Original Article
Lack of association between bcl-2 expression and prognosis of osteosarcoma: a meta-analysis

Tao Fu[1*], Chengyan Xia[1*], Zonghuan Li[2], Hua Wu[1]

[1]Department of Orthopedics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China; [2]Department of Orthopedics, Zhongnan Hospital of Wuhan University, Wuhan 430071, China. *Equal contributors and co-first authors.

Abstract: Several studies investigated the bcl-2 in prognosis of osteosarcoma, but no consistent conclusions were achieved. This meta-analysis was conducted to determine the prognostic role of bcl-2 in osteosarcoma. Databases including Pubmed, Embase, Cochrane library, Google scholar, Wanfang and CNKI were searched systematically up to March 1, 2014. Cohort studies assessing the prognostic role of bcl-2 expression in patients with osteosarcoma were included. Pooled odds ratio (OR) with 95% confidence intervals (95% CI) was adopted. Sensitivity analysis was also performed. Five studies with a total of 202 patients were included in final analysis. Compared with positive bcl-2 expression, negative bcl-2 expression was associated with better 3-year overall survival (OR=0.21, 95% CI 0.07-0.65, P=0.007). No significant difference was achieved with respect to 5-year overall survival (OR=0.76, 95% CI 0.42-1.38, P=0.264) and diseases-free survival (OR=1.20, 95% CI 0.47-3.06, P=0.709). Sensitivity analysis indicates the conclusion was stable. This meta-analysis suggests that the bcl-2 expression may be independent with the prognosis for patients with osteosarcoma. Nevertheless, additional well-designed studies with larger sample size are needed to further confirm the results.

Keywords: Bcl-2, osteosarcoma, prognosis, meta-analysis

Introduction

Osteosarcoma is the most common primary bone malignancy in children and adolescents [1]. Since the 1980's, the regimen consists of a multi-drug chemotherapy followed by limb-sparing surgery has been recommended for the treatment of osteosarcoma [2], which leads to significant improvement for patients survival [2]. However, the 5-year survival rates are 70% for patients with non-metastatic osteosarcoma [3], and only 20%-30% for patients with metastatic osteosarcoma [4]. Therefore, Identification of prognostic factors effective on treatments selection for patients with osteosarcoma will be vital. Bcl-2 is a main member of bcl-2 family and it is also suggested to play an important role in anti-apoptosis [5]. It promotes cell survival and is considered as an oncogene for most cancers [5]. However, previous studies have suggested that positive bcl-2 is associated with better prognosis in lung cancer [6] and breast cancer [7]. Although there are several studies on bcl-2 in the prognosis of osteosar-
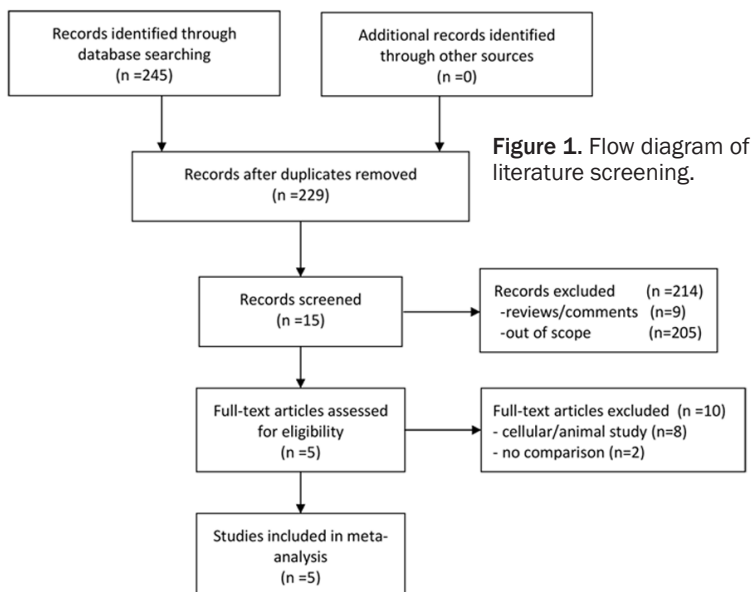
coma [8-12], but the conclusion are still are controversial. Some studies suggested that positive expression of bcl-2 may be a potential biomarker for predicting the poor prognosis [8, 9, 11], while other researches demonstrated that bcl-2 is not a reliable prognostic marker [10, 12]. Therefore, we performed a meta-analysis on the published studies to evaluate the prognostic role of bcl-2 in osteosarcoma.

Materials and methods

Search strategy

We systematically searched databases (Pubmed, Embase, Cochrane library, Google scholar, Wanfang Data and CNKI) from their establishment to March 1, 2014 for cohort studies on the prognosis role of bcl-2 in patients with osteosarcoma. For English database search, terms used were "osteosarcoma" or "bone sarcoma" or "osteogenic sarcoma" or "osteogenic sarcoma" and "bcl-2" or "B-cell leukemia -2" or "B-cell lymphoma-2". For Chinese database

Figure 1. Flow diagram of literature screening.

excluding one study a time. A P value less than 0.05 was considered statistically significant.

Results

Study characteristics

As showed in **Figure 1**, a total of 245 studies were retrieved from the database search and reference list check and only 15 articles remained by titles and abstracts screening. After full-text evaluation, 5 studies [8-12] met the eligibility criteria and were included in final analysis. Four studies were published in English [8-11] and one in Chinese [12]. All included studies [8-12] were retrospective cohort studies with a total of 202 patients. The general characteristics of included studies were showed in **Table 1**. Two studies provided data on 3-year overall survival rate [10, 12]. Four studies provided data on 5-year overall survival rate [8, 9, 11, 12]. Disease-free survival rate was reported in two studies [8, 9]. Among the 202 patients, 84 (41.6%) patients had positive bcl-2 expression, while the remaining 118 (58.4%) patients had negative bcl-2 expression.

Positive bcl-2 expression and 3-year OS in osteosarcoma

Three-year OS was reported in two studies [10, 12]. Between-study heterogeneity was negligible (I^2=18%), and the fixed-effects model was used to pool the estimations. The results indicated that patients with positive bcl-2 expression had lower 3-year OS (OR=0.21, 95% CI 0.07-0.65, *P*=0.007, **Figure 2A**). Sensitivity analysis suggested patients with positive bcl-2 expression presented a tendency of worse 3-year OS, though it did not achieve statistically significance (**Figure 2B**).

Positive bcl-2 expression and 5-year OS in osteosarcoma

Five-year OS was reported in four studies [8, 9, 11, 12]. Between-study heterogeneity existed and was negligible (I^2=24.6%), and the fixed-effects model was used to pool the results. The pooled estimations suggested that no differ-

search, terms were translated into corresponding Chinese. Reference lists of relevant studies were also checked for eligible studies.

Eligibility criteria

The eligibility criteria in this meta-analysis were the following: (1) patients diagnosed with osteosarcoma pathologically; (2) different bcl-2 expression levels (negative and positive) were reported; (3) outcomes including overall survival rate (OS) and disease-free survival rate (DFS) were reported.

Data extraction

Two authors extracted the data independently. The general information (first author, published year, location, sample size, average age, male/female ratio, detection method) and outcomes (overall survival rate and disease-free survival rate) were extracted.

Statistical analysis

Statistical analysis was performed with Stata 12.0 (StataCorp LP, College Station, TX, USA). Odds ratio (OR) with 95% confidence intervals (95% CI) was used to assess the prognostic role of bcl-2 in osteosarcoma. Statistical heterogeneity was estimated with I^2 value. Namely, when I^2<25%, heterogeneity could be neglected and the fixed-effects model was used. Otherwise the randomized-effects model was used. Sensitivity analysis was performed to evaluate the stability of pooled estimation by

Table 1. General characteristics of included studies

Included study	Country	Cases (M/F)[a]	Age (years)	Detection method	Outcomes
Kaseta 2008	Greece	35 (19/16)	30 (14-67)	IHC	5-year DFS, 5-year OS
Nedelcu 2008	Austria	29 (19/10)	22 (9-57)	IHC	5-year DFS, 5-year OS
Wu 2012	China	56 (36/20)	13-37	IHC	3-year OS
Feng 2013	China	36 (17/19)	4-12	IHC	3-year OS, 5-year OS
Trieb 2013	Austria	49 (28/21)	22 (9-53)	IHC	5-year OS

[a]The number of males or females; IHC: Immunohistochemistry; DFS: disease free survival; OS: overall survival.
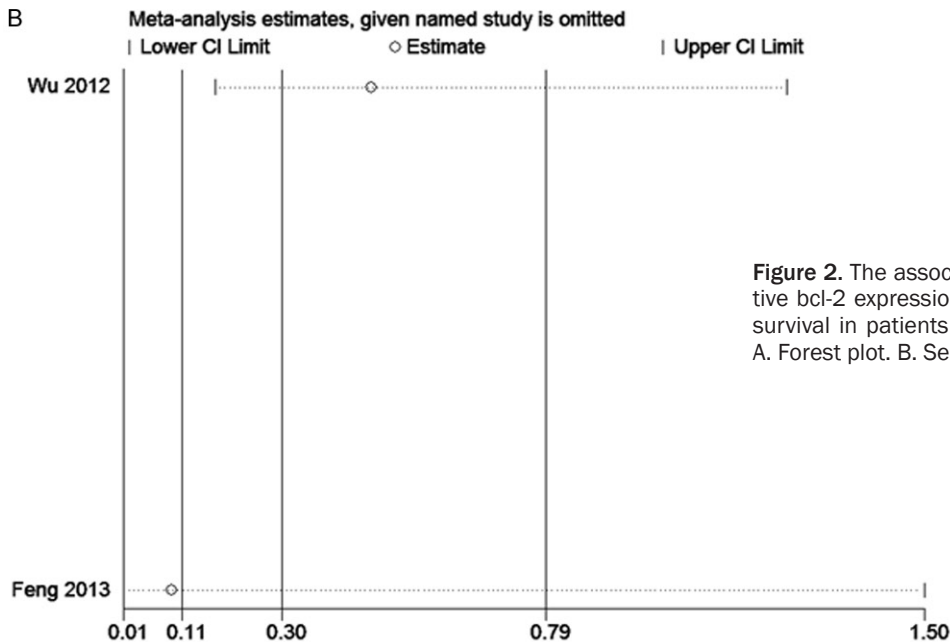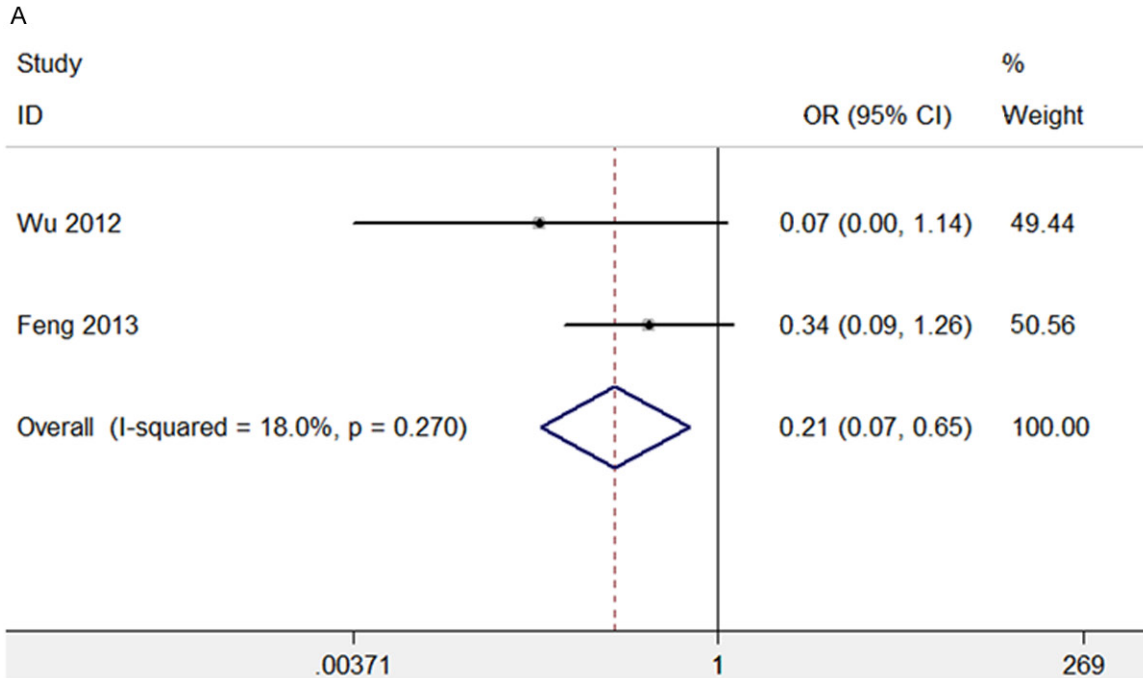

Figure 2. The association between positive bcl-2 expression and 3 year overall survival in patients with osteosarcoma. A. Forest plot. B. Sensitive analysis.

A

Study

ID

OR (95% CI)   %
              Weight

Kaseta 2008   1.25 (0.28, 5.53)   39.07

Nedelcu 2008   1.16 (0.35, 3.90)   60.93

Overall (I-squared = 0.0%, p = 0.940)   1.20 (0.47, 3.06)   100.00

.181   1   5.53

B

Meta-analysis estimates, given named study is omitted

| Lower CI Limit   ○ Estimate | Upper CI Limit

Kaseta 2008 |⋯⋯⋯⋯○⋯⋯⋯⋯⋯⋯⋯|

Nedelcu 2008   ⋯⋯⋯○⋯⋯⋯⋯⋯⋯⋯⋯⋯|

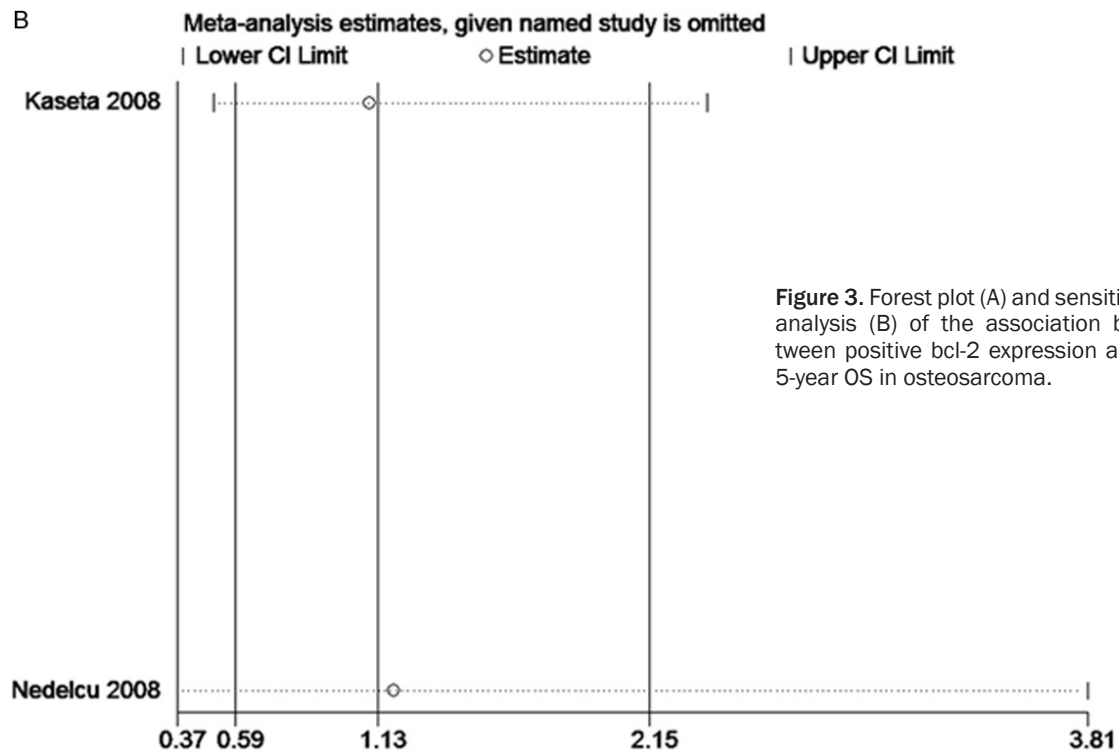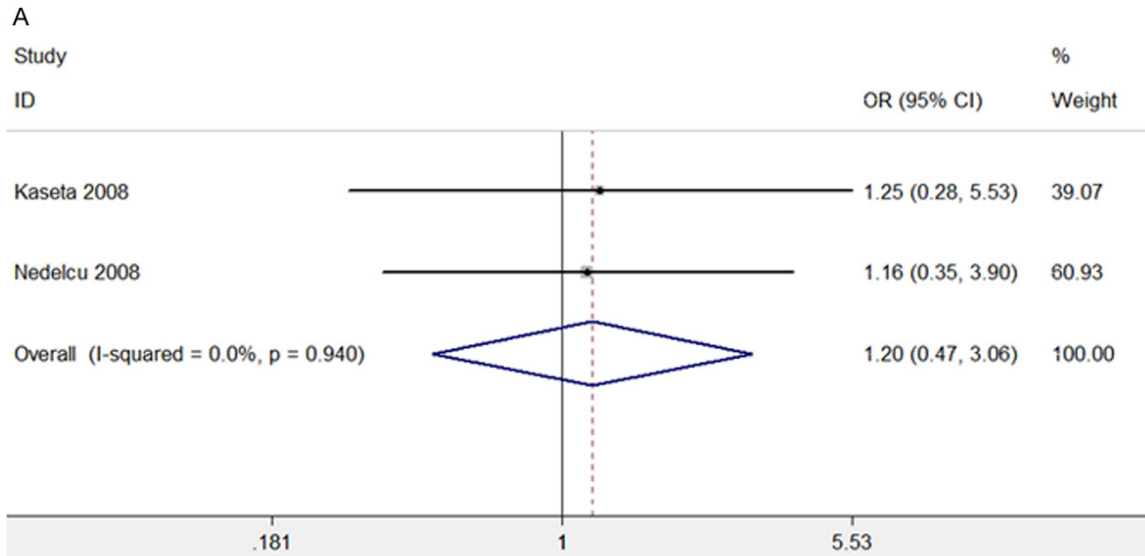0.37 0.59   1.13   2.15   3.81

Figure 3. Forest plot (A) and sensitive analysis (B) of the association between positive bcl-2 expression and 5-year OS in osteosarcoma.

ence was detected between the positive and negative bcl-2 expression with respect to 5-year OS (OR=0.76, 95% CI 0.42-1.38, *P*=0.264, **Figure 3A**). The results of sensitivity analysis were in accordance with the pooled results (**Figure 3B**).

Positive bcl-2 expression and DFS in osteosarcoma

DFS was reported in two studies [8, 9]. There was also no between-study heterogeneity

(I^2=0%), and the fixed-effects model was used to pool the results. No difference was detected between the positive and negative bcl-2 expression with respect to DFS (OR=1.20, 95% CI 0.47-3.06, *P*=0.709, **Figure 4A**). The results of sensitivity analysis were in accordance with the pooled results (**Figure 4B**).

Discussion

The prognostic role of bcl-2 in patients with osteosarcoma was controversial. Our meta-

A

Study ID	OR (95% CI)	% Weight
Kaseta 2008	1.20 (0.30, 4.74)	15.00
Nedelcu 2008	1.12 (0.35, 3.59)	21.50
Feng 2013	0.11 (0.01, 0.97)	29.17
Trieb 2013	0.89 (0.33, 2.37)	34.33
Overall (I−squared = 24.6%, p = 0.264)	0.76 (0.42, 1.38)	100.00

.0129　　　　1　　　　77.4

B

Meta−analysis estimates, given named study is omitted

| Lower CI Limit　　　○ Estimate　　　| Upper CI Limit

Kaseta 2008

Nedelcu 2008

Feng 2013

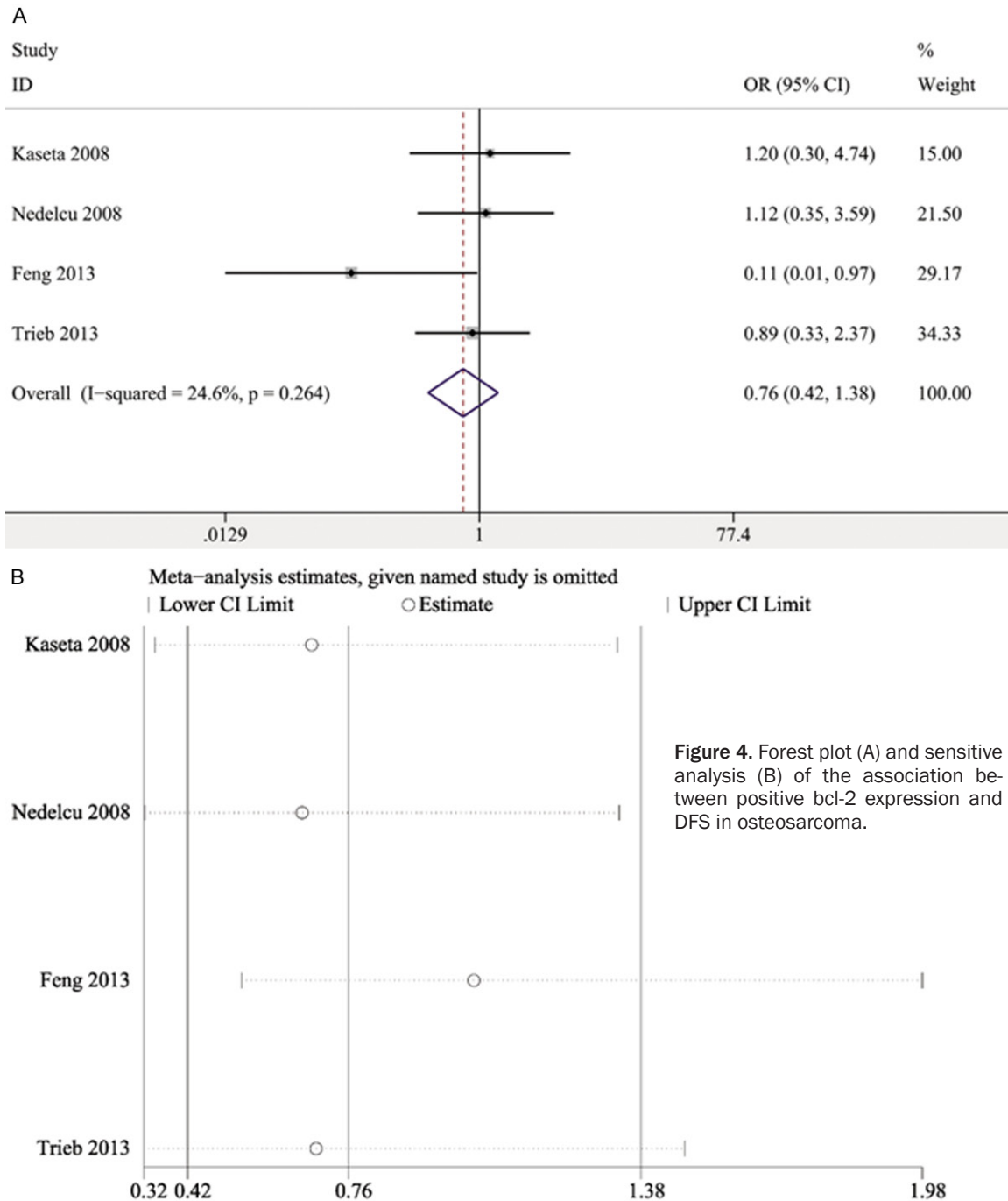Trieb 2013

0.32 0.42　　　0.76　　　1.38　　　1.98

Figure 4. Forest plot (A) and sensitive analysis (B) of the association between positive bcl-2 expression and DFS in osteosarcoma.

analysis revealed that positive bcl-2 expression predicted poor 3-year OS in patients with osteo-sarcoma. However, bcl-2 expression in patients with osteosarcoma was independent with 5-year OS and DFS.

Bcl-2 is an important member in bcl-2 family and it has a variety effects in cancer [13]. Accumulated evidence shows that bcl-2 inhibits

apoptosis and promotes cell survival [5]. It is overexpressed in many cancers and contributes to tumorigenesis, progression and resistance to therapy [5]. Down-regulated bcl-2 expression enhances sensitivity to anticancer drugs and improves in vivo survival [14]. However, a meta-analysis on bcl-2 and lung cancer demonstrated that bcl-2 expression was associated with better prognosis in non-

small cell lung cancer [6]. The similar association was also observed in breast cancer [7] and distal colorectal adenocarcinomas [15]. An immunohistochemical study demonstrated the bcl-2 expression was moderate-to-strong in 81% patients with osteosarcoma [16]. The results of our meta-analysis suggested that bcl-2 expression may not be correlated with the prognosis of osteosarcoma, although pooled estimation exhibited that patient with bcl-2 positive expression had lower 3-year OS in osteosarcoma.

Our findings showed that patient with positive bcl-2 expression predicted poor 3-year OS in osteosarcoma, while no difference was detected with respect to 5-year OS and DFS. Here are several explanations: First, only five studies which carried out in different regions met the included criteria for our meta-analysis. Among them, two were conducted in China, two in Austria and one in Greece. Bcl-2 expression in different human population may be not exactly the same, and the role of the same molecule in different populations may be various. The results of 3-year OS were based on two studies from China, while the rest was not. Moreover, the small number of included studies and sample size might also contribute to the inconsistency.

The current meta-analysis was based on an aggregation of data from published studies. There were some limitations that we should take into consideration. First, the number of both included studies and patients were relatively small, which might lead to false negative. Namely, bcl-2 expression might do have a role in prognosis of patients with osteosarcoma. Our small sample size might mast such effects. Thus, large sample size, more well-designed, prospective studies were needed to confirm the role of bcl-2. Second, due to limited included studies, we are unable to perform the subgroup analysis and publication bias. Furthermore, various molecules functioned as a network both intracellularly and extracellularly [17]. Bcl-2 family not only consists of many anti-apoptosis molecules (bcl-2, bcl-xl), also haslots of pro-apoptosis molecules (bax, bak) and both play a crucial role in apoptosis [5]. Therefore, the importance of single expression abnormality of bcl-2 might be limited. A combined analysis of all these factors may be more meaningful.

In conclusion, there is a lack of evidence that bcl-2 expression is associated with prognosis of osteosarcoma. Although the sensitivity analysis showed that the estimations in our meta-analysis were stable, the conclusion still needs support from more well-designed and prospective study.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hua Wu, Department of Orthopedics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China. E-mail: wuhua360@aliyun.com

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