, Hangzhou 310009, P. R. China

Received January 27, 2016; Accepted February 9, 2018; Epub May 15, 2018; Published May 30, 2018

Abstract: Background: The prognostic value of Her-2/ErbB-2 in patients with high-grade osteosarcoma remains controversial. Hence, we aimed to investigate the effects of Her-2 overexpression on histological response and survival risks in this population. Material and methods: From inception to December 2015, we searched for potentially relevant studies published in English using the following databases: PubMed, Science Direct, the Cochrane Library and EBSCO. Eligible studies should provide information about expression levels of Her-2. The primary outcome measures included histological response, 5-year event-free survival (EFS) and overall survival (OS). Results: 10 studies were in accordance with inclusion criteria. Her-2 immunohistochemical staining indicated that overexpression was not associated with histological response (n = 7, RR = 0.74, 95% CI: 0.45 to 1.21, P = 0.23). Compared with low or undetectable levels of expression, Her-2 overexpression had nonpredictive effect on EFS or OS (EFS: n = 7, RR = 0.82, 95% CI: 0.60 to 1.14, P = 0.24; OS: n = 8, RR = 0.79, 95% CI: 0.58 to 1.08, P = 0.14). By subgroup analyses, we found that whether metastasis and local disease were included together or not had a significant influence on histological response, EFS and OS. Conclusions: Our meta-analysis suggests that Her-2 expression is not associated with histological response, EFS or OS in high-grade osteosarcoma. And Her-2 expression is associated with tumor metastasis which can influence chemotherapy response and prognosis. Although Her-2 still cannot be used as a prognostic factor, further large-scale prospective studies are warranted to confirm our findings.

Keywords: Osteosarcoma, Her-2/ErbB2, prognosis, meta-analysis

Introduction

Osteosarcoma is the fifth most common malignancy among adolescents and young adults [1]. And high-grade osteosarcoma is the classical or conventional form comprising nearly 80% of osteosarcoma [2]. Until the seventies, osteosarcoma had a very poor prognosis. Major progress was made with the discovery of several active chemotherapeutic agents. Although the event-free survival (EFS) probability of high-grade localized osteosarcoma has improved up to approximately 50%-80% [3-5], the prediction of survival benefit for individual is still limited at present. Moreover, it seems that survival rates have reached a plateau. Thus, clinical and biological factors for predicting prognosis need developing.

The Her-2 gene (also known as ErbB-2) encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. The amplification and/or overexpression of Her-2 has been reported in numerous cancers, such as breast [6], ovarian [7] and lung cancers. In some of these cancers, Her-2 overexpression was associated with resistance to chemotherapeutic agents. And Her-2 overexpression was a significant predictor of both overall survival (OS) and EFS.

A number of studies have showed that osteosarcomas can express Her-2 [8-10]. However, the levels of Her-2 expression in high-grade osteosarcoma are rather controversial, ranging from studies in which Her-2 overexpression was detected in 15%-70% [8, 11] of patients to oth-

ers in which it was uncommon [12, 13] or even absent [14-16]. A majority of studies used immunohistochemical staining to define Her-2 status: grading 2+ and 3+ were considered positive expression. While in these studies, variability still existed in methodology, including the antibody utilized and the grading system.

Although the prognostic significance of Her-2/ ErbB-2 in high-grade osteosarcoma has been investigated, the results were inconsistent across studies. Several prior studies suggested that increased level of Her-2 expression at diagnosis was associated with a worse outcome since Onda [9] first described Her-2 expression in osteosarcoma. For example, study by Zhou [10] showed that Her-2 expression was associated with shorter metastasis-free survival, and reported that the survival differences were significant. Conversely, some other studies showed insignificant or opposite results [8, 17]. All the studies discussed above included a relatively small sample size because osteosarcoma was a rare tumor with a low incidence of about 3 per 1 million [18]. Therefore, there is not enough evidence to draw comprehensive conclusions.

A recent meta-analysis by L Y [19] demonstrated that Her-2/ErbB-2 positive status was significantly associated with a worse 5-year OS in osteosarcoma. Unfortunately, L Y neglected several studies meeting their inclusion criteria and chose several duplicate studies so that there was an unavoidable selection bias. And there were many other major flaws as L F [20] and X Y [21] described, so definitive conclusions remained elusive. Furthermore, none of previous meta-analysis had evaluated the question whether Her-2 overexpression was associated with the resistance to chemotherapeutic agents in osteosarcomas. Last but not the least, our target population was only limited to patients with high-grade osteosarcoma as little progress had been made in the treatment in the last two decades.

As mentioned above, we conducted this meta-analysis to provide a comprehensive evaluation of the effects of Her-2/ErbB-2 on histological response, and 5-year EFS and OS in patients with high-grade osteosarcoma.

Materials and methods

Search strategy

Two of the authors (J M and X T) simultaneously and independently screened relevant studies in the following databases: PubMed, Science Direct, Cochrane Library and EBSCO. The retrieval time was set as from inception to December 2015. The following keywords were used to identify possible publications: “Her-2”, “Her2”, “ErbB-2”, “ErbB2”, “osteosarcoma”, and “osteogenic sarcoma”. Studies that did not obviously conform to our criteria were excluded; examples of such included studies about patients with low grade osteosarcoma. The most recent or the largest sample size publication was included when the authors published several studies using the same subjects. Studies about Her-2 in high-grade osteosarcomas were further evaluated, and any differences that arose were resolved by one of the other authors (L S). We reported the meta-analysis according to the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) statement [22].

Quality assessment

Two of the authors (J M and H Y) independently completed quality assessments on the basis of the Newcastle-Ottawa Quality Assessment Scale (NOS) [23], which was validated for cohort studies in a meta-analysis. The NOS criteria included three aspects: (1) Subject selection: 0-4; (2) Comparability of subject: 0-2; (3) Clinical outcome: 0-3. NOS scores ranged from 0 to 9; and a score ≥ 7 indicated a good quality. Any differences were resolved by a third investigator (H G).

Inclusion and exclusion criteria

In this meta-analysis, only published clinical studies on humans were included, and the language restriction was set to English. Studies were included if they fulfilled the following inclusion criteria: (1) Patients with a definite diagnosis of high-grade osteosarcoma, and (2) Who received standard (neo-adjuvant and/or adjuvant) chemotherapy and definitive surgery, (3) The studies should provide sufficient information about expression levels of Her-2, histologi-

Prognostic role of Her-2 in osteosarcoma

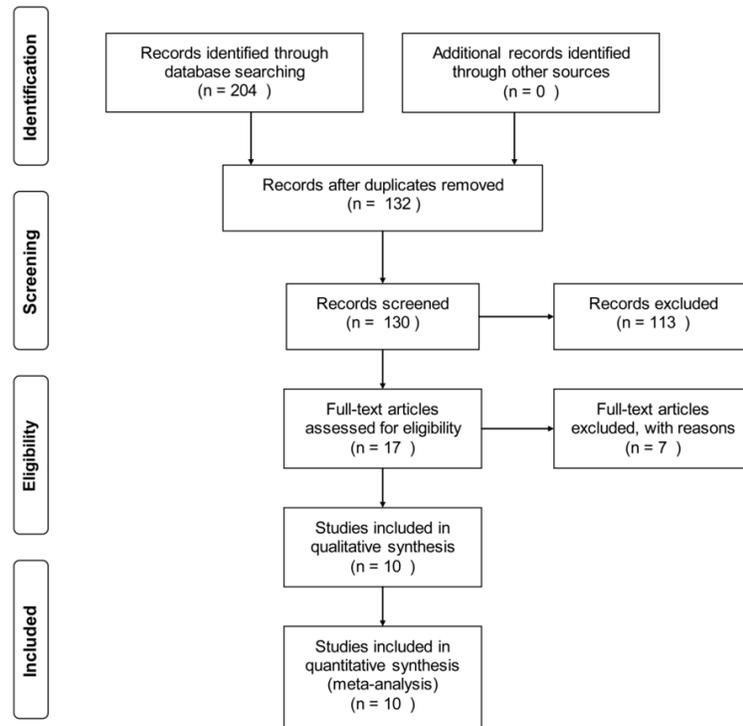


Figure 1. Flow diagram of study selection.

cal response to chemotherapy and long-term outcomes (5-year EFS and/or OS).

Data extraction

The outcomes that we primarily focused on were histological response and clinical outcomes (5-year EFS and/or OS). The essential data were extracted from each study in a unified format that included the first author's name, year of publication, country, patient number, metastasis, mean age, study design, method, antibody, cut-off point and positive rate (%). The numbers of patients that experienced good histological response, 5-year EFS and OS were extracted directly from each study. We extracted the risk ratios (RRs) and 95% confidence intervals (95% CI) to evaluate the baseline comparison, histological response and survival effects on 5-year EFS and OS.

Statistical analysis

We comprehensively evaluated histological response and 5-year EFS and/or OS using STATA 12.0 (Stata Corp, College Station, Texas). We then calculated RRs and corresponding

95% CIs for dichotomous data. A fixed-effect model was used acquiescently. In our opinion, there was substantial heterogeneity between studies and therefore we used a random-effect model in cases where differences between groups ($P < 0.05$ and $I^2 > 60\%$) existed simultaneously. Subgroup analysis was performed to further identify possible reasons, whenever appropriate. The Egger's test was used to reveal publication bias [24]. For all analysis, a two-tailed P -value < 0.05 was considered significant.

Results

Characteristics of the included studies

We identified 204 articles from the databases as stated above, and 17 [8-11, 17, 25-36] of these articles were included based on our search criteria. Upon further review, 7 studies were excluded: 2 were eliminated due to low grade osteosarcomas [25, 28]; 2 were duplicate studies on the same population [26, 30]; one was a study just focused on patients with first recurrence in the lung [29]; one was with a rate of lost to follow-up as high as 60% [31] and another one study was only with 3 Her-2 overexpression patients [27]. Eventually, total of 10 studies [8-11, 17, 32-36] that published between 1996 and 2014, were found to be in accordance with our inclusion criteria. Our search strategy and the steps used to select eligible studies are summarized in the flow diagram which is shown in **Figure 1**.

A total of 650 patients were included in our meta-analysis, of which 253 (39.0%) were Her-2 overexpression, and 397 were not. As to the method for determining Her-2 status, 9 studies [8, 10, 11, 17, 32-36] used immunohistochemistry (IHC) and the other one [9] used immunoblotting (IB) for protein levels. In each study, the IHC cut-off values of Her-2 appeared to be different. Most of the patients with high-grade

Prognostic role of Her-2 in osteosarcoma

Table 1. Characteristics of included studies

| Study | Year | Country | No. | Metastasis | Mean Age (year) | Study Design | Method | IHC Antibody | IHC cut-off | Positive rate | NOS |
|------------|------|---------|-----|------------|-----------------|--------------|--------|--------------|-------------|---------------|-----|
| Akatsuka | 2002 | Japan | 81 | 0 | 15.7 | Retro | IHC | CB11 | 31% | 0.63 | 9 |
| Ebb | 2012 | USA | 96 | 96 | 14.5 | Retro | IHC | CB11 | 50% | 0.43 | 8 |
| Gorlick | 2014 | USA | 135 | 0 | 14 | Retro | IHC | CB11 | 51% | 0.13 | 7 |
| Kilpatrick | 2001 | USA | 27 | 0 | 29 | Retro | IHC | CB11 | 10% | 0.67 | 7 |
| Morris | 2002 | USA | 73 | 10 | 16.9 | Retro | IHC | 3B5 | 26% | 0.45 | 9 |
| Munoz | 2009 | Spain | 26 | 0 | 10 | Retro | IHC | Rabbit | ND | 0.31 | 8 |
| Onda | 1996 | Japan | 25 | 6 | 19.9 | Retro | IB | CB11 | 1+ | 0.42 | 9 |
| Scotlandi | 2005 | Italy | 83 | 0 | <40 | Retro | IHC | TAB250 | 26% | 0.33 | 8 |
| Yalcin | 2008 | Turkey | 79 | 8 | 13 | Retro | IHC | CB11 | 31% | 0.45 | 9 |
| Zhou | 2003 | USA | 25 | 6 | 14 | Retro | IHC | Ab3 | 26% | 0.44 | 8 |

Abbreviation: Metastasis: metastasis at diagnosis; Retro: retrospective study; IHC: immunohistochemistry; IB: immunoblotting; ND: no data; NOS: Newcastle-Ottawa Quality Assessment Scale.

Table 2. Baseline characteristics

| Outcome | Studies | No. | RR | LL | UL | Heterogeneity | | | P |
|-------------|---------|-----|------|------|------|---------------|----------------|------|------|
| | | | | | | Chi-squared | I ² | p | |
| Age (<14 y) | 6 | 291 | 0.73 | 0.56 | 0.96 | 2.65 | 0 | 0.75 | 0.02 |
| Gender (M) | 7 | 387 | 0.92 | 0.78 | 1.10 | 9.24 | 35% | 0.16 | 0.38 |
| ALP (high) | 3 | 155 | 1.08 | 1.08 | 1.46 | 1.80 | 0 | 0.41 | 0.62 |

Abbreviation: M: male; RR: relative risk; LL: lower limit; UL: upper limit.

osteosarcoma in the included studies were treated with neo-adjuvant chemotherapy associated with surgery. The clinical features of the 10 included studies were summarized in **Table 1**.

Most of the patients in the included studies were evaluated 5-year survivals. The exceptions included the study by Ebb [17] that evaluated the use of Trastuzumab in combination with neo-adjuvant/adjuvant chemotherapy in the experimental group for newly diagnosed, high-grade metastatic osteosarcomas. And another study conducted by Kilpatrick [32] presented the individual patient data of immunohistochemical staining by 2 antibodies simultaneously in patients with high-grade osteosarcoma. The main drawback of these two studies was that they were with follow-ups as short as 2.5 years. Eventually, we reserved these 2 studies in order to reduce heterogeneity as much as possible.

It is additionally worth noting that, in total, out of all 6 studies [8-10, 32, 34, 35] that were included in our meta-analysis, only 30 patients stained positive for overexpression of Her-2,

which is a relatively small sample size.

Methodological quality of the studies

We presented results of the assessment of the methodological quality in **Table 1**. Ten included studies were all with high quality (score ≥ 7) according to the NOS scores.

Baseline comparison

More than 3 studies provided baseline information about age, gender and ALP level, so we investigated the correlations of Her-2/ErbB-2 expression with these 3 factors. The median age of the included patients was 11.3 years. 44% of the included patients were ≤ 14 years, and there were significant differences between groups that were with Her-2 overexpression versus those that were not ($n = 6$, RR = 0.73, 95% CI: 0.56 to 0.96, $P = 0.02$). Although 57% of the patients were male, no significant differences were found between the two groups ($n = 7$, RR = 0.92, 95% CI: 0.78 to 1.10, $P = 0.38$). Only three of these included studies [9, 33, 36] with 155 patients investigated the serum ALP level. And there were no significant differences between the groups that were with Her-2 overexpression versus those that were not ($n = 3$, RR = 1.08, 95% CI: 0.80 to 1.46, $P = 0.62$). The baseline analysis details were shown in **Table 2**.

Prognostic role of Her-2 in osteosarcoma

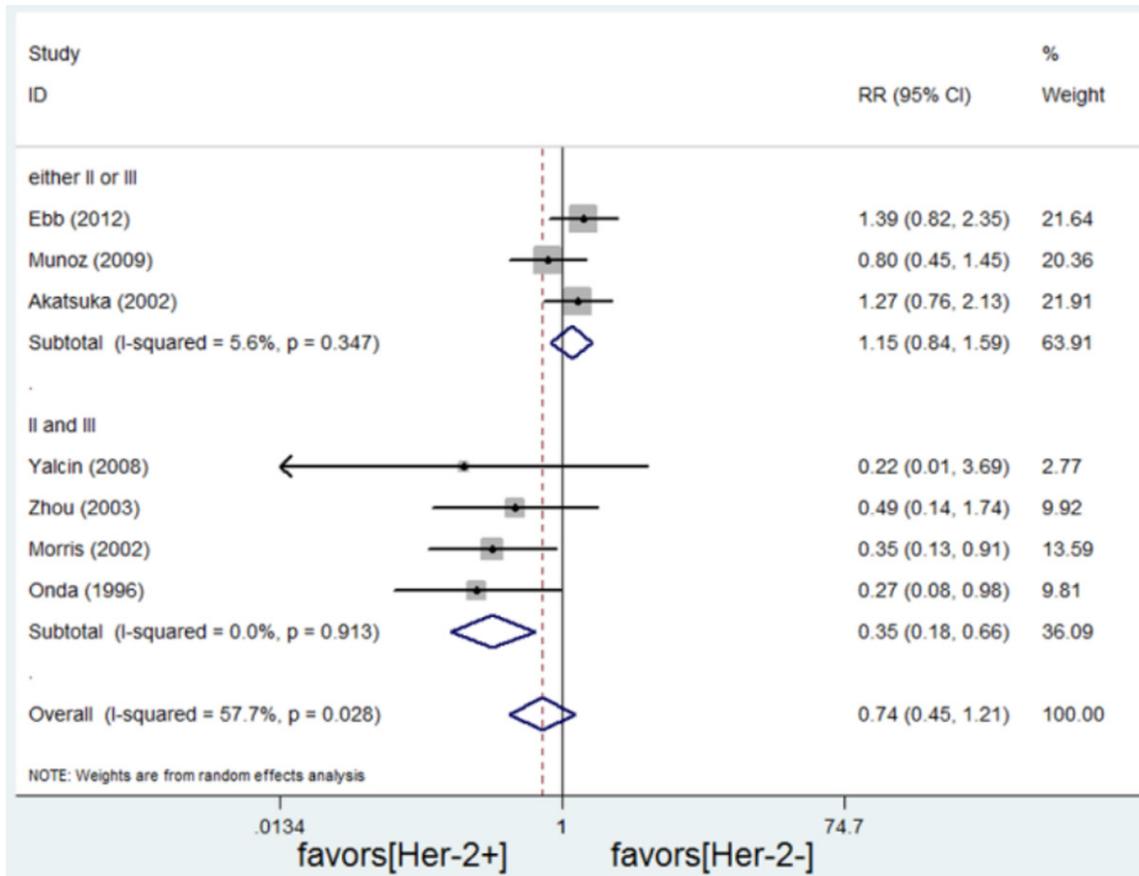


Figure 2. Her-2 expression and histological response to chemotherapy in high-grade osteosarcomas. RR = Risk ratio; CI = Confidence interval; Stage II: localized; Stage III: metastasis.

Table 3. Subgroup analyses of histological response, event-free survival (EFS) and overall survival (OS)

| Outcome | Studies | No. | RR | LL | UL | Heterogeneity | | | P |
|------------------|---------|-----|------|------|------|---------------|----------------|-------|--------|
| | | | | | | Chi-squared | I ² | p | |
| Response (good) | 7 | 291 | 0.74 | 0.45 | 1.21 | 2.12 | 58% | 0.03 | 0.23 |
| Either II or III | 3 | 169 | 1.15 | 0.84 | 1.59 | 0.53 | 6% | 0.35 | 0.39 |
| II and III | 4 | 122 | 0.35 | 0.18 | 0.66 | 14.18 | 0 | 0.91 | 0.001 |
| EFS | 7 | 552 | 0.82 | 0.60 | 1.14 | 22.08 | 73% | 0.001 | 0.24 |
| Either II or III | 4 | 395 | 1.07 | 0.82 | 1.38 | 6.78 | 56% | 0.08 | 0.63 |
| II and III | 3 | 157 | 0.49 | 0.33 | 0.71 | 0.31 | 0 | 0.86 | <0.001 |
| OS | 8 | 502 | 0.79 | 0.58 | 1.08 | 24.99 | 72% | 0.001 | 0.14 |
| Either II or III | 4 | 326 | 1.10 | 0.93 | 1.30 | 3.49 | 14% | 0.32 | 0.28 |
| II and III | 4 | 176 | 0.46 | 0.32 | 0.67 | 0.96 | 0 | 0.81 | <0.001 |

Abbreviation: RR: relative risk; LL: lower limit; UL: upper limit; Stage II: localized; Stage III: metastasis.

Impact of Her-2 overexpression on histological response

The data from 7 retrieved studies including 291 patients [9, 10, 11, 17, 33, 34, 36] were

pooled and no statistical significance had been found in histological response to preoperative chemotherapy between patients with Her-2 overexpression versus those that were not (n = 7, RR = 0.74, 95% CI: 0.45 to 1.21, P = 0.23, see **Figure 2**). There was a significant degree of heterogeneity between these

studies, so we chose a random-effect model (P = 0.02 and I² = 61.9%). By subgroup analyses, studies including patients with both stage II and stage III osteosarcomas showed that overexpression of Her-2 was correlated

Prognostic role of Her-2 in osteosarcoma

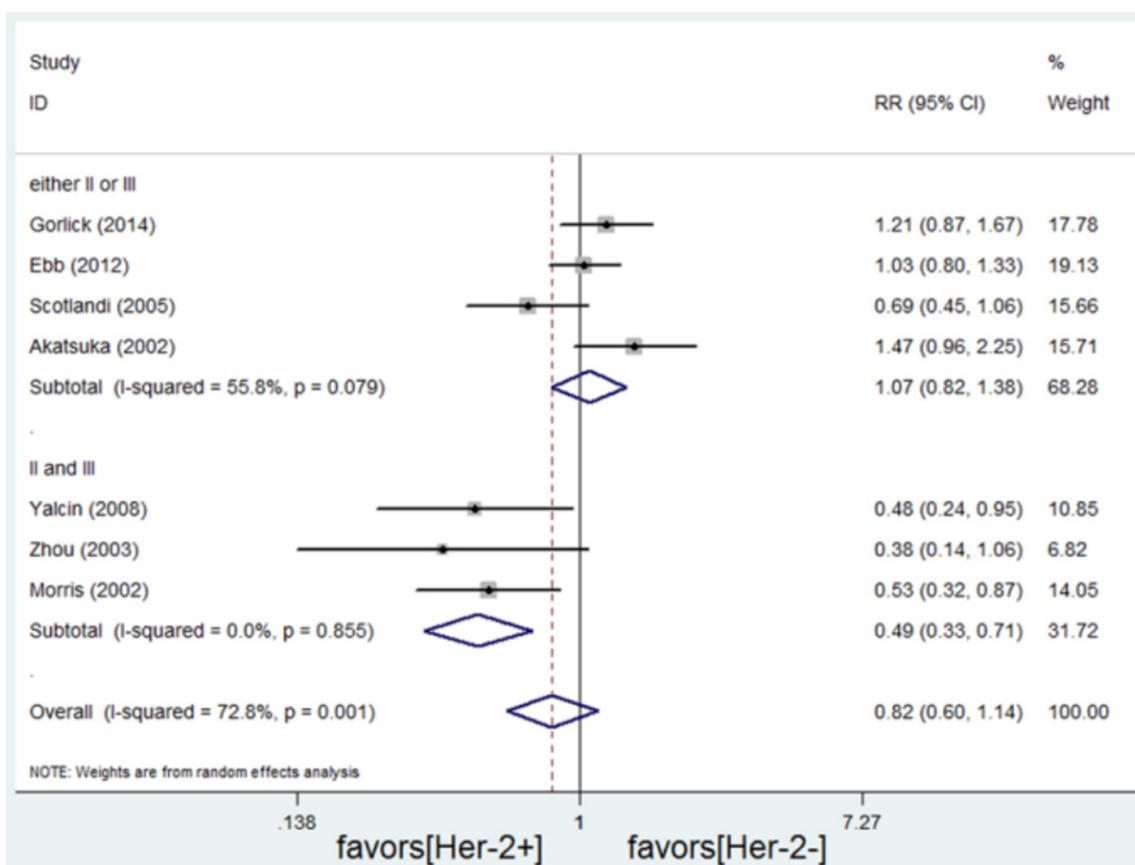


Figure 3. Her-2 expression and event-free survival in high-grade osteosarcomas. RR = Risk ratio; CI = Confidence interval; Stage II: localized; Stage III: metastasis.

with a significantly worse *histological* response ($n = 3$, RR = 0.35, 95% CI: 0.18 to 0.66, $P = 0.001$). And studies including patients with either stage II or stage III osteosarcomas demonstrated that there was no relation between the level of expression of Her-2 and response ($n = 4$, RR = 1.15, 95% CI: 0.84 to 1.59, $P = 0.53$, see **Table 3**).

Impact of Her-2 overexpression on EFS and OS

Event-free survival (EFS): Seven studies [8, 10, 11, 17, 33, 35, 36] with a total of 552 high-grade osteosarcoma patients dealing with Her-2 overexpression and EFS were meta-analyzed. Here, we adopted a random-effect model because of the heterogeneity ($P = 0.001$ and $I^2 = 73\%$). Her-2 overexpression had nonpredictive effect on EFS ($n = 7$, RR = 0.82, 95% CI: 0.60 to 1.14, $P = 0.24$). By subgroup analyses, studies including patients with both stage II and stage III osteosarcomas showed that overexpression of Her-2 was correlated with a significantly worse EFS ($n = 3$, RR = 0.49, 95% CI:

0.33 to 0.71, $P < 0.001$). And studies including patients with either stage II or stage III osteosarcomas demonstrated that there was no relation between the level of expression of Her-2 and EFS ($n = 4$, RR = 1.07, 95% CI: 0.82 to 1.38, $P = 0.63$, see **Figure 3** and **Table 3**).

Overall survival (OS): A total of 8 studies [8-11, 17, 32, 33, 36] provided RR values about OS. We chose a random-effect model to evaluate the differences between the above studies ($P = 0.001$ and $I^2 = 72\%$). Her-2 overexpression had nonpredictive effect on OS ($n = 8$, RR = 0.79, 95% CI: 0.58 to 1.08, $P = 0.14$). By subgroup analyses, the result indicated that Her-2 expression was not correlated with OS in either stage II or stage III osteosarcomas ($n = 4$, RR = 1.10, 95% CI: 0.93 to 1.30, $P = 0.28$). The studies including patients with both stage II and stage III osteosarcomas showed that high levels of Her-2 correlated significantly with decreased EFS ($n = 4$, RR = 0.46, 95% CI: 0.32 to 0.67, $P < 0.001$, see **Figure 4** and **Table 3**).

Prognostic role of Her-2 in osteosarcoma

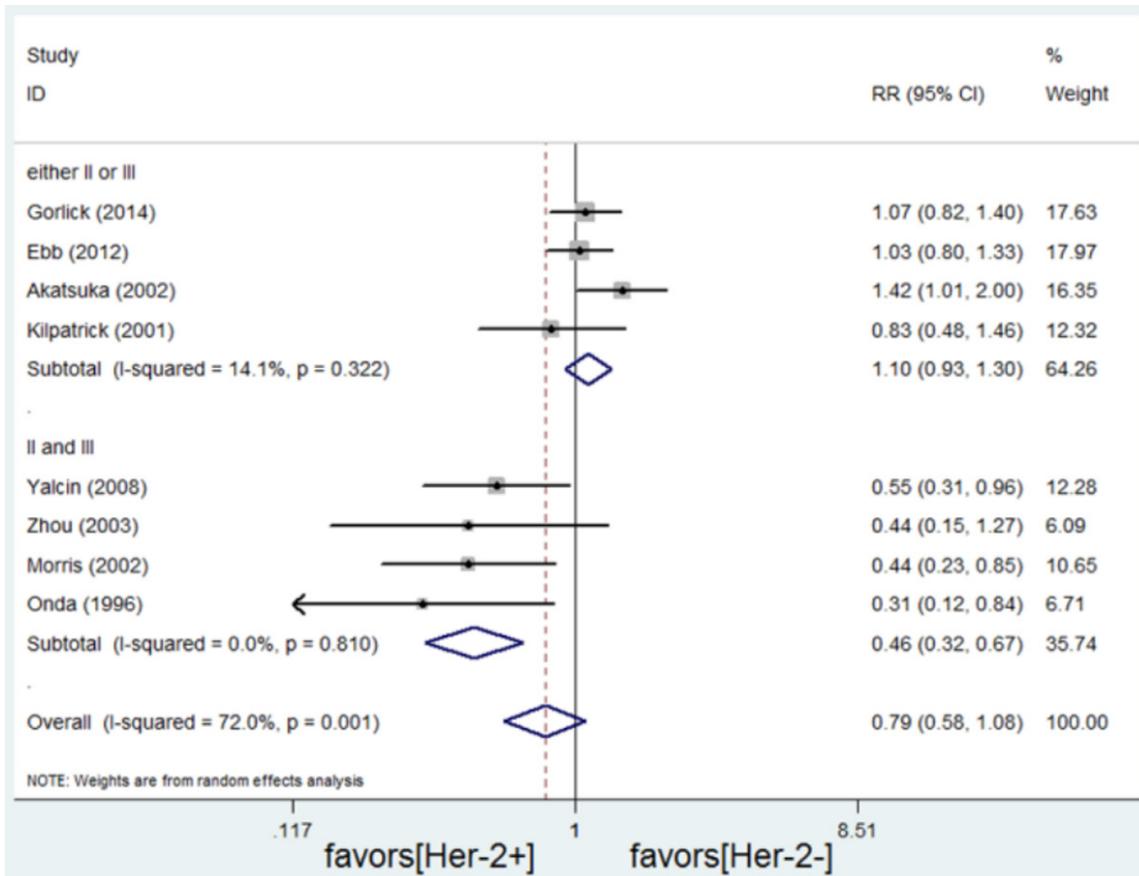


Figure 4. Her-2 expression and overall survival in high-grade osteosarcomas. RR = Risk ratio; CI = Confidence interval; Stage II: localized; Stage III: metastasis.

Publication bias: There was no evidence of asymmetry in the funnel plots. Simultaneously, formal evaluation using Egger's test failed to any reveal evidence for significant publication bias in the histological response ($P = 0.108$), EFS ($P = 0.298$) and OS ($P = 0.635$).

Discussion

Her-2 has been shown to be overexpressed in many human cancer, including breast, ovaries, lung, stomach, and salivary gland [7]. And it has been the most thoroughly evaluated in breast cancer. While the prognostic value of Her-2 status in patients with high-grade osteosarcoma is still controversial in the literature. The conflict results might be partly attributed to the small patient numbers in prior studies. Furthermore, in the studies examining the levels of expression of Her-2 by immunohistochemistry, there are differences in the antibodies being used, differences in the cut-offs of positive staining, and differences in the grading

systems used to define overexpression. As inherent limitations and inconsistent conclusions were found among previous studies, it was therefore necessary for us to synthesize as many studies as possible to summarize the most credible evidence.

Almost all the studies included in our meta-analysis were of high-quality. The majority of them were conducted in the United States [8, 10, 17, 32, 33], and the remaining were conducted in various European countries and Japan. The studies were published between 1996 and 2014; thus, they provided relatively new data. The standard treatment did not substantially change over this period of time, and worldwide measures of 5-year EFS and OS also were not significantly improved [37].

In the included studies, there were no significant differences in regard of gender and ALP levels, which might influence the outcomes. In addition, three studies offered individual

patient data so that we could ensure these included data with definite diagnose and long-enough follow-ups, and excluded the individual who was not accordant with our inclusion criteria. Finally, both subgroup analysis and Egger's test were conducted to identify possible reasons and ensure our results were more robust. All of the above made this meta-analysis more comprehensive and credible.

It was confusing that the younger patients were with lower rate of Her-2 overexpression. It may be partly due to early diagnosis. Previous meta-analysis by Collins [38] suggested that there was a significant association of age with toxicity of chemotherapy. So there also might be a potential relation between age and Her-2 overexpression, and we couldn't rule out the confounding effect of age on toxicity and/or survival.

There's no doubt that histological response to neo-adjuvant chemotherapy significantly correlated with subsequent treatment and prognosis in high-grade osteosarcoma [39]. This meta-analysis confirmed that Her-2 status was not associated with resistance to chemotherapeutic agents. Then the primary results suggested that Her-2 expression was not associated with 5-year EFS or OS in patients with high-grade osteosarcoma. It is well established that the presence of metastasis at diagnosis is an important prognostic factor. By subgroup analyses, we found the high heterogeneity above could be partly explained by whether metastasis and local disease were included together or not. Her-2 expression was associated with Tumor metastasis which could influence chemotherapy response and prognosis. Further large-scale prospective studies are warranted to confirm this correlation, and it's urgent to develop novel molecular biology markers in non-metastatic high-grade osteosarcomas.

Nonetheless, it should be circumspect to make a verdict of the association with Her-2 and high-grade osteosarcoma, because there are still several issues should be considered. First, a consensus should be reached regarding the most reliable and reproducible methods for determining Her-2 status. Second, more well-designed clinical studies with large samples of high-grade osteosarcoma should be performed in the future to validate the relationship between Her-2 expression level and prognosis.

Third, the high levels of statistical heterogeneity among included studies made some conclusions cautiously even we conducted subgroup analyses. Finally, only one study used targeted drug in combination with standard chemotherapy in patients with high-grade metastatic osteosarcoma simultaneously, we were not able to evaluate the impact of targeted drug delivery on prognosis. Understanding the underlying mechanisms would lend confidence to the clinical application of targeted therapy based on Her-2 pathway in high-grade osteosarcoma in the further.

In conclusion, this meta-analysis has shown that the Her-2/ErbB2 overexpression is not significantly associated with histological response, EFS or OS in patients with high-grade osteosarcoma. And Her-2 expression is associated with tumor metastasis which can influence chemotherapy response and prognosis. Further multicenter, well designed prospective studies with larger patient cohorts will be required to confirm these findings and more clinical studies should be carried out before Her-2/ErbB2 can be used as a prognostic factor or in the treatment of high-grade osteosarcoma.

Disclosure of conflict of interest

None.

Address correspondence to: Peijian Tong, Department of Orthopedics, The First Affiliated Hospital of Zhejiang Chinese Medicine University, Hangzhou 310009, P. R. China. E-mail: szytj@163.com

References

- [1] ESMO/European Sarcoma Network Working Group. Bone sarcomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2012; 23 Suppl 7: vii100-vii109.
- [2] Marina N, Gebhardt M, Teot L and Gorlick R. Biology and therapeutic advances for pediatric osteosarcoma. *Oncologist* 2004; 9: 422-441.
- [3] Bielack SS, Kempf-Bielack B, Delling G, Exner GU, Flege S, Helmke K, Kotz R, Salzer-Kuntschik M, Werner M, Winkelmann W, Zoubek A, Jürgens H and Winkler K. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. *J Clin Oncol* 2002; 20: 776-790.
- [4] Smeland S, Müller C, Alvegard TA, Wiklund T, Wiebe T, Björk O, Stenwig AE, Willén H, Holm-

Prognostic role of Her-2 in osteosarcoma

- ström T, Follerås G, Brosjö O, Kivioja A, Jons-son K, Monge O and Sæter G. Scandinavian Sarcoma Group Osteosarcoma Study SSG VIII: prognostic factors for outcome and the role of replacement salvage chemotherapy for poor histological responders. *Eur J Cancer* 2003; 39: 488-494.
- [5] Kim MS, Cho WH, Song WS, Lee SY and Jeon DG. Time dependency of prognostic factors in patients with stage II osteosarcomas. *Clin Orthop Relat Res* 2007; 463: 157-165.
- [6] Pegram M and Slamon D. Biological rationale for HER2/neu (c-erbB2) as a target for monoclonal antibody therapy. *Semin Oncol* 2000; 27: 13-19.
- [7] Hynes NE. Amplification and overexpression of the erbB-2 gene in human tumors: its involvement in tumor development, significance as a prognostic factor, and potential as a target for cancer therapy. *Semin Cancer Biol* 1993; 4: 19-26.
- [8] Gorlick S, Barkauskas DA, Krailo M, Piperdi S, Sowers R, Gill J, Geller D, Randall RL, Janeway K, Schwartz C, Grier H, Meyers PA, Gorlick R, Bernstein M and Marina N. HER-2 expression is not prognostic in osteosarcoma; a Children's Oncology Group prospective biology study. *Pediatr Blood Cancer* 2014; 61: 1558-1564.
- [9] Onda M, Matsuda S, Higaki S, Iijima T, Fukushima J, Yokokura A, Kojima T, Horiuchi H, Kurokawa T and Yamamoto T. ErbB-2 expression is correlated with poor prognosis for patients with osteosarcoma. *Cancer* 1996; 77: 71-78.
- [10] Zhou H, Randall RL, Brothman AR, Maxwell T, Coffin CM and Goldsby RE. Her-2/neu expression in osteosarcoma increases risk of lung metastasis and can be associated with gene amplification. *J Pediatr Hematol Oncol* 2003; 25: 27-32.
- [11] Akatsuka T, Wada T, Kokai Y, Kawaguchi S, Isu K, Yamashiro K, Yamashita T, Sawada N, Yamawaki S and Ishii S. ErbB2 expression is correlated with increased survival of patients with osteosarcoma. *Cancer* 2002; 94: 1397-1404.
- [12] Bakhshi S, Gupta A, Sharma MC, Khan SA and Rastogi S. Her-2/neu, p-53, and their co-expression in osteosarcoma. *J Pediatr Hematol Oncol* 2009; 31: 245-251.
- [13] Tsai JY, Aviv H, Benevenia J, Chang VT, Patterson F, Aisner S and Hameed M. HER-2/neu and p53 in osteosarcoma: an immunohistochemical and fluorescence in situ hybridization analysis. *Cancer Invest* 2004; 22: 16-24.
- [14] Anninga JK, van de Vijver MJ, Cleton-Jansen AM, Kristel PM, Taminiau AH, Nooij M, Egeler RM and Hogendoorn PC. Overexpression of the HER-2 oncogene does not play a role in high-grade osteosarcomas. *Eur J Cancer* 2004; 40: 963-970.
- [15] Somers GR, Ho M, Zielenska M, Squire JA and Thorner PS. HER2 amplification and overexpression is not present in pediatric osteosarcoma: a tissue microarray study. *Pediatr Dev Pathol* 2005; 8: 525-532.
- [16] Thomas DG, Giordano TJ, Sanders D, Biermann JS and Baker L. Absence of HER2/neu gene expression in osteosarcoma and skeletal Ewing's sarcoma. *Clin Cancer Res* 2002; 8: 788-793.
- [17] Ebb D, Meyers P, Grier H, Bernstein M, Gorlick R, Lipshultz SE, Krailo M, Devidas M, Barkauskas DA, Siegal GP, Ferguson WS, Letson GD, Marcus K, Goorin A, Beardsley P and Marina N. Phase II trial of trastuzumab in combination with cytotoxic chemotherapy for treatment of metastatic osteosarcoma with human epidermal growth factor receptor 2 overexpression: a report from the children's oncology group. *J Clin Oncol* 2012; 30: 2545-2551.
- [18] Mirabello L, Troisi RJ and Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. *Cancer* 2009; 115: 1531-1543.
- [19] Liu Y, Ma YH, Sun ZZ, Rui YJ, Yin QD, Song S, Wei XM, Liu J, Liu XG and Hu KJ. Effect of c-erbB2 overexpression on prognosis in osteosarcoma: evidence from eight studies. *Tumour Biol* 2014; 35: 8939-8943.
- [20] Liu F, Zhang Q, Li J, Li Z, Jia H and Zhou D. Comments on Liu Yet al. Effect of c-erbB2 overexpression on prognosis in osteosarcoma: evidence from eight studies. *Tumour Biol* 2015; 36: 2211-2212.
- [21] Yin X, Deng F and Liao G. Question about Liu Y et al. entitled "Effect of c-erbB2 overexpression on prognosis in osteosarcoma: evidence from eight studies". *Tumor Biol* 2015; 1-2.
- [22] Moher D, Liberati A, Tetzlaff J and Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg* 2010; 8: 336-41.
- [23] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010; 25: 603-605.
- [24] Egger M, Smith GD, Schneider M and Minder C. Bias in meta-analysis detected by a simple, graphical test. *Bmj* 1997; 315: 629-634.
- [25] Abdou AG, Kandil M, Asaad NY, Dawoud MM, Shahin AA and Abd Eldayem AF. The Prognostic Role of Ezrin and HER2/neu Expression in Osteosarcoma. *Appl Immunohistochem Mol Morphol* 2016; 24: 355-363.
- [26] Akatsuka T, Wada T, Kokai Y, Sawada N, Yamawaki S and Ishii S. Loss of ErbB2 expression in pulmonary metastatic lesions in osteosarcoma. *Oncology* 2001; 60: 361-366.

Prognostic role of Her-2 in osteosarcoma

- [27] Becker RG, Galia CR, Morini S and Viana CR. Immunohistochemical expression of vegf and her-2 proteins in osteosarcoma biopsies. *Acta Ortop Bras* 2013; 21: 233-238.
- [28] Boulytcheva IV, Soloviev YN, Kushlinskii NE and Mahson AN. Expression of molecular markers in the tumor and survival prognosis in osteosarcoma. *Bull Exp Biol Med* 2010; 150: 237-242.
- [29] Ferrari S, Bertoni F, Zanella L, Setola E, Bacchini P, Alberghini M, Versari M and Bacci G. Evaluation of P-glycoprotein, HER-2/ErbB-2, p53, and Bcl-2 in primary tumor and metachronous lung metastases in patients with high-grade osteosarcoma. *Cancer* 2004; 100: 1936-1942.
- [30] Gorlick R, Huvos AG, Heller G, Aledo A, Beardsley GP, Healey JH and Meyers PA. Expression of HER2/erbB-2 correlates with survival in osteosarcoma. *J Clin Oncol* 1999; 17: 2781-2788.
- [31] Rakesh Kumar V, Gupta N, Kakkar N and Sharma SC. Prognostic and predictive value of c-erbB2 overexpression in osteogenic sarcoma. *J Cancer Res Ther* 2006; 2: 20-23.
- [32] Kilpatrick SE, Geisinger KR, King TS, Sciarrotta J, Ward WG, Gold SH and Bos GD. Clinicopathologic analysis of HER-2/neu immunorepression among various histologic subtypes and grades of osteosarcoma. *Mod Pathol* 2001; 14: 1277-1283.
- [33] Morris CD, Gorlick R, Huvos G, Heller G, Meyers PA and Healey JH. Human epidermal growth factor receptor 2 as a prognostic indicator in osteogenic sarcoma. *Clin Orthop Relat Res* 2001; 59-65.
- [34] Munoz A, Alfaro J, Pardo N, Garcia-Miguel P, Quintero V, Gros L, Melero C, Antuna MJ, Ocete G, de Las Heras J, Ruiz del Portal M, Huguet R and Maldonado MS. Long-term results of the Spanish Protocol SO-95 for the treatment of non-metastatic high-grade osteosarcoma of the extremities in children. *Clin Transl Oncol* 2009; 11: 387-392.
- [35] Scotlandi K, Manara MC, Hattinger CM, Benini S, Perdichizzi S, Pasello M, Bacci G, Zanella L, Bertoni F, Picci P and Serra M. Prognostic and therapeutic relevance of HER2 expression in osteosarcoma and Ewing's sarcoma. *Eur J Cancer* 2005; 41: 1349-1361.
- [36] Yalcin B, Gedikoglu G, Kutluk T, Varan A, Akyuez C and Bueyuekpamukcu M. C-erbB-2 expression and prognostic significance in osteosarcoma. *Pediatr Blood Cancer* 2008; 51: 222-227.
- [37] Craft AW. Osteosarcoma: the European Osteosarcoma Intergroup (EOI) perspective. *Cancer Treat Res* 2009; 152: 263-274.
- [38] Collins M, Wilhelm M, Conyers R, Herschtal A, Whelan J, Bielack S, Kager L, Kuhne T, Sydes M, Gelderblom H, Ferrari S, Picci P, Smeland S, Eriksson M, Petrilli AS, Bleyer A and Thomas DM. Benefits and adverse events in younger versus older patients receiving neoadjuvant chemotherapy for osteosarcoma: findings from a meta-analysis. *J Clin Oncol* 2013; 31: 2303-2312.
- [39] Whelan JS, Jinks RC, McTiernan A, Sydes MR, Hook JM, Trani L, Uscinska B, Bramwell V, Lewis IJ, Nooij MA, van Glabbeke M, Grimer RJ, Hogendoorn PC, Taminiu AH and Gelderblom H. Survival from high-grade localised extremity osteosarcoma: combined results and prognostic factors from three European Osteosarcoma Intergroup randomised controlled trials. *Ann Oncol* 2012; 23: 1607-1616.