

Review Article

The prognostic value of ezrin expression in bone and soft tissue sarcomas: a meta-analysis

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Abstract: Previous studies have indicated an association between ezrin expression and prognosis for patients with bone and soft tissue sarcomas. However, conclusions from these studies were remain controversial. Thus, we examine the association of ezrin expression with prognosis for patients with bone and soft tissue sarcomas. The association of ezrin expression with bone and soft tissue sarcomas prognosis was searched from various databases including PubMed, Cochrane Library, Embase, China Biology Medicine disc (CBM), China National Knowledge Infrastructure (CNKI) up to July 10th, 2017 and the meta-analysis was performed with STATA software. Relative ratios (RRs) with 95% confidence intervals (CIs) were calculated to evaluate the strength of the correlations. Besides, different subgroup-analyses and a publication bias test were performed. Through a systematic literature search, 15 published studies comprising 1070 patients were identified in this meta-analysis. Ezrin expression in bone and soft tissue sarcomas was associated with unfavorable overall survival (RR=1.85, 95% CI: 1.34-1.57, P<0.001). Likewise, similar results were found in different subgroup-analyses of country, test method, analysis method, sample source and size. Additionally, ezrin expression in bone and soft tissue sarcomas was linked with poor metastasis-free survival (RR=2.91, 95% CI: 1.56-5.41, P<0.001) and event-free survival (RR=2.35, 95% CI: 1.02-5.41, P<0.001). In summary, this meta-analysis towards ezrin expression has indicated poor prognosis of bone and soft tissue sarcomas. Future studies comprising larger cohort size from multicenter are required to confirm our conclusions.

Keywords: Ezrin, overall survival, metastasis-free survival, event-free survival, sarcomas

Introduction

Sarcomas approximately account for 1% of adult tumors and 15% of paediatric tumors [1]. Generally, sarcomas are a heterogeneous neoplasms that can be grouped into two types: soft tissue sarcoma (STS) and primary bone sarcoma (BS) [2]. Primary bone sarcomas mainly consist of osteosarcoma, Ewing's sarcoma and chondrosarcoma; soft tissue sarcomas mainly include synovial sarcoma, liposarcoma, leiomyosarcoma and angiosarcoma. With the emergence of effective chemotherapy regimens and the development of surgical techniques, the 5-year disease-free survival rate for these patients approaches about 50-70% [3, 4]. However, metastasis still occurs in 20-55% of these patients, which ultimately may be the leading cause of sarcoma-specific death [5]. Identification of effective prognostic factors is of great importance to predict the survival outcomes of STS and BS, and also helpful to devel-

op novel and effective therapeutic approaches.

Ezrin is a membrane cytoskeletal linker protein which is encoded by the VIL2 gene, and it is a vital member of ezrin, radixin and moesin (ERM) family of proteins [6, 7]. ERM proteins occur in the cytoplasm in an inactive closed conformation with N-terminal to C-terminal associations within the protein or with the other ERM members. Upon threonine and tyrosine phosphorylation, ezrin assumes an active open conformation, moves to the cell membrane and tethers F-actin directly or indirectly to the cell membrane [8]. It is also related to the Rho mediated signal transduction pathway and to the Akt mediated apoptotic pathway [9]. Previous studies have detected that ezrin was actively involved in the metastatic process of cancer cells, and even was regarded as a potential prognostic marker [10-12]. Recently, gene expression and proteomic studies also have

suggested the possible role of ezrin in metastasis in STS and BS [13, 14]. The identification of ezrin expression is a necessary component in sarcomas and it could be valuable for exploring its prognostic marker. However, conclusions for its prognostic value were still controversial, which could be explained by the relatively small sample size in each published study. Meta-analysis can explore the authentic and comprehensive results through incorporating all available evidences to get a more precise and accurate estimation by using statistical software [15]. Herein, we conduct the present meta-analysis to analyze the prognosis of ezrin expression in STS and BS.

Materials and methods

Identification and eligibility of relevant studies

Literature resources including PubMed, Cochrane Library, Embase, CBM and CNK were searched for eligible literatures, using the terms (“osteosarcoma OR sarcoma OR sarcomas OR chondrosarcoma OR Ewing sarcoma OR leiomyosarcoma OR angiosarcoma OR synovial sarcoma OR malignant fibrous OR histiocytoma OR liposarcoma OR rhabdomyosarcoma”), (“survival OR prognosis OR prognostic”) and (“ezrin”). Last search of current investigation was updated on July 10th, 2017. Additionally, the publication language was only limited to English and Chinese. In case of omission, we identified the reference lists of the relevant articles and review articles to seek for the potentially relevant studies. We did not contact the corresponding authors if the relevant data was unavailable.

Inclusion and exclusion criteria

Studies followed the following criteria could be identified: (1) clinical study about the association of ezrin with STS and BS prognostic value; (2) we can get relevant available data of RRs and 95% CIs to evaluate its associations.

Studies met the following four criteria were excluded: (1) the available data regarding about associations was absent; (2) similar or duplicate study (When the same or similar cohort was applied, after careful examination, the most complete information was included); (3) other types of articles including reviews or abstracts; (4) studies were involved with cells lines or animal model researches.

Data extraction

In the light of inclusion and exclusion criteria, we extracted the relevant information from each eligible publication. If disagreements were noticed, we are clearly open to discussion by each other (K. Xie and J. Yang), or reviewed by a third author (S. Fang). The data on first author, publication year, study country, age, cancer type, sample source, test method, follow-up year, sample size, survival outcome, analysis method, RR (95% CI), the cut-off value and the Newcastle-Ottawa Scale (NOS) scores were collected by two authors independently. We did not contact any authors of the original researches even though the essential information could not be available. Besides, study country came from Asia, Europe and others. Sample source was stratified into tissue, formalin-fixed and paraffin-embedded (FFPE) and tissue microarray (TMA); Test method included immunohistochemistry (IHC), and reverse transcription-polymerase chain reaction (RT-PCR); Sample size was separated into ≥ 70 and < 70 ; Cancer type included osteosarcoma and others. Analysis method was divided into univariate and multivariate analysis. Patients prognostic outcomes included overall survival (OS), event-free survival (EFS), metastasis-free survival (MFS).

Quality score assessment

In addition, two reviewers (K. Xie and J. Yang) independently assessed the quality of the included studies according to the NOS. The NOS consisted of selection, comparability of the groups and ascertainment of exposure was introduced to evaluate the included publication's quality. The NOS scores 0 to 10 stars. If an included study obtained no less than six stars, it could be regarded as a good quality.

Statistical analysis

We explored the association of ezrin expression with STS and BS prognostic value by applying STATA software (Version 12.0, Stata Corporation, College Station, TX). RR and 95% CI were collected for assessing the prognostic value of ezrin expression in STS and BS. Meanwhile, the heterogeneity has been assessed via chi-square-based Q and I^2 test across studies (no heterogeneity $I^2 < 25\%$, moderate heterogeneity $I^2 = 25\% - 50\%$, extreme heterogeneity $I^2 > 50\%$) [16]. In case of extreme heterogeneity

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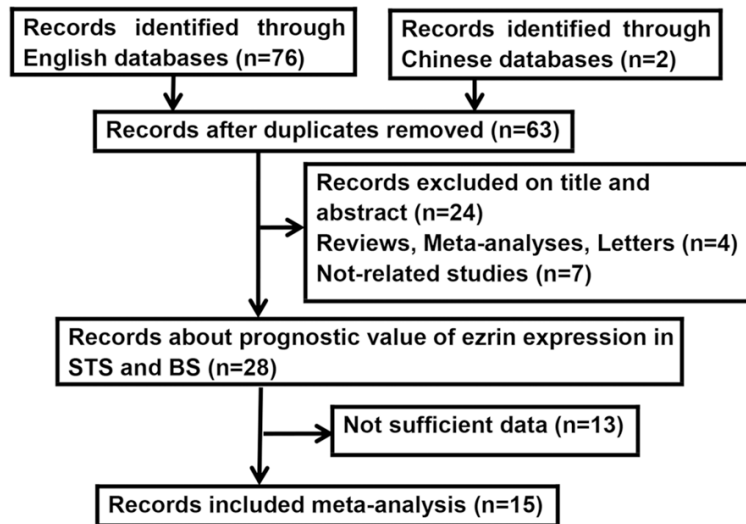


Figure 1. Flow diagram of the study selection process in the meta-analysis.

($I^2 > 50\%$ or $P < 0.01$ for Q test), we used random-effects (DerSimonian and Laird method) model [17]. Otherwise, fixed-effects (Mantel-Haenszel method) model was introduced [18].

One-way sensitivity analyses individually removed publications in meta-analysis were conducted to assess results' stability. It mainly explore the impact of a specific study upon mixed RR.

The Egger and Begg's funnel plots where logRR was plotted against SE. P value less than 0.05 indicated that there was a bias of study [19]. Additionally, different subgroups consisted of country, test method, sample source, sample size and cancer type were conducted.

Results

Characteristics of eligible studies

As a result, 15 studies consisted of 1070 samples satisfied the eligible studies [20-34] (**Figure 1**). The principal characteristics of the included studies were summarized in **Table 1**. Among these studies, 13 were written in English and two were published in Chinese. The sample sizes ranged from 25 to 256. The tumor types contained are as follows: 10 osteosarcoma, two soft tissue sarcomas, two myxofibrosarcomas and one synovial sarcoma. Meanwhile, two RT-PCR and 13 IHC in test method were applied. According to the sample source, there were seven formalin-fixed and paraffin-embedded (FFPE), three tissue and five tissue microarray (TMA). As for the survival outcomes, 15 eligible

studies were divided into 23 datasets: 12 for OS, seven for MFS and four for RFS. However, the cut-off value for ezrin expression was somewhat inconsistent among these included studies. Additionally, as shown in **Table 1**, all the included studies had high quality.

Quantitative synthesis

Meta-analysis for ezrin expression with OS

12 studies were involved in the current meta-analysis to assess the prognostic value of ezrin expression in STS and BS [20-23, 26, 27, 29-34]. As

apparent heterogeneity was found ($I^2 = 87.8\%$, $P^h = 0.000$), random effects model was applied to calculate the combined RR and 95% CI. Consequently, ezrin expression in STS and BS was associated with unfavorable OS (RR=1.85, 95% CI: 1.34-1.57, $P < 0.001$) (**Figure 2**). Besides, it seemed that there were certain associations via subgroup-analyses regarding country, sample source, sample size and cancer type (**Table 2**).

Meta-analysis for ezrin expression with MFS

There are six studies to detect the association between ezrin expression and MFS in STS and BS [22, 24, 28, 32-34]. Among them, one study was involved in univariate and multivariate analysis [24]. As apparent heterogeneity was found ($I^2 = 78.6\%$, $P^h = 0.000$), random effects model was applied to calculate the combined RR and 95% CI. As a result, the result suggested that ezrin expression was linked with poor MFS (RR=2.91, 95% CI: 1.56-5.41, $P < 0.001$) (**Figure 3**).

Meta-analysis for ezrin expression with EFS

Only four studies were included to explore the prognostic value of ezrin expression in STS and BS [25, 28, 29, 31]. Ultimately, we found that ezrin expression was associated with poor EFS (RR=2.35, 95% CI: 1.02-5.41, $P < 0.001$) (**Figure 4**).

Sensitivity analysis

Each single study included in the meta-analysis was deleted at a time to assess the specific

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Table 1. Main characteristics of the eligible studies

First author	Year	Country	Age	Test method	Cancer type	Sample source	Sample size	Follow-up, Median (range)	Outcome	Analysis method	RR (95% CI)	Cut-off value	NOS
Palmerini E [20]	2015	Italy	37 (11-63)	IHC	Synovial sarcoma	FFPE	88	72 (12-360)	OS	M	2.00 (1.64-2.45)	>1	8
Abdou AG [21]	2015	Egypt	19 (4-75)	IHC	Osteosarcoma	FFPE	57	6-156	OS	U	1.32 (0.79-2.22)	>1	7
Le Guellec S [22]	2013	France	18 (8-57)	IHC	Osteosarcoma	TMA	36	48 (12-228)	OS/MFS	M	0.66 (0.15-2.89)/1.22 (0.98-1.51)	>1	8
Mi DL [23]	2012	China	19 (7-62)	IHC	Osteosarcoma	FFPE	82	42 (3-88)	OS	M	1.83 (1.47-2.27)	≥1	7
Carneiro A [24]	2011	Sweden	66 (21-96)	IHC	Soft tissue sarcomas	TMA	256	48 (12-228)	MFS	U/M	1.8 (1.1-2.8)/1.8 (0.9-3.7)	>1	8
Wang YF [25]	2010	China	16 (4-56)	RT-PCR	Osteosarcoma	Tissue	25	22.6 (5.5-28.1)	EFS	U	6.355 (1.447-27.907)	>50%	8
Yang JZ [26]	2010	China	21.43 (12-49)	IHC	Osteosarcoma	Tissue	51	NA	OS	M	1.00 (0.43-2.32)	>1	7
Boldrini E [27]	2010	Brazil	15.9 (7-25)	IHC	Osteosarcoma	FFPE	34	28.2 (9-69)	OS	U	1.63 (0.32-8.28)	>1	6
Huang HY [28]	2010	China	61 (16-84)	RT-PCR	Myxofibrosarcomas	TMA	78	53.7 (2-201)	EFS/MFS	M	4.537 (2.916-40.888)/4.083 (1.907-13.449)	>1	8
Kim C [29]	2009	Korea	15.7 (3.8-64.4)	IHC	Osteosarcoma	FFPE	70	59.9	OS/EFS	M	2.24 (0.98-5.12)/2.28 (1.11-4.70)	>1	8
Ferrari S [30]	2008	Italy	47 (10-115)	IHC	Osteosarcoma	FFPE	95	16 (4-39)	OS	U	2.85 (1.24-6.55)	>1	7
Salas S [31]	2007	France	15	IHC	Osteosarcoma	Tissue	37	54 (10-150)	OS/EFS	M	1.071 (1.024-1.120)/1.070 (1.033-1.108)	>1	8
Kim MS [32]	2007	Korea	19.4 (4-58)	IHC	Osteosarcoma	TMA	64	78.2 (12-137)	OS/MFS	M	30.3 (4.0-228.3)/35.9 (4.8-268.5)	>1	8
Kim MS [33]	2007	Korea	61 (21-79)	IHC	Myxofibrosarcomas	TMA	47	50 (9-218)	OS/MFS	M	8.0 (1.8-35.4)/35.90 (4.80-268.49)	>1	7
Weng WH [34]	2005	Sweden	60 (19-81)	IHC	soft tissue sarcomas	FFPE	50	90 (50-134)	OS/MFS	M	2.54 (1.52-4.23)/2.80 (0.53-14.76)	>1	9

NA, Not available; IHC, Immunohistochemistry; RT-PCR, Reverse transcription-polymerase chain reaction; FFPE, Formalin-fixed and paraffin-embedded; TMA, Tissue microarray; OS, Overall survival; EFS, event-free survival; MFS, metastasis-free survival; U, Univariate analysis; M, Multivariate analysis; RR, Relative ratio; CI, Confidence interval; NOS, The Newcastle-Ottawa Scale.

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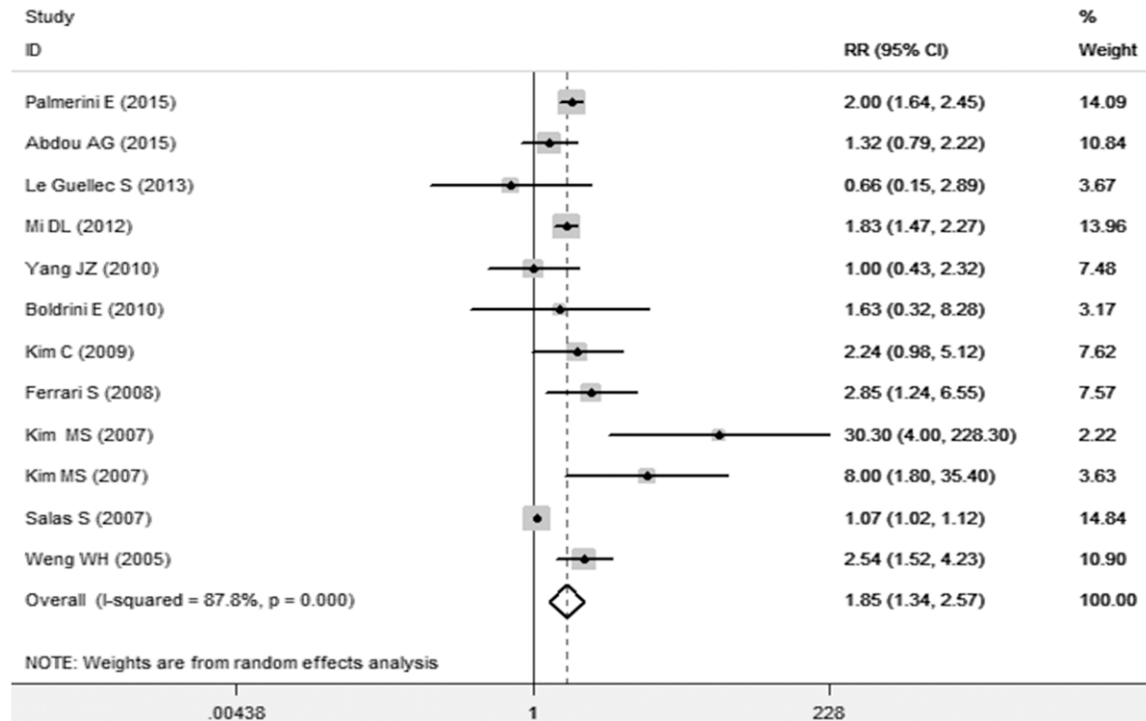


Figure 2. Forest plot for the meta-analysis of the association between ezrin expression and OS with random-effects model. The squares and horizontal lines correspond to the study-specific RR and 95% CI. The area of the squares reflects the weight. The diamond represents the summary RR and 95% CI. CI, confidence interval; RR, relative ratio.

Table 2. Stratified analysis of the ezrin expression and overall survival

Categories	Subgroups	No of datasets	RR (95% CI)	P-value	I ²	P ^h
All		12	1.85 (1.34-1.57)	0.000	87.8%	0.000
Country	Asia	6	2.08 (1.48-2.94)	0.000	62.8%	0.020
	Europe	4	1.62 (0.84-3.11)	0.148	81.8%	0.001
	Others	2	1.35 (0.82-2.20)	0.237	0.0%	0.809
Cancer type	Osteosarcoma	9	1.59 (1.11-2.29)	0.011	80.9%	0.000
	Others	3	2.11 (1.75-2.54)	0.000	48.1%	0.146
Sample source	FFPE	7	1.93 (1.69-2.21)	0.000	0.0%	0.604
	TMA	3	5.04 (0.58-43.92)	0.143	80.7%	0.006
	Tissue	2	1.07 (1.02-1.12)	0.003	0.0%	0.873
Sample size	<70	4	1.95 (1.69-2.25)	0.000	0.0%	73.0%
	>70	8	1.75 (1.06-2.88)	0.028	76.3%	0.000
Analysis method	Univariate	3	1.63 (1.07-2.50)	0.023	15.7%	0.305
	Multivariate	9	1.88 (1.29-2.75)	0.001	90.6%	0.000

RT-PCR, Reverse transcription-polymerase chain reaction; IHC, Immunohistochemistry; ISH, In situ hybridization; FFPE, Formalin-fixed and paraffin-embedded; TMA, Tissue microarray; RR, Relative ratio; CI, Confidence interval; P^h, P-value of heterogeneity test.

effect of the individual data on the pooled RRs, and one-way sensitivity analysis suggested pooled results were relatively stable in OS (Figure 5).

Publication bias evaluation

The funnel plot was used to evaluate the publication bias. Begg's test indicated that publica-

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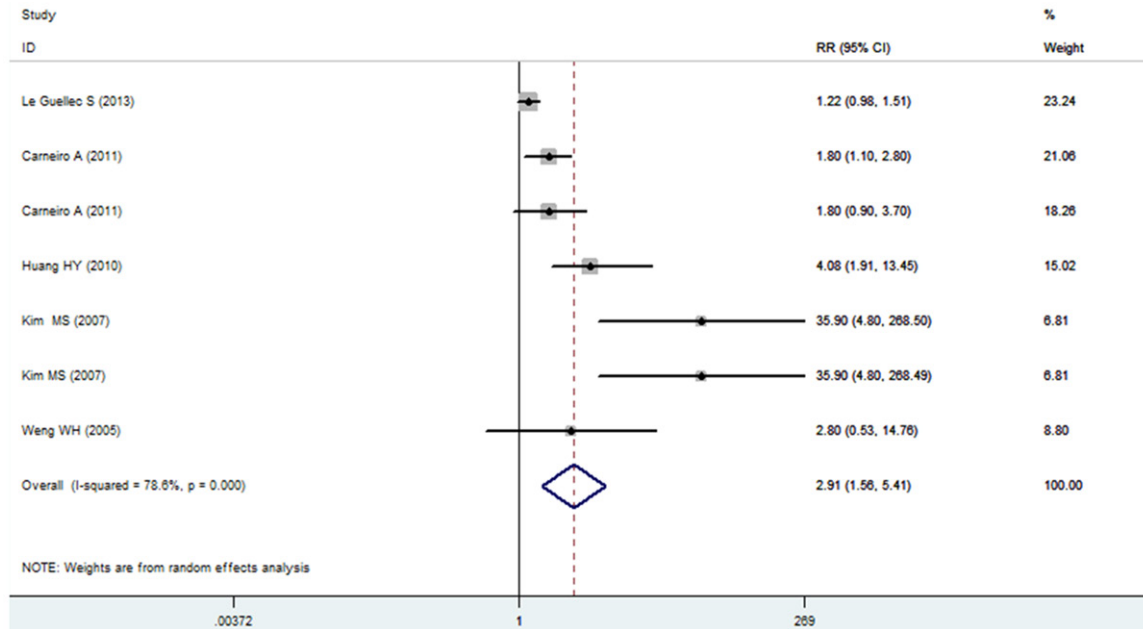


Figure 3. Forest plot for the meta-analysis of the association between ezrin expression and MFS with random-effects model. The squares and horizontal lines correspond to the study-specific RR and 95% CI. The area of the squares reflects the weight. The diamond represents the summary RR and 95% CI. CI, confidence interval; RR, relative ratio.

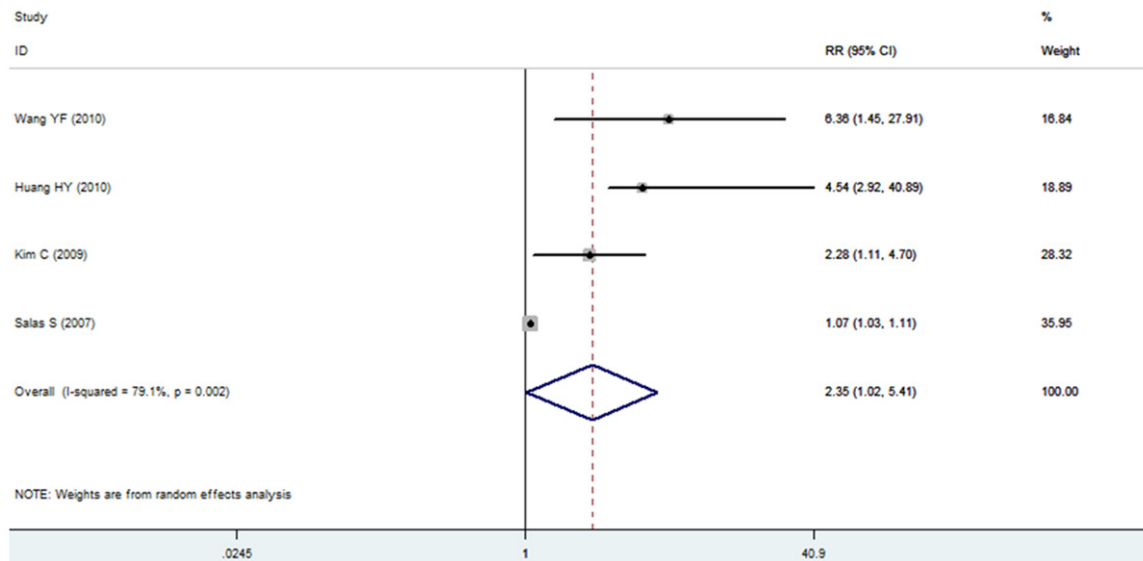


Figure 4. Forest plot for the meta-analysis of the association between ezrin expression and EFS with random-effects model. The squares and horizontal lines correspond to the study-specific RR and 95% CI. The area of the squares reflects the weight. The diamond represents the summary RR and 95% CI. CI, confidence interval; RR, relative ratio.

tion bias was not found in OS ($P=0.631$) (**Figure 6**). Meanwhile, no publication bias was found in each subgroup for meta-analysis.

Discussion

Sarcomas are relatively uncommon and comprise a wide variety of different entities. The

development of optimal therapeutic strategies for sarcomas has been greatly complicated by the large number of subtypes, the heterogeneity in their biological behaviors, and the small number of patients with particular subtypes enrolled in trials [35]. Thus, the prognosis was poor for patients with sarcomas. Different potential independent prognostic factors with

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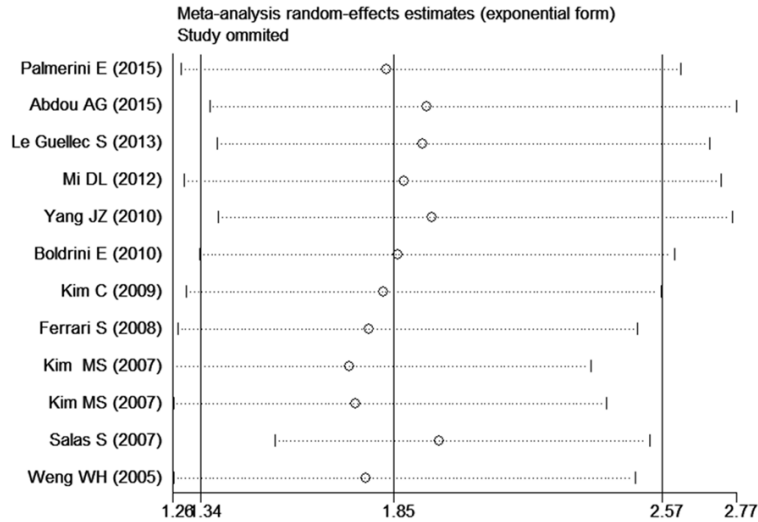


Figure 5. One-way sensitivity analysis of ezrin expression with OS. The studies individually removed and the stable results were confirmed.

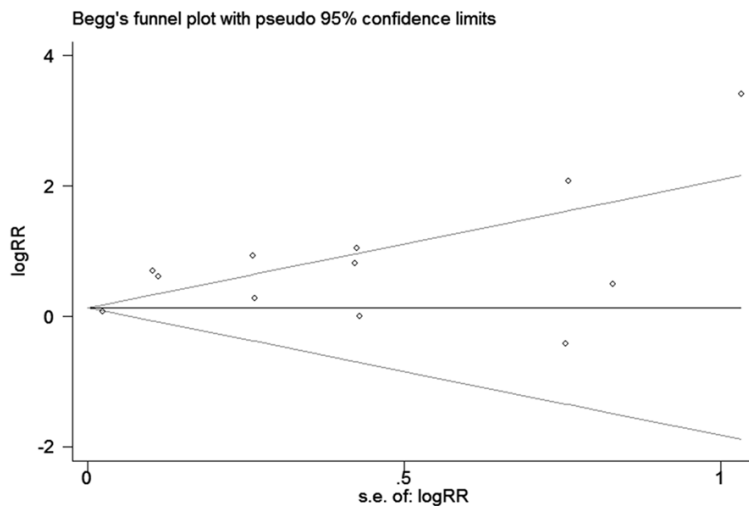


Figure 6. Begg's funnel plot for publication bias test. The x-axis is $\log(RR)$, and the y-axis is natural logarithm of RR . The horizontal line in the figure represents the overall estimated $\log(RR)$. The two diagonal lines indicate the pseudo 95% confidence limits of the effect estimate. $\log(RR)$, log-transformed RR , RR , relative ratio.

potency for OS have been explored in recent years. To date, these factors included clinical characteristics and molecular markers which might be used as novel kind of biomarkers for cancer diagnosis or prognosis prediction [36].

Ezrin, one of the promising key components in metastasis, has been identified as an independent prognostic factor in several human malignancies. It belongs to the ERM protein family that acts as membrane organizers and linkers

between plasma membrane and cytoskeleton. It is involved in cell adhesion to the extracellular matrix as well as in cell-cell interactions, receptor tyrosine-kinase signaling (cmet/hepatocyte growth factor pathway), signal transduction through Rho GTPase, and interactions with the Akt mediated cellular apoptotic machinery [37-39]. It has been reported that high levels of ezrin expression were linked with metastatic behavior in different tumor types [40]. Recent studies focused on the role of ezrin as a prognostic marker in sarcomas. Among sarcomas, ezrin expression can induce a highly metastatic state in rhabdomyosarcoma cell lines [41]. Ezrin overexpression was also correlated to clinical stage in children with embryonal rhabdomyosarcoma [41]. Moreover, its expression also could potentially be used to identify high-risk sarcoma patients who would benefit from postoperative chemotherapy [24]. Notably, ezrin expression is a potential marker to predict survival outcome for sarcoma patients, which results could be explained by that positive ezrin expression was correlated to the prognostic factors grade, the extent of tumour necrosis and infiltrative growth pattern [42, 43].

Until now, investigations focused on the correlation between ezrin expression with prognosis of STS and BS were inconclusive. As small sample-sized studies lacking statistical power often resulted in apparently contradictory conclusions. Meta-analysis is an useful tool for providing convincing evidence as it could present inconsistent results from different investigations to get a relatively precise estimation. Correspondingly, the current meta-analysis is conducted to comprehensively assess the correla-

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tion of ezrin expression with prognosis of STS and BS. Meanwhile, the potential associations were explored in different subgroups. Consequently, of particular interest is the finding of significant correlations between ezrin expression and unfavorable OS/MFS/EFS in STS and BS. All of the subgroup analyses also indicated that ezrin expression was associated with poor OS. Actually, these results require further investigation to conclude a definite relationship.

However, our meta-analysis has its limitations. Firstly, only published studies could not provide sufficient evidences in this meta-analysis. Additionally, our conclusion was checked by crude estimation rather than adjusted data. Therefore, other risk factors could also need to be taken into consideration in advanced researches. Meanwhile, the heterogeneity suggested there were potential or undiscovered factors including adjustment for surgery, chemotherapy, socioeconomic status, tumor characteristics and so on. Whereas, in spite of aforementioned limitations, certain relationships of ezrin expression in prognostic value were found in our meta-analysis.

In conclusion, the current study is the first original meta-analysis to address the correlation between the ezrin expression and prognosis for patients with sarcomas. Marginally significant associations were explored in overall population as well as the corresponding subgroups. It presented that ezrin expression might be associated with poor OS/MFS/EFS to some extent. Further prospective studies are required to confirm the prognostic value of ezrin expression in a large cohort of patients with sarcomas to confirm the usefulness in clinical practice.

Disclosure of conflict of interest

None.

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References

[1] Doyle LA. Sarcoma classification: an update based on the 2013 world health organization classification of tumors of soft tissue and bone. *Cancer* 2014; 120: 1763-1774.

- [2] Skubitz KM, D'Adamo DR. Sarcoma. *Mayo Clin Proc* 2007; 82: 1409-1432.
- [3] Youn P, Milano MT, Constine LS, Travis LB. Long-term cause-specific mortality in survivors of adolescent and young adult bone and soft tissue sarcoma: a population-based study of 28,844 patients. *Cancer* 2014; 120: 2334-2342.
- [4] Biau DJ, Weiss KR, Bhumbra RS, Davidson D, Brown C, Griffin A, Wunder JS, Ferguson PC. Monitoring the adequacy of surgical margins after resection of bone and soft-tissue sarcoma. *Ann Surg Oncol* 2013; 20: 1858-1864.
- [5] Nakamura T, Matsumine A, Yamakado K, Matsumura T, Takaki H, Nakatsuka A, Takeda K, Abo D, Shimizu T, Uchida A. Lung radio-frequency ablation in patients with pulmonary metastases from musculoskeletal sarcomas. *Cancer* 2009; 115: 3774-3781.
- [6] Hunter KW. Ezrin, a key component in tumor metastasis. *Trends Mol Med* 2004; 10: 201-204.
- [7] Bretscher A, Edwards K, Fehon RG. ERM proteins and merlin: integrators at the cell cortex. *Nat Rev Mol Cell Biol* 2002; 3: 586-99.
- [8] Vaheri A, Carpén O, Heiska L, Helander TS, Jääskeläinen J, Majander-Nordenswan P, Sainio M, Timonen T, Turunen O. The ezrin protein family: membrane-cytoskeleton interactions and disease associations. *Curr Opin Cell Biol* 1997; 9: 659-666.
- [9] Khanna C, Khan J, Nguyen P, Prehn J, Caylor J, Yeung C, Trepel J, Meltzer P, Helman L. Metastasis-associated differences in gene expression in a murine model of osteosarcoma. *Cancer Res* 2001; 61: 3750-3759.
- [10] Köbel M, Langhammer T, Hüttelmaier S, Schmitt WD, Kriese K, Dittmer J, Strauss HG, Thomssen C, Hauptmann S. Ezrin expression is related to poor prognosis in FIGO stage I endometrioid carcinomas. *Mod Pathol* 2006; 19: 581-587.
- [11] Li Q, Wu M, Wang H, Xu G, Zhu T, Zhang Y, Liu P, Song A, Gang C, Han Z, Zhou J, Meng L, Lu Y, Wang S, Ma D. Ezrin silencing by small hairpin RNA reverses metastatic behaviors of human breast cancer cells. *Cancer Lett* 2008; 261: 55-63.
- [12] Elzagheid A, Korkeila E, Bendardaf R, Buhmeida A, Heikkilä S, Vaheri A, Syrjänen K, Pyrhönen S, Carpén O. Intense cytoplasmic ezrin immunoreactivity predicts poor survival in colorectal cancer. *Hum Pathol* 2008; 39: 1737-1743.
- [13] Li YR, Akbari E, Tretiakova MS, Hart J, Akbari M, Urbanski SJ, Gao ZH. Primary hepatic malignant fibrous histiocytoma: clinicopathologic characteristics and prognostic value of ezrin expression. *Am J Surg Pathol* 2008; 32: 1144-1158.

Roles of ezrin in bone and soft tissue sarcomas prognosis

- [14] Zhao DH, Zhu J, Wang WB, Dong F, Zhang Q, Fan HW, Zhang JZ, Wang YM. Correlations of ezrin expression with pathological characteristics and prognosis of osteosarcoma: a meta-analysis. *ScientificWorldJournal* 2014; 2014: 837543.
- [15] Munafo MR, Flint J. Meta-analysis of genetic association studies. *Trends Genet* 2004; 20: 439-444.
- [16] 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.
- [17] Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; 21: 1539-1558.
- [18] DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; 7: 177-188.
- [19] Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959; 22: 719-748.
- [20] Palmerini E, Benassi MS, Quattrini I, Pazzaglia L, Donati D, Benini S, Gamberi G, Gambarotti M, Picci P, Ferrari S. Prognostic and predictive role of CXCR4, IGF-1R and Ezrin expression in localized synovial sarcoma: is chemotaxis important to tumor response? *Orphanet J Rare Dis* 2015; 10: 6.
- [21] Abdou AG, Kandil M, Asaad NY, Dawoud MM, Shahin AA, Abd Eldayem AF. The prognostic role of Ezrin and HER2/neu expression in osteosarcoma. *Appl Immunohistochem Mol Morphol* 2016; 24: 355-363.
- [22] Le Guellec S, Moyal EC, Filleron T, Delisle MB, Chevreau C, Rubie H, Castex MP, de Gauzy JS, Bonneville P, Gomez-Brouchet A. The $\beta 5$ /focal adhesion kinase/glycogen synthase kinase 3 β integrin pathway in high-grade osteosarcoma: a protein expression profile predictive of response to neoadjuvant chemotherapy. *Hum Pathol* 2013; 44: 2149-2158.
- [23] Mi DL, Shen Z, Lin F, Xu XL, Huang WT, Yao Y. Clinical significances of Runx2 and Ezrin expressions in osteosarcoma. *China Oncology* 2012; 22: 685-689.
- [24] Carneiro A, Bendahl PO, Åkerman M, Domanski HA, Rydholm A, Engellau J, Nilbert M. Ezrin expression predicts local recurrence and development of metastases in soft tissue sarcomas. *J Clin Pathol* 2011; 64: 689-694.
- [25] Wang YF, Shen JN, Xie XB, Wang J, Huang G. Expression change of ezrin as a prognostic factor in primary osteosarcoma. *Med Oncol* 2011; 28 Suppl 1: S636-643.
- [26] Yang JZ, Sun LX, Liu JR, Wang Y, Ding Y, Zhang XH. Expression and significance of Ezrin protein in osteosarcoma. *Canc Res Prev Treat* 2010; 37: 818-821.
- [27] Boldrini E, Peres SV, Morini S, de Camargo B. Immunoexpression of Ezrin and CD44 in patients with osteosarcoma. *J Pediatr Hematol Oncol* 2010; 32: e213-217.
- [28] Huang HY, Li CF, Fang FM, Tsai JW, Li SH, Lee YT, Wei HM. Prognostic implication of ezrin overexpression in myxofibrosarcomas. *Ann Surg Oncol* 2010; 17: 3212-3219.
- [29] Kim C, Shin E, Hong S, Chon HJ, Kim HR, Ahn JR, Hong MH, Yang WI, Roh JK, Rha SY. Clinical value of ezrin expression in primary osteosarcoma. *Cancer Res Treat* 2009; 41: 138-144.
- [30] Ferrari S, Zanella L, Alberghini M, Palmerini E, Staals E, Bacchini P. Prognostic significance of immunohistochemical expression of ezrin in non-metastatic high-grade osteosarcoma. *Pediatr Blood Cancer* 2008; 50: 752-756.
- [31] Salas S, Bartoli C, Deville JL, Gaudart J, Fina F, Calisti A, Bollini G, Curvale G, Gentet JC, Duffaud F, Figarella-Branger D, Bouvier C. Ezrin and alpha-smooth muscle actin are immunohistochemical prognostic markers in conventional osteosarcomas. *Virchows Arch* 2007; 451: 999-1007.
- [32] Kim MS, Song WS, Cho WH, Lee SY, Jeon DG. Ezrin expression predicts survival in stage IIB osteosarcomas. *Clin Orthop Relat Res* 2007; 459: 229-236.
- [33] Kim MS, Cho WH, Song WS, Lee SY, Jeon DG. Prognostic significance of ezrin expression in pleomorphic malignant fibrous histiocytoma. *Anticancer Res* 2007; 27: 1171-1178.
- [34] Weng WH, Ahlén J, Aström K, Lui WO, Larsson C. Prognostic impact of immunohistochemical expression of ezrin in highly malignant soft tissue sarcomas. *Clin Cancer Res* 2005; 11: 6198-6204.
- [35] Cormier JN, Pollock RE. Soft tissue sarcomas. *CA Cancer J Clin* 2004; 54: 94-109.
- [36] Baird K, Davis S, Antonescu CR, Harper UL, Walker RL, Chen Y, Glatfelter AA, Duray PH, Meltzer PS. Gene expression profiling of human sarcomas: insights into sarcoma biology. *Cancer Res* 2005; 65: 9226-9235.
- [37] Bretscher A, Edwards K, Fehon RG. ERM proteins and merlin: integrators at the cell cortex. *Nat Rev Mol Cell Biol* 2002; 3: 586-599.
- [38] Gautreau A, Pouillet P, Louvard D, Arpin M. Ezrin, a plasma membrane-microfilament linker, signals cell survival through the phosphatidylinositol 3-kinase/Akt pathway. *Proc Natl Acad Sci U S A* 1999; 96: 7300-7305.
- [39] Wang JW, Peng SY, Li JT, Wang Y, Zhang ZP, Cheng Y, Cheng DQ, Weng WH, Wu XS, Fei XZ, Quan ZW, Li JY, Li SG, Liu YB. Identification of

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- metastasis-associated proteins involved in gallbladder carcinoma metastasis by proteomic analysis and functional exploration of chloride intracellular channel 1. *Cancer Lett* 2009; 281: 71-81.
- [40] Hunter KW. Ezrin, a key component in tumor metastasis. *Trends Mol Med* 2004; 10: 201-204.
- [41] Yu Y, Khan J, Khanna C, Helman L, Meltzer PS, Merlino G. Expression profiling identifies the cytoskeletal organizer ezrin and the developmental homeoprotein six-1 as key metastatic regulators. *Nat Med* 2004; 10: 175-181.
- [42] Machado I, Navarro S, Giner F, Alberghini M, Bertoni F, Llombart-Bosch A. Ezrin immunohistochemical expression in chondrosarcomas, osteosarcomas and ewing sarcoma family of tumors. *Virchows Arch* 2010; 457: 87-89.
- [43] Rydholm A, Gustafson P. Should tumor depth be included in prognostication of soft tissue sarcoma? *BMC Cancer* 2003; 3: 17.