

Meta-analysis of serum lactate dehydrogenase and prognosis for osteosarcoma

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Abstract

Backgrounds: A large number of studies have reported the relationships between serum lactate dehydrogenase (LDH) and prognosis of osteosarcoma. However, the result is still controversial and no consensus has been reached. Therefore, we performed a meta-analysis to evaluate the prognostic role of serum LDH in osteosarcoma patients.

Methods: We performed the systematic computerized search for eligible articles from PubMed, Embase, and Cochrane databases until December 21, 2017. The pooled hazard ratio (HR) and 95% confidence intervals (CIs) of overall survival (OS) and event-free survival (EFS) were obtained to assess the prognostic value of serum LDH.

Results: A total of 18 studies with 2543 osteosarcoma patients were included. Overall, 15 studies assessed the elevated serum LDH level on OS and the pooled HR was 1.87 (95% CI= 1.58–2.20). Meanwhile, the pooled HR to evaluate the relationship between serum LDH and EFS in 9 studies was 1.78 (95% CI= 1.51–2.10). The same results were acquired when these studies were stratified by Enneking stage, geographic region, and sample size. No heterogeneity existed between these subgroups ($P > .05$). Begg's funnel plot and Egger's test (OS: $P = .04$; EFS: $P = .34$) showed that possible publication bias might exist in OS studies. Sensitivity analysis suggested the pooled HR was robust.

Conclusions: This meta-analysis demonstrates that elevated serum LDH level is apparently associated with lower EFS rate and serum LDH could be a prognostic biomarker for osteosarcoma patients.

Abbreviations: ALP = alkaline phosphatase, AMPK = adenosine monophosphate-activated protein kinase, CI = confidence interval, DFS = disease-free survival, EFS = event-free survival, HIF-1 alpha = hypoxia-inducible factor-1 alpha, HR = hazard ratio, LDH = lactate dehydrogenase, MSQA = mean stars of quality assessment, mTOR = mammalian target of rapamycin, NAD⁺ = oxidized form nicotinamide adenine dinucleotide, NADH = reduced form of nicotinamide adenine dinucleotide, NOS = Newcastle–Ottawa scale, OS = overall survival, PFS = progression-free survival.

Keywords: biomarker, lactate dehydrogenase, meta-analysis, osteosarcoma, prognosis

1. Introduction

Osteosarcoma, one of the most common primary bone malignant tumors, has the annual incidence of nearly 3/10⁶ in population. The peak age of onset is 15 to 25 and males are more frequently seen.^[1] Before the 1970s, all patients were only treated by amputation and almost 80% to 90% of them were died in earlier

phases due to micro-metastasis.^[2] The micrometastasis often appeared because cancer cells tended to colonize selective distant organs with a favorable microenvironment and interaction between them determined the formation of metastatic carcinomas. It was known as the theory of “seed and soil,” which was first mentioned by Paget in 1889.^[3] With the development of treatment, especially the complete resection of osteosarcoma combined with adjuvant and neoadjuvant chemotherapy, the 5-year survival rate of patients had raised to >60%.^[4] However, a large number of patients still face the frustrating outcome since the development of metastasis and local relapse.^[5] Therefore, identifying a valuable prognostic factor is important for predicting high-risk patients and multimodal treatment can start earlier to improve the prognosis.

In our body, normal cells depend on aerobic oxidation to supply energy, while cancer cells prefer to glycolysis to meet great demands for energy, which is known as the Warburg effect.^[6] During the process of glycolysis, glucose is transformed into pyruvate and oxidized form nicotinamide adenine dinucleotide (NAD⁺) is converted to a reduced form of nicotinamide adenine dinucleotide (NADH).^[7] Lactate dehydrogenase, known as the NAD⁺-dependent enzyme, catalyzes the reversible reaction of pyruvate to lactate accompanies with the reproduction of NAD⁺, maintaining the generation of ATP, and continuing glycolysis.^[8] It is regarded as a biomarker indicates the tumor burden and its prognostic role has been demonstrated in several tumors.^[9]

Up to now, numerous researches had shown that serum LDH level was associated with the prognosis of osteosarcoma patients,

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while some hold the opposite view. Thus, the consistency of the results had not been reached and it was unclear whether these differences were caused by the limitation of sample size or genuine heterogeneity. Therefore, we searched for all relevant studies and performed a meta-analysis to explore the prognostic value of serum LDH in osteosarcoma.

2. Materials and methods

2.1. Search strategy

We searched on PubMed, Embase, and Cochrane databases for relevant literature until December 21, 2017. The search terms were combined as follows: lactate dehydrogenase (or LDH) and osteosarcoma (or osteogenic sarcoma, bone sarcoma). Only published articles written in English were considered.

2.2. Inclusion and exclusion criteria

Studies met the following criteria that were eligible for meta-analysis: retrospective or prospective cohort study; tumors were confirmed as osteosarcoma by histology; studies reported the relation between serum LDH level and prognosis of osteosarcoma patients; studies provided sufficient information to estimate HR as well as 95% CI of EFS and OS. The exclusion criteria were: duplicated studies searched from different databases; studies unpublished or published in non-English; when different studies reported the same or overlapping patients, only the latest or most complete was included. All studies were searched and extracted by 2 reviewers independently. Disagreements were solved by discussion and consensus was reached in the end.

2.3. Data extraction

The required data were extracted from all eligible studies, including first author's family name, publication time, country, number of all patients in studies, age, tumor stage (Enneking stage), follow-up time and range, LDH cut-off level, population of patients reported LDH levels, prognostic indicators OS, EFS including disease-free survival (DFS), and progression-free survival (PFS). The HR and 95% CI of studies were obtained by 3 methods: directly acquired from articles without any adjustments; calculated from the number of elevated and normal LDH level patients, total dead populations and log-rank test's *P* values; estimated the data by using Engauge Digitizer software to analyze Kaplan–Meier survival curves, then combined with maximal and minimal follow-up time to calculate HR.^[10]

2.4. Quality assessment

Two reviewers assessed the methodological quality of all included studies by Newcastle–Ottawa scale (NOS) independently.^[11] The maximum of 9 stars was applied to evaluate the selection, comparability as well as exposure and outcome of each study. Studies with mean stars of quality assessment (MSQA) ≥ 7 stars were considered as high quality.

2.5. Statistical analysis

We measured the effects of serum LDH level in OS and EFS rates by pooled HR and 95% CI. Heterogeneity was assessed by I^2 test.^[12] The random effect model was used for analysis when heterogeneity existed ($I^2 > 50\%$). If not ($I^2 \leq 50\%$), the fixed effect model was used.^[13] When pooled HR and 95% CI were > 1 , it

demonstrated that patients with higher level of serum LDH had lower survival rate. We also performed subgroup analyses by dividing patients into different subgroups according to clinical variables such as Enneking stage, geographic region, and sample size. Publication bias was examined by Begg's funnel plot and Egger's test.^[14] To evaluate the influence of each study on HR, the sensitivity analysis was performed. $P < .05$ was considered as statistically significant. All the above analyses were conducted by STATA 12.0 software (Stata Corporation, College Station, TX).

3. Results

3.1. Study characteristics and quality assessment

We initially identified 689 articles according to the search strategies described previously. However, 155 articles were excluded due to duplicate. Around 505 articles were excluded after reading the titles or abstracts and 11 articles were eliminated after the full text review (4 articles with partially overlapped patients, 7 articles without sufficient data for extraction). In the end, 18 articles met the selection criteria were included in this meta-analysis.^[15–32] Figure 1 showed the flow diagram of this selection process. These 18 studies were conducted in 15 countries or districts and published between 1991 and 2017. A total of 2543 patients were included in this study after excluding those which did not test ALP level and the amount of patient was from 28 to 860. The major characteristics of these articles are shown in Table 1.

HRs and 95% CIs of OS were extracted from 15 articles and 6 of 15 articles hypothesized that high serum LDH level had no impact on OS rates. We checked the description of event in EFS and discovered it was defined as recurrence, metastasis, or death, which accorded with the event in DFS and PFS. Therefore, we regarded DFS and PFS as EFS and extracted HRs and 95% CIs from these studies. In the end, nine studies evaluated the relationship between serum LDH level and different effect size including EFS, DFS, and PFS. Three of them indicated that high level of serum LDH had no relations with prognosis (Table 2). Two independent reviewers assessed the quality of articles by NOS and the average star was 6.95, which implied that all 18 articles included were moderate quality.

3.2. Serum LDH level and OS or EFS

The heterogeneity of 15 studies included for assessing the relationship between OS and serum LDH level did not exist ($I^2 = 32.1\%$), so the fixed effect model was used. The pooled HR was 1.87 (95% CI = 1.58–2.20), indicating that higher serum LDH level was obviously associated with poorer OS in osteosarcoma patients (Fig. 2). Using the same method, we also found there was no heterogeneity ($I^2 = 49.3\%$) existed in 9 studies of EFS and serum LDH level. Therefore, the fixed effect model was applied and the combined HR was 1.78 (95% CI = 1.51–2.10), suggesting that patients with elevated serum LDH level had lower EFS rate (Fig. 3).

3.3. Subgroup analyses

The studies were divided into different subgroups by Enneking stage, geographic region, and sample size. The pooled HRs, 95% CIs, and *P* values for heterogeneity between different subgroups were shown in Tables 3 and 4. All subgroups' HRs and 95% CIs were > 1 , which indicated that osteosarcoma patients with higher serum LDH level had a poorer prognosis regardless of different

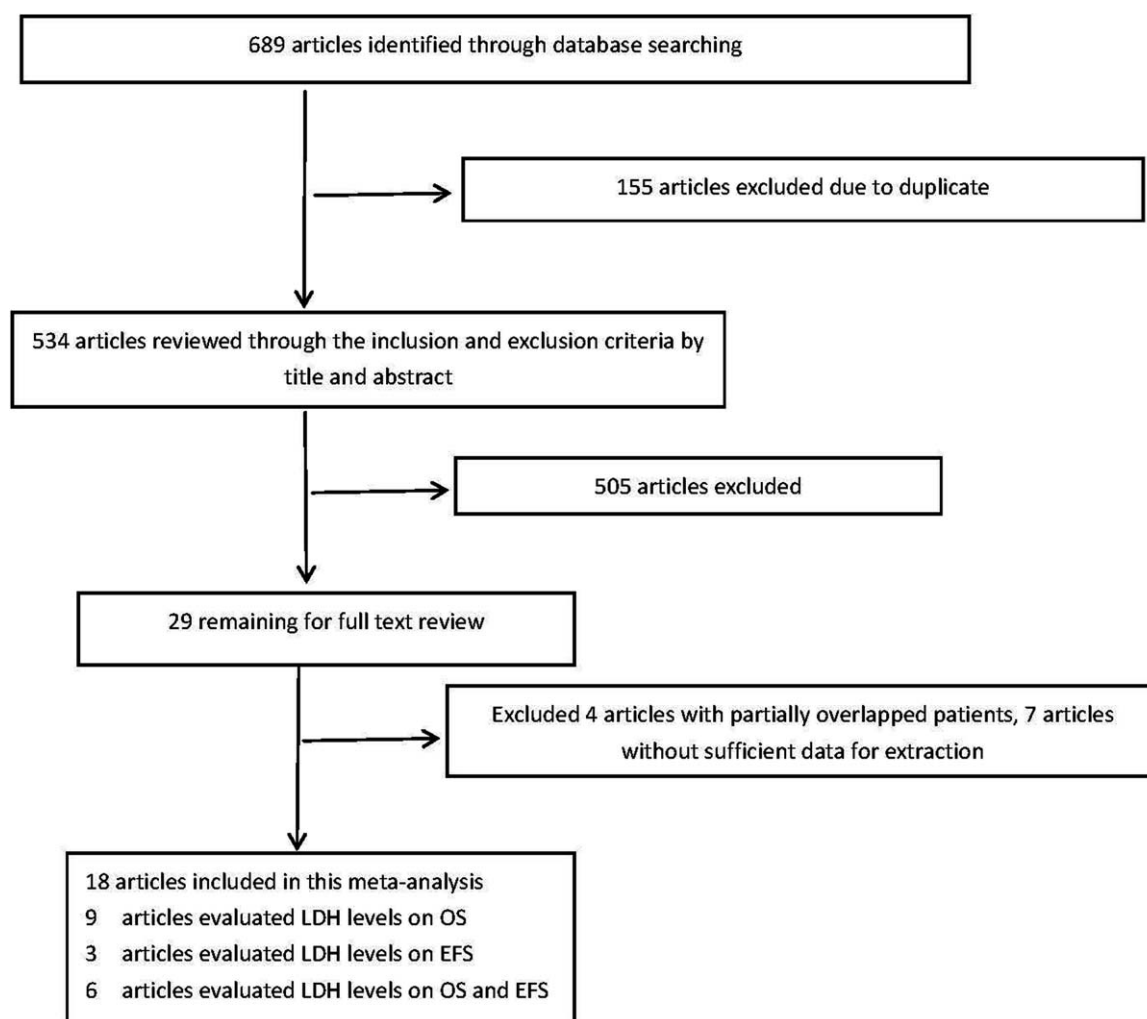


Figure 1. Flow diagram showed the selection process of meta-analysis.

Table 1

Main characteristics of eligible studies.

Study, year	Location	No. of patients (M/F)	Age (years)	Enneking stage	No. with LDH (E/N)	LDH Cut-off Level (IU/L)	Survival analysis	Follow-up (months) (range)	MSQA
Hu et al, 2017	China	106 (62/44)	19* (7–53)	II	106 (36/70)	210	OS	52* (7–80)	8
Vasquez et al, 2016	Peru	73 (45/28)	14* (5–17)	II–III	73 (57/16)	NR	OS	30* (1.5–152)	7
Berner et al, 2015	Norway	424 (246/178)	NR	II–III	N	A [‡]	OS/EFS	NR	8
Durmali et al, 2013	Turkey	240 (153/87)	25.1† (13–74)	II–III	182 (81/101)	NR	OS	30.5† (0.5–213)	7
González-Billalabeitia et al, 2009	Spain	66 (64/2)	15.5† (3–60)	II–III	66 (14/52)	NR	OS/PFS	100† (NR)	7
Hagleitner et al, 2011	Netherlands	102 (55/47)	17.8† (4.5–39.5)	II	85 (74/11)	NR	OS	67† (29–360)	5.5
Wu et al, 2009	Taiwan	202 (128/74)	20.2* (5–84)	II–III	91 (65/26)	213	OS	58.2* (2–233)	7
Bacci et al, 2004	Italy	1421 (819/602)	NR	II–III	860 (158/702)	240	DFS	180* (36–324)	7
Fellenberg et al, 2007	Germany	35 (19/16)	19.9† (8–51)	II–III	28 (25/3)	A [§]	OS	40.8* (3.6–102)	6.5
Ilic et al, 2004	Croatia	36 (21/15)	14.4† (5–24)	II–III	36 (2/34)	A	OS	47.5† (6–144)	8
Ferrari et al, 2012	Italy	246 (146/100)	14.4† (4–39)	II	198 (62/136)	NR	OS/EFS	66† (1–104)	6
Tomer et al, 1999	Israel	35 (17/18)	13.3 (NR)	II	35 (25/10)	250	DFS	88† (NR)	8
Aparicio et al, 1999	Spain	35 (19/16)	17.7† (12–42)	II	32 (6/26)	225	OS/DFS	96† (60–156)	7
Pochanugool et al, 1997	Thailand	130 (72/58)	NR	II–III	130 (45/85)	300	OS	NR	7
Chou et al, 2009	USA	91 (56/35)	NR	III	91 (53/38)	NR	OS/EFS	89† (1–141)	7
Link et al, 1991	USA	165 (91/74)	NR	II	125 (39/86)	NR	EFS	NR	6
Rech et al, 2004	Brazil	50 (34/16)	13 (3–22)	II–III	44 (6/38)	1000	OS	36 (6–126)	7
Nataraj et al, 2016	India	102 (78/24)	18.9† (8–48)	III	75 (64/11)	NR	OS/EFS	23# (5–108)	6

* = mean.

† = median.

‡ = LDH cut-off level: 0–10 years 400 IU/L; 11–70 years 205 IU/L; >70 years 255 IU/L.

§ = LDH cut-off level: males and females < 15 years 300 IU/L; males > 15 years 225 IU/L; females > 15 years: 214 IU/L.

|| = LDH cut-off level: girls < 12 years 573 IU/L; boys < 12 years 544 IU/L; boys and girls between 13 and 14 years of age 497 IU/L; boys > 14 years 441 IU/L; girls > 14 years 427 IU/L.

M = male, F = female, E = elevated, N = normal, OS = overall survival, NR = not reported, EFS = event-free survival, PFS = progression-free survival, DFS = disease-free survival; MSQA = mean stars of quality assessment.

N = Number with LDH (E/N): OS:286 (126/160), EFS:224 (139/85).

Table 2**Results of eligible studies for HR and 95% CI.**

Study, year	Event*	HR	95%CI (LL-UL)
Hu et al, 2017	OS	3.00	1.37–6.55
Vasquze et al, 2016	OS	2.13	0.63–6.67
Berner et al, 2015	OS	1.70	1.28–2.26
Berner et al, 2015	EFS	1.50	1.07–2.10
Dumali et al, 2013	OS	1.80	1.16–2.81
González-Billalabeitia et al, 2009	OS	9.38	1.73–50.74
González-Billalabeitia et al, 2009	EFS	8.62	1.71–43.37
Hagleitner et al, 2011	OS	1.15	0.44–2.99
Wu et al, 2009	OS	1.54	0.86–2.74
Bacci et al, 2004	EFS	1.68	1.29–2.18
Fellenberg et al, 2007	OS	12.06	1.22–87.24
Ilic et al, 2004	OS	9.08	1.53–53.97
Ferrari et al, 2012	OS	1.46	0.82–2.61
Ferrari et al, 2012	EFS	1.76	1.09–2.83
Tomer et al, 1999	EFS	1.63	0.51–5.19
Aparicio et al, 1999	OS	2.79	0.73–10.68
Aparicio et al, 1999	EFS	1.84	0.56–6.01
Pochanugool et al, 1997	OS	1.90	1.11–3.26
Chou et al, 2009	OS	2.72	1.52–4.89
Chou et al, 2009	EFS	2.42	1.42–4.12
Link et al, 1991	EFS	4.30	2.14–8.64
Rech et al, 2004	OS	3.61	1.27–10.23
Nataraj et al, 2016	OS	0.80	0.30–1.70
Nataraj et al, 2016	EFS	0.80	0.30–1.60

HR=hazard ratio, CI=confidence interval, LL=lower limit, UL=upper limit, OS=overall survival, EFS=event-free survival.

* DFS and PFS belong to EFS, so both of them were replaced by EFS here.

Enneking stage, geographic region or sample size. All *P* values of heterogeneity in subgroups were $>.05$, suggesting no heterogeneity existed in these subgroups.

3.4. Publication bias and sensitivity analysis

The Begg's funnel plot and Egger's test were used to evaluate the publication bias of studies. For studies in OS, the Begg's funnel plot was not symmetry (Fig. 4) and the *P* value of Egger's test was .04. It indicated the possibility of publication bias might exist. On the contrary, the Begg's funnel plot was almost symmetry (Fig. 5) and the *P* value of Egger's test was .34 in EFS studies, which meant the possibility of publication bias was excluded.

The sensitivity analysis was also performed to assess each study's effect on pooled HR. Figures 6 and 7 showed when removing any study in this research, no significant change was achieved. It indicated that the consequence of this meta-analysis was stable.

4. Discussion

Nowadays, more and more studies focused on the biomarkers to improve the early diagnosis and prognosis of cancer. For osteosarcoma, one of the most common bone malignant tumors, a large number of researchers found that over-expression of some biomarkers, such as ALP, VEGF and CD44V6, were associated with the poorer prognosis.^[33–35] LDH was one of the most common clinical test indexes that could be easily measured in blood and hardly increased in normal tissues. Some studies had

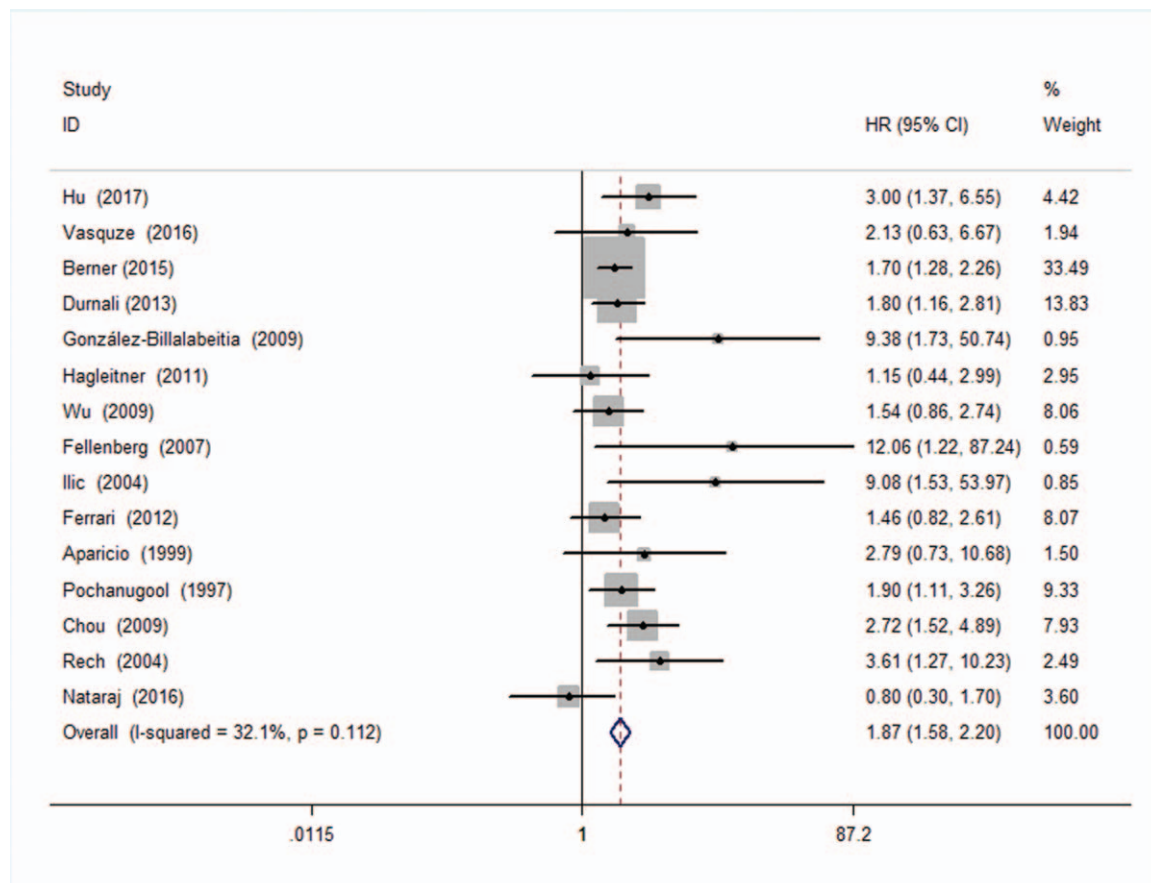


Figure 2. Forest plot showed the relationship between serum LDH level and OS rate: 15 studies were included and the fixed effect model was used. The pooled HR was 1.87 (95% CI=1.58–2.20). CI=confidence interval, LDH=lactate dehydrogenase, OS=overall survival.

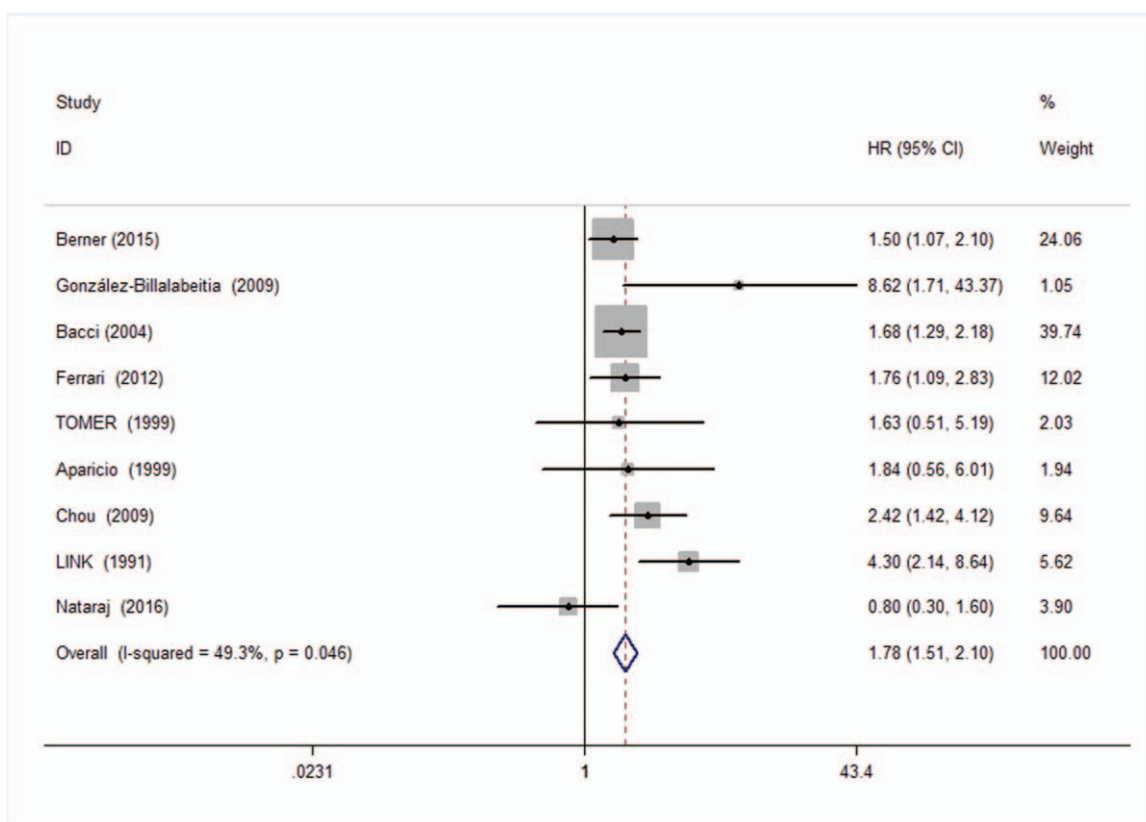


Figure 3. Forest plot showed the relationship between serum LDH level and EFS rate: 9 studies were included and the fixed effect model was used. The pooled HR was 1.78 (95% CI=1.51–2.10). CI =confidence interval, EFS=event-free survival, HR=hazard ratio, LDH=lactate dehydrogenase.

demonstrated serum LDH could be an effective biomarker to predict the prognosis of small cell lung cancer, renal cell carcinoma and colorectal cancer.^[36–38] In an animal study, Nakamura and Kitagawa^[39] transplanted the human osteosarcomas to nude mice and found LDH could be a biomarker to predict the prognosis of these mice. Some cohort studies also reported serum LDH was an indicator of prognosis in osteosarcoma patients. The lower level of serum LDH, usually accompanied by other biomarkers such as alkaline phosphatase (ALP), was associated with a better prognosis.^[32,40] However, some researches revealed serum LDH was not a prognostic indicator for osteosarcoma and the importance of serum LDH in osteosarcoma was still controversy.

So we systematically searched the literature online and did this comprehensive meta-analysis.

Based on 18 articles involved in this study, we found patients with elevated serum LDH level had worse OS or EFS rate. This result would not change when any study was omitted for sensitivity analysis. For patients with different Enneking stage, the effect of high level serum LDH on survival was consistent and no heterogeneity existed. We acquired the same results when articles were stratified by sample size and geographic region. In the end, we got the conclusion that serum LDH was a prognosis biomarker for osteosarcoma patients and it had a negative correlation with OS and EFS rates.

Table 3
The subgroup analysis of serum LDH and OS in osteosarcoma patients.

	Number of studies	HR (95%CI)	P* value
Sample size			
<100	10	2.09 (1.55–2.81)	.378
>100	5	1.78 (1.46–2.17)	
Enneking stage			.972
II	4	1.79 (1.20–2.67)	
II–III	9	1.89 (1.56–2.29)	
III	2	1.86 (1.14–3.01)	
Geographic region			.537
European	8	1.79 (1.46–2.21)	
Non-European	7	2.00 (1.53–2.61)	

LDH=lactate dehydrogenase, OS=overall survival.
* P value refers to the heterogeneity between groups.

Table 4
The subgroup analysis of serum LDH and EFS in osteosarcoma patients.

	Number of studies	HR (95%CI)	P* value
Sample size			.67
<100	5	1.92 (1.31–2.81)	
>100	4	1.75 (1.45–2.10)	
Enneking stage			.38
II	4	2.21 (1.55–3.16)	
II–III	3	1.65 (1.35–2.03)	
III	2	1.76 (1.12–2.76)	
Geographic region			.15
European	6	1.67 (1.39–2.01)	
Non-European	3	2.29 (1.57–3.34)	

EFS=event-free survival, LDH=lactate dehydrogenase.
* P value refers to the heterogeneity between groups.

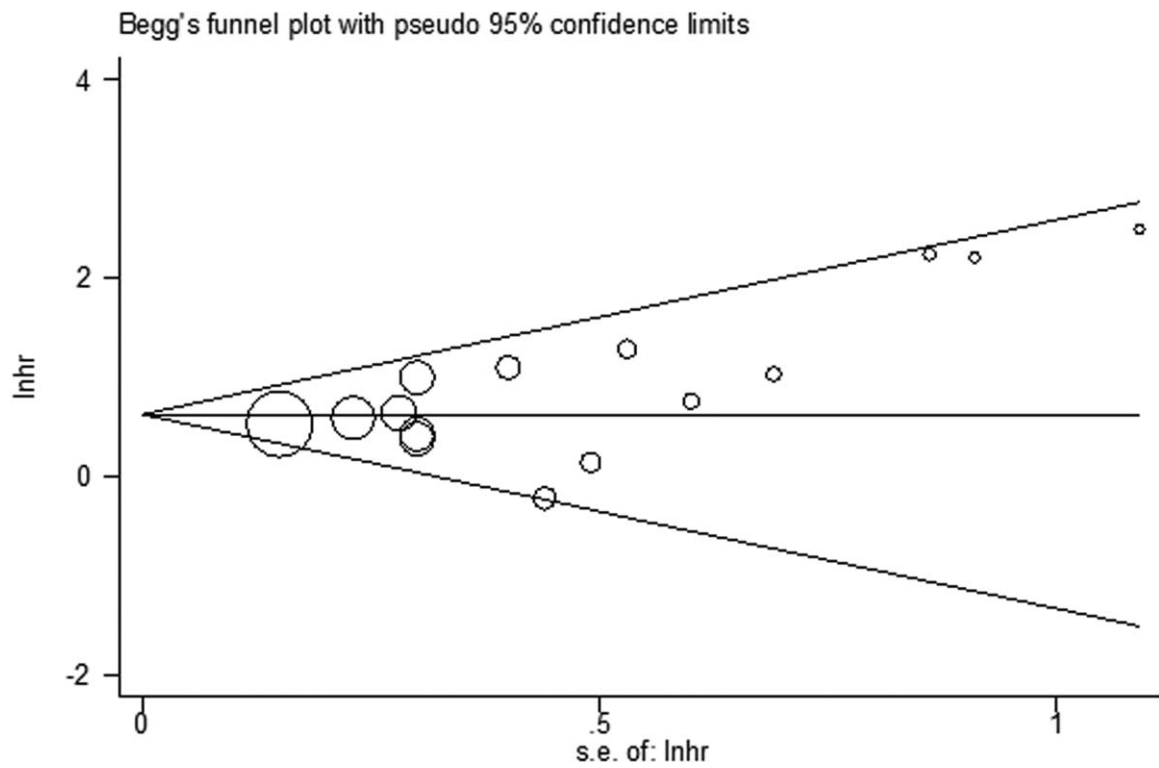


Figure 4. Begg's funnel plot to assess the publication bias for OS. OS=overall survival.

However, the mechanism of LDH's role in osteosarcoma was still unknown. Some researchers had demonstrated that cancer cells depended on glycolysis to get sufficient energy for cellular proliferation and these cells could manage this process by

regulating the uptake of substrate, as well as some enzymes related to glycolysis. In addition, the regulation of adenosine monophosphate-activated protein kinase (AMPK) signal transduction, a key sensor that managed cellular metabolism, was also

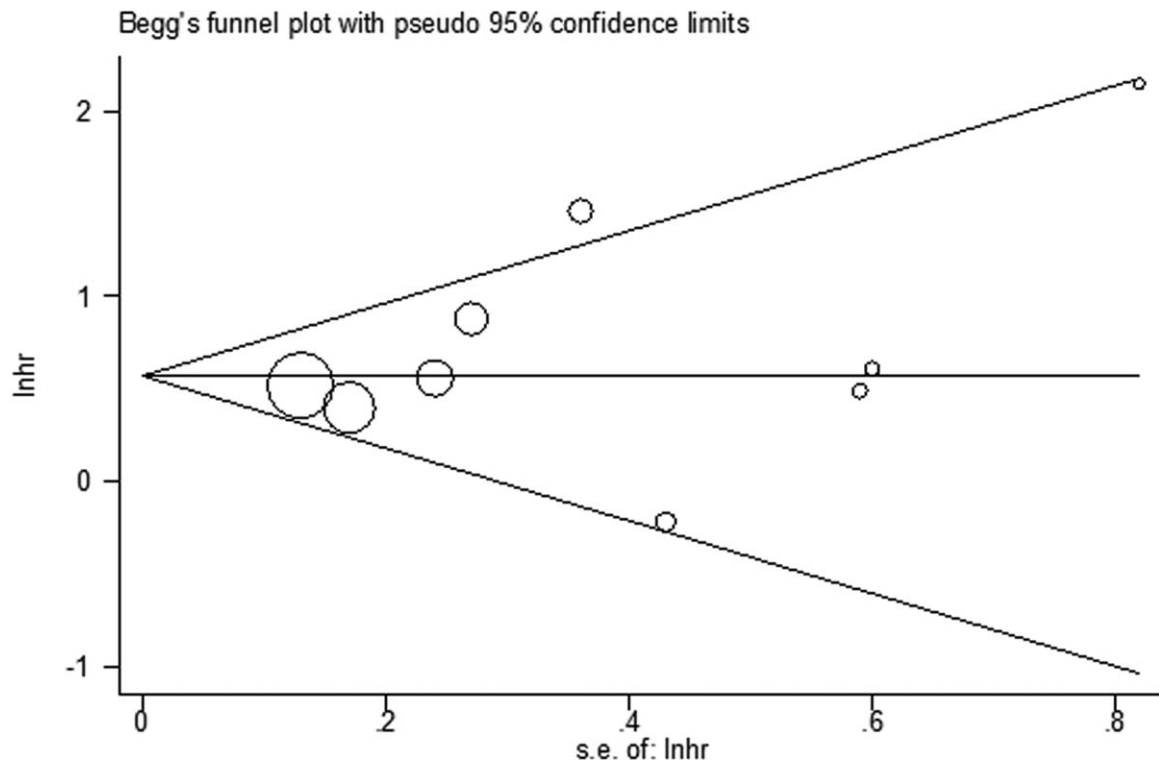


Figure 5. Begg's funnel plot to assess the publication bias for EFS. EFS=event-free survival.

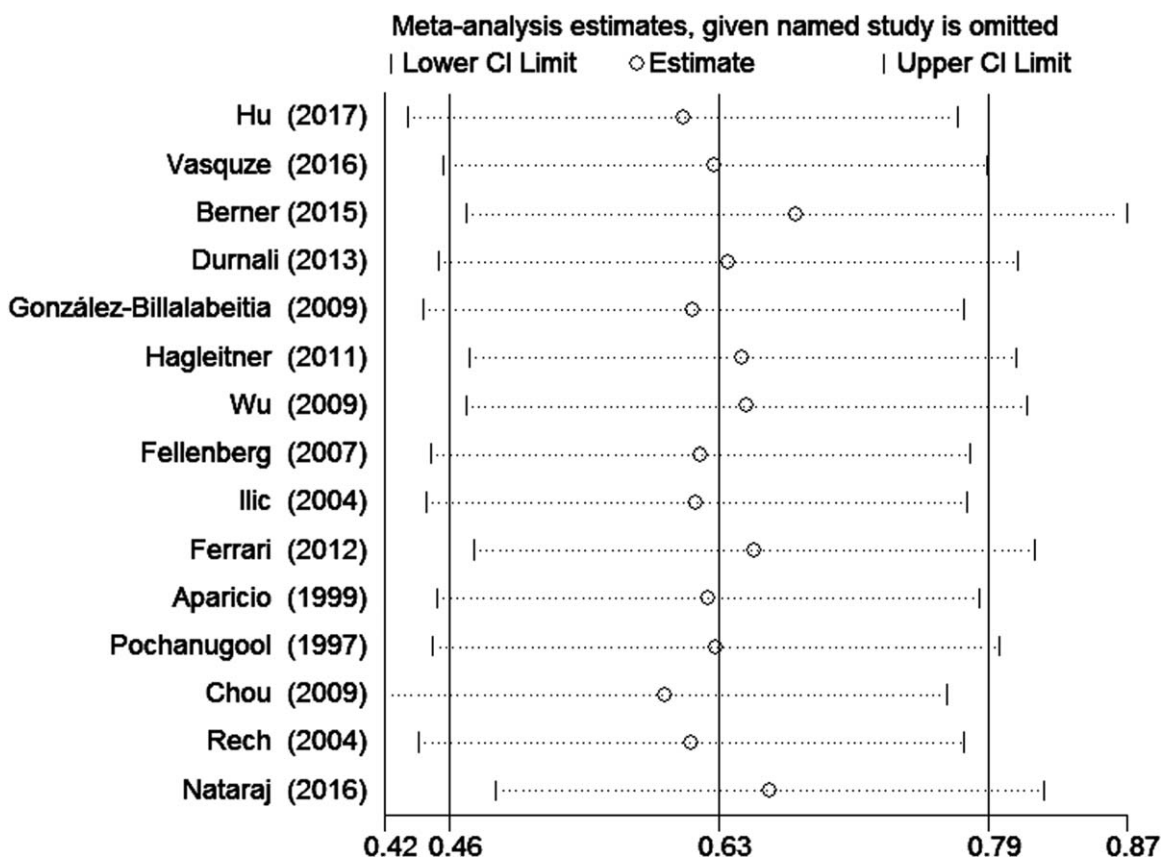


Figure 6. Forest plot for the sensitivity analysis in OS. OS=overall survival.

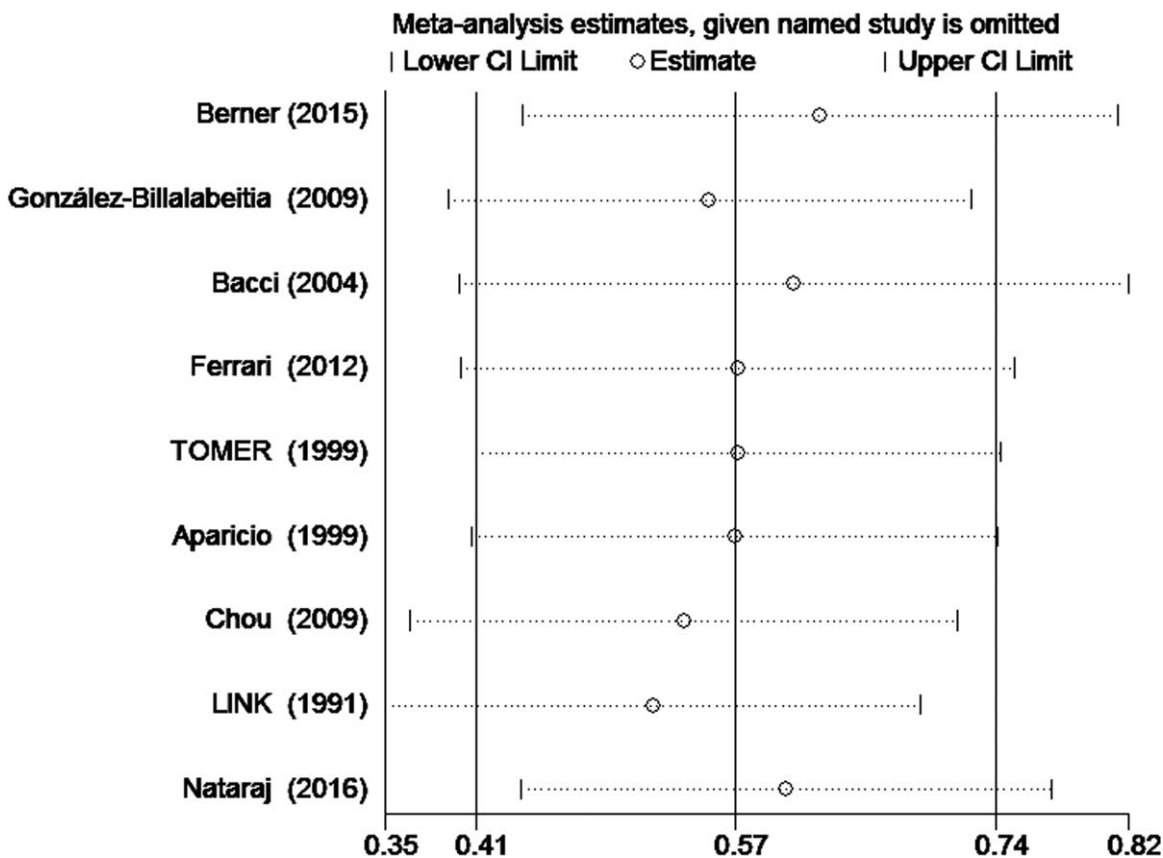


Figure 7. Forest plot for the sensitivity analysis in EFS. EFS=event-free survival.

related to energy synthesis in cancer cells. What is more, genetic excision of AMPK activated mammalian target of rapamycin (mTOR) signal with ectopic expression of hypoxia-inducible factor-1 alpha (HIF-1 alpha), which could activate some oncogenes to encode essential enzymes involved in glycolysis.^[41,42] LDH was one of these enzymes that involved in the conversion of pyruvate to lactate. It had at least 6 isoenzymes and in clinical practice, the activity of LDH was mainly measured by total amount in blood. Many researchers thought higher serum LDH level meant heavier osteosarcoma burden, which implied worse prognosis. Numerous studies also found the ability of proliferation and metastasis in malignant tumors was decreased when LDH activity was suppressed.^[43]

At the same time, some limitations and disadvantages might exist in this meta-analysis. First, perhaps the publication bias was induced because one of our inclusion criteria was studied that should be published and written in English, which meant some unpublished or non-English literature met the other criteria were ignored. This might narrow the searching range of studies. Besides, researchers tend to publish positive results over negative findings in most cases, which might also bring some bias. Second, this meta-analysis included 18 studies of 2543 patients. The sample size was relatively moderate and this might increase the risk of bias. Third, there was not a recognized or precise definition of elevated serum LDH level in osteosarcoma patients, thus patients were divided into different groups by various LDH cut-off values, which might cause some heterogeneity. What is more, the normal serum LDH level in different age was diverse and it was not considered in some studies, which might make the result less accurate. Fourth, we used 2 methods mentioned before to extract the HRs and 95% CIs due to they were not directly shown in all studies. As a result, a slight risk of bias was probably produced between original data and calculated one, whereas it would not affect the final conclusion. Finally, the study design and clinical features of patients were different in each research, which would increase the heterogeneity of meta-analysis. Moreover, with the development of new drugs and surgical methods, the treatment of osteosarcoma was changed in recent decades. Therefore, the therapeutic protocols used in different studies were not always the same, which might also generate heterogeneity.

In conclusion, although there are some limitations described before, our meta-analysis demonstrates the higher level of serum LDH is associated with lower EFS rate in osteosarcoma patients. Serum LDH is a fast, affordable and simple clinical parameter which could be used as a favorable biomarker in predicting the prognosis of osteosarcoma patients. Moreover, LDH might be considered as a potential therapeutic target to improve the prognosis of malignant tumor patients. In the future, more professionally-designed multi-center prospective study should be carried out to validate the conclusion of this meta-analysis.

Author contributions

Data curation: Tao Lan.

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Software: Wei Yu.

Supervision: Hongliu Cai, Anwei Lu.

Validation: Anwei Lu.

Visualization: Anwei Lu.

Writing – original draft: Yucheng Fu.

Writing – review & editing: Yucheng Fu, Wei Yu.

References

- Picci P. Osteosarcoma (osteogenic sarcoma). *Orphanet J Rare Dis* 2007;2:6.
- Dahlin DC, Unni KK. Osteosarcoma of bone and its important recognizable varieties. *Am J Surg Pathol* 1977;1:61–72.
- Fokas E, Engenhardt-Cabillic R, Daniilidis K, et al. Metastasis: the seed and soil theory gains identity. *Cancer Metastasis Rev* 2007;26:705–15.
- Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. *CA Cancer J Clin* 2008;58:71–96.
- Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. *Cancer* 2009;115:1531–43.
- Zhang J, Yao YH, Li BG, et al. Prognostic value of pretreatment serum lactate dehydrogenase level in patients with solid tumors: a systematic review and meta-analysis. *Scient Rep* 2015;5:9800.
- Liao AC, Li CF, Shen KH, et al. Loss of lactate dehydrogenase B subunit expression is correlated with tumour progression and independently predicts inferior disease-specific survival in urinary bladder urothelial carcinoma. *Pathology* 2011;43:707–12.
- Krieg AF, Rosenblum LJ, Henry JB. Lactate dehydrogenase isoenzymes a comparison of pyruvate-to-lactate and lactate-to-pyruvate assays. *Clin Chem* 1967;13:196–203.
- Walenta S, Mueller-Klieser WF. Lactate: mirror and motor of tumor malignancy. *Semin Radiat Oncol* 2004;14:267–74.
- Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Statist Med* 1998;17:2815–34.
- 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;25:603–5.
- Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ (Clin Res ed)* 2003;327:557–60.
- Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959;22:719–48.
- Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ (Clinical Res ed)* 1997;315:629–34.
- Berner K, Hall KS, Monge OR, et al. Prognostic factors and treatment results of high-grade osteosarcoma in norway: a scope beyond the “classical” patient. *Sarcoma* 2015;2015:516843.
- Chou AJ, Kleinerman ES, Krailo MD, et al. Addition of muramyl tripeptide to chemotherapy for patients with newly diagnosed metastatic osteosarcoma: a report from the Children’s Oncology Group. *Cancer* 2009;115:5339–48.
- Durnali A, Alkis N, Cangur S, et al. Prognostic factors for teenage and adult patients with high-grade osteosarcoma: an analysis of 240 patients. *Med Oncol* 2013;30:624.
- Fellenberg J, Bernd L, Delling G, et al. Prognostic significance of drug-regulated genes in high-grade osteosarcoma. *Mod Pathol* 2007;20:1085–94.
- Ferrari S, Ruggieri P, Cefalo G, et al. Neoadjuvant chemotherapy with methotrexate, cisplatin, and doxorubicin with or without ifosfamide in nonmetastatic osteosarcoma of the extremity: an Italian sarcoma group trial ISG/OS-1. *J Clin Oncol* 2012;30:2112–8.
- González-Billalabeitia E, Hitt R, Fernández J, et al. Pre-treatment serum lactate dehydrogenase level is an important prognostic factor in high-grade extremity osteosarcoma. *Clin Transl Oncol* 2009;11:479–83.
- Hagleitner MM, Hoogerbrugge PM, van der Graaf WT, et al. Age as prognostic factor in patients with osteosarcoma. *Bone* 2011;49:1173–7.
- Nataraj V, Rastogi S, Khan SA, et al. Prognosticating metastatic osteosarcoma treated with uniform chemotherapy protocol without high dose methotrexate and delayed metastasectomy: a single center experience of 102 patients. *Clin Transl Oncol* 2016;18:937–44.
- Vasquez L, Tarrillo F, Oscanoa M, et al. Analysis of prognostic factors in high-grade osteosarcoma of the extremities in children: a 15-year single-institution experience. *Front Oncol* 2016;6:22.
- Wu PK, Chen WM, Chen CF, et al. Primary osteogenic sarcoma with pulmonary metastasis: clinical results and prognostic factors in 91 patients. *Jpn J Clin Oncol* 2009;39:514–22.
- Hu K, Wang Z, Lin P, et al. Three hematological indexes that may serve as prognostic indicators in patients with primary, high-grade, appendicular osteosarcoma. *Oncotarget* 2017;8:43130–9.
- Ilic I, Manojlovic S, Cepulic M, et al. Osteosarcoma and Ewing’s sarcoma in children and adolescents: retrospective clinicopathological study. *Croat Med J* 2004;45:740–5.

- [27] Tomer G, Cohen IJ, Kidron D, et al. Prognostic factors in non-metastatic limb osteosarcoma: a 20-year experience of one center. *Int J Oncol* 1999;15:179–85.
- [28] Aparicio J, Segura A, Montalar J, et al. Long-term results after combined modality treatment for non-metastatic osteosarcoma. *Med Oncol* 1999;16:255–60.
- [29] Pochanugool L, Subhadharaphandou T, Dhanachai M, et al. Prognostic factors among 130 patients with osteosarcoma. *Clin Orthop Rel Res* 1997;345:206–14.
- [30] Link MP, Goorin AM, Horowitz M, et al. Adjuvant chemotherapy of high-grade osteosarcoma of the extremity. Updated results of the Multi-Institutional Osteosarcoma Study. *Clin Orthop Rel Res* 1991;270:8–14.
- [31] Rech A, Castro CG Jr, Mattei J, et al. Clinical features in osteosarcoma and prognostic implications. *J Pediatr* 2004;80:65–70.
- [32] Bacci G, Longhi A, Ferrari S, et al. Prognostic significance of serum lactate dehydrogenase in osteosarcoma of the extremity: experience at Rizzoli on 1421 patients treated over the last 30 years. *Tumori* 2004;90:478–84.
- [33] Zhang Y, Ding C, Wang J, et al. Prognostic significance of CD44V6 expression in osteosarcoma: a meta-analysis. *J Orthop Surg Res* 2015;10:187.
- [34] Hao H, Chen L, Huang D, et al. Meta-analysis of alkaline phosphatase and prognosis for osteosarcoma. *Eur J Cancer Care* 2017;26:e12536.
- [35] Chen D, Zhang YJ, Zhu KW, et al. A systematic review of vascular endothelial growth factor expression as a biomarker of prognosis in patients with osteosarcoma. *Tumour Biol* 2013;34:1895–9.
- [36] Shen J, Chen Z, Zhuang Q, et al. Prognostic value of serum lactate dehydrogenase in renal cell carcinoma: a systematic review and meta-analysis. *PLoS One* 2016;11:e0166482.
- [37] Li G, Wang Z, Xu J, et al. The prognostic value of lactate dehydrogenase levels in colorectal cancer: a meta-analysis. *BMC Cancer* 2016;16:249.
- [38] Zhang X, Guo M, Fan J, et al. Prognostic significance of serum LDH in small cell lung cancer: a systematic review with meta-analysis. *Cancer Biomarkers* 2016;16:415–23.
- [39] Nakamura T, Kitagawa T. Anticancer drug screening test with LDH in nude mouse bearing bone and soft part sarcoma. *Cancer* 1985;56:1112–6.
- [40] Marais LC, Bertie J, Rodseth R, et al. Pre-treatment serum lactate dehydrogenase and alkaline phosphatase as predictors of metastases in extremity osteosarcoma. *J Bone Oncol* 2015;4:80–4.
- [41] Yoshida GJ. Therapeutic strategies of drug repositioning targeting autophagy to induce cancer cell death: from pathophysiology to treatment. *J Hematol Oncol* 2017;10:67.
- [42] Yoshida GJ. Metabolic reprogramming: the emerging concept and associated therapeutic strategies. *J Exp Clin Cancer Res* 2015;34:111.
- [43] Augoff K, Hryniewicz-Jankowska A, Tabola R. Lactate dehydrogenase 5: an old friend and a new hope in the war on cancer. *Cancer Lett* 2015;358:1–7.