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Original Article

Serum lactate dehydrogenase as a prognostic biomarker in patients with Ewing's sarcoma: a meta-analysis

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Abstract: Purpose: The prognostic value of serum Lactate dehydrogenase (sLDH) in Ewing sarcoma (ES) has been studied worldwide during these years and provided un-uniformed conclusions. Methods: Comprehensive literature was selected from PUBMED, EMBASE and WEB OF KNOWLEDGE. Clinical studies which reported analysis of survival data about sLDH in ES were included. Stata 12.0 was used for performing a meta-analysis on evaluating the relation between LDH and clinical staging, overall survival (OS) and disease free survival (DFS). Results: A total of 13 articles, including 2395 patients who satisfied inclusion criteria were analyzed. The result showed that high concentration of sLDH was related to a bad OS (HR = 1.93, 95% CI 1.68-2.22) and a extremely worse DFS (HR = 5.96, 95% CI 3.37-10.54). The subgroup analysis on different location of ES suggested the prognosis of extremity group (HR = 1.91, 95% CI 1.67-2.34) was better than axial skeleton group (HR = 2.03, 95% CI 1.67-2.48). Another subgroup analysis suggested that it was even worse prognosis (HR = 2.11, 95% CI 1.74-2.55) when distant metastasis percents > 30% than distant metastasis percents < 30% (HR = 1.96, 95% CI 1.69-2.28). Conclusions: Our findings suggest that sLDH can be regarded as a poor prognostic maker for ES and may represent a important new therapeutic target.

Keywords: Serum lactate dehydrogenase, meta-analysis, Ewing's sarcoma, prognosis analysis, overall survival

Introduction

Ewing's sarcoma or Ewing sarcoma (ES) is a malignant tumor which occurs most frequently in teenagers and young adults [1]. The prognosis of ES is reported to be poor with metastases and/or recurrences in about 30% to 50% cases [2]. Patients with recurrence have a 5-year survival of 13% [3]. Lactate dehydrogenase (LDH) is an important enzyme for the interconversion of lactate and pyruvate, also involved in the oxidation of long-chain fatty acid and can provide NAD+ for continued glycolysis in active muscle [4]. With these features, serum LDH (sLDH) level is now widely used in clinical, even as a blood chemistry indicator. In recent years, sLDH has attracted broad interests and discussions because of an enlarging view on what LDH does on the prognosis value of many tumors. A great deal of studies have reported serum LDH level could predict the prognosis of several tumors, including lung cancer, rectal cancer, pancreatic cancer, prostate cancer and even osteosarcoma [5-7].

A great number of studies have investigated the role of LDH level in patients with ES but have yielded inconsistent and inconclusive results. Patrick J. reported that ES children with normal LDH (≤ 250 IU/L) are more likely to survive from metastases and/or recurrences [8]. Gaetano Bacci found that ES patients with normal LDH level at presentation have a better 10 years overall survival (OS) than those with elevated LDH level [9]. In contrast, other researchers reported that the initial LDH level was found to have no prognostic value [10, 11]. Therefore, it is still unclear and controversial whether serum LDH level at presentation could reflect the prognosis of ES.

In this study, we attempted to conduct a meta-analysis to estimate the relationship between serum LDH level at presentation and OS and

disease free survival (DFS) among patients with ES. And we sought to find out whether serum LDH level could provide helpful guidance in the treatment and prognosis of ES.

Materials and methods

Search strategy

Literature selected from PubMed (MEDLINE), EMBASE and WEB OF KNOWLEDGE was conducted by combining search terms "lactate dehydrogenase","ldh","lacticdehydrogenase","dehydrogenase", "lactate dehydrogenase", "lactic acid dehydrogenase" with "ewing sarcoma", "ewing' sarcoma", "ewing's sarcoma", "ewing", "ewing's", "askintumour", "Peripheral neuroectodermaltumour (PNET)", "Primitive peripheral neuroectodermal (PPNET)". The deadline was June 21st, 2015. To prevent the omission of any research via electronic search strategy, reference lists from identified primary studies and review articles were also searched [12].

Study inclusion or exclusion criteria

Inclusion criteria for the study were as follows: (1) confirmed diagnosis of ES in humans; (2) literature was published in English; (3) clinical trials investigating the association between LDH and the prognosis of ES patients, not basic research and animal experiments; (4) reviews, articles published in a book and only summaries of the literature were excluded; (5) clinical research association of LDH with overall survival, and/or disease free survival (DFS); (6) no duplicate data. The names of all authors and medical centers involved for each article was examined by us to avoided duplication of data. Authors that published multiple reports on the same sample were included once; (7) having survival data about LDH; (8) literature must provide prognostic hazard ratio (HR) or sufficient information that can calculate HR value. Incomplete information was also excluded. The quality sores were assessed by Newcastle-Ottawa Scale ,low quality studies were removed [13].

Data extraction

Two authors of us (HW and JQC) used a standard information collection form to extract the following items: (1) article information including first author's name, publication date and country of origin; (2) demographic data including number, gender structure, mean age, follow-up period, and percentage of serum LDH level positive; (3) ES information including tumor location, percentage of distant metastasis; (4) survival data including OS and DFS; (5) technology of LDH measurement, cut-off value used for assessing LDH positivity; Any differences between the two authors in the data extraction were resolved together by our review team.

Quality assessment

The risk of bias in our included studies was assessed by two independent reviewers of us (WH and JQC) by the Newcastle-Ottawa Scale (NOS). According to the Cochrane Collaboration, the quality of the nonrandomized studies like our including studies were assessed by using NOS with some modifications to match the needs. The quality of including studies was evaluated by using the following three items: selection, comparability and assessment of outcome and the quality of each study in our meta-analysis was graded as two levels: level one (0 to 4 points) and level two (5 to 9 points). Any discrepancy about the judgment in the quality assessment was resolved by discussion.

Data synthesis

We calculated the value of hazard ratios (HR) with its corresponding 95% confidence interval (95% CI) to evaluate the relationship between serum LDH level and OS/DFS. For those HRs were not reported in published data, we calculated the HR with the available data via the methods described by Freels S [14]. If the only available data in the included articles were survival curves, we analysis them via Engauge Digitizer version 4.1 and extracted survival rate from them to calculate the HR, 95% CI and its standard error (SE) [12, 15]. All the data were analyzed by Stata version 12.0 (Stata Corporation, College Station, TX, USA). We assessed H-tests and *P*-values to estimate the effect of between-study heterogeneity in our meta-analysis. When there was a significant heterogeneity existed across the included studies we carefully selected (I squared > 50% or P < 0.10), the random effects model (the DerSimonian-Laird method) was used for our meta-analysis [16]. Otherwise, the fixed effects model was used to calculate the

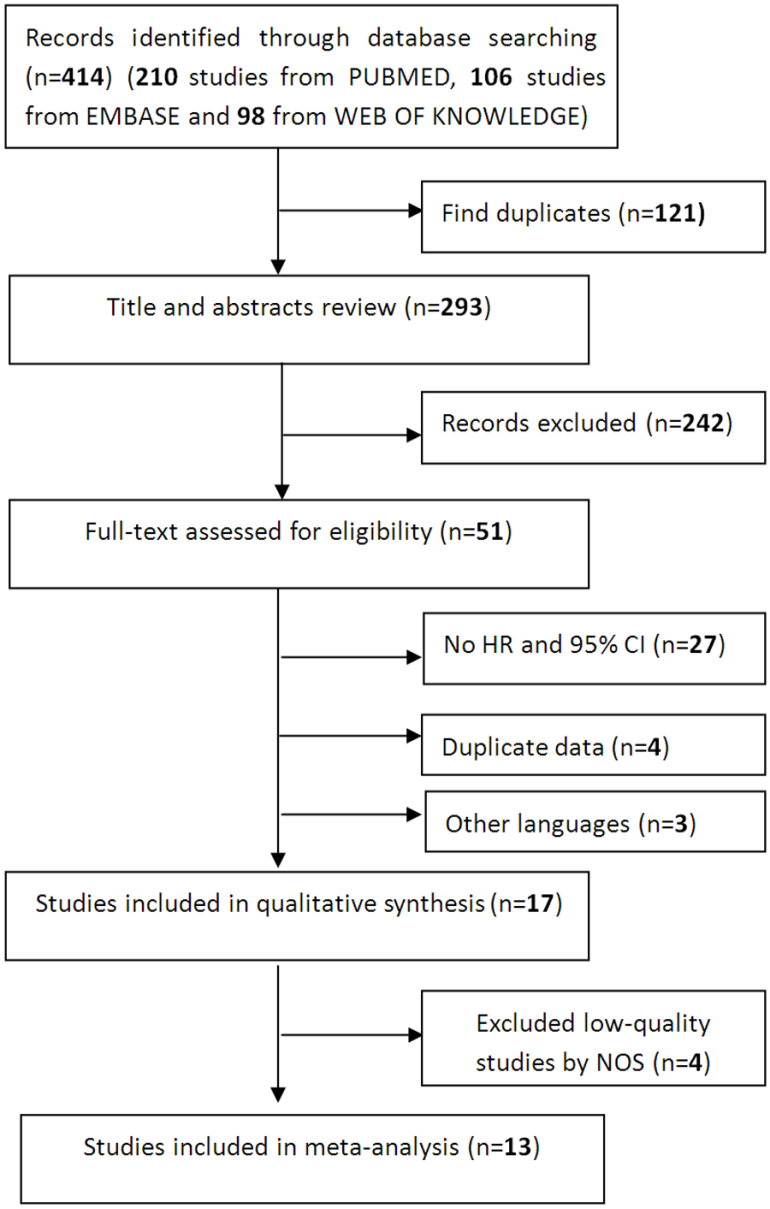


Figure 1. Methodological flow chart of the literature search and selection of included studies.

HR and its 95% CI according to the method of Mantel and Haenszel [17].

Results

Literature search and study design characteristics

By searching in PubMed, EMBASE and WEB OF KNOWLEDGE databases, a total of 414 primary studies were yielded and we evaluated 51 possible candidate literatures in full text. By further articles review, twenty-seven articles were excluded because of no prognostic analysis or no HR. Three articles were excluded because they were not published in English. Five articles whose author were Gaetano, had duplicate data because of the same research institute, the crossing follow-up time and the analogous methods, we chose the most valuable one to add in our meta-analysis by selection method told by Whitehead A [18]. At last we excluded 4 articles because of low-quality studies (less than 4 points) by using the Newcastle-Ottawa Scale (NOS) (**Figure 1**). Finally, A total of 13 articles [8, 11, 19-29] including 2395 patients who satisfied the inclusion criteria were analyzed. The results are shown on **Table 1**. The publication date ranged from 1975 to 2014. Five reports originated from America, three from Italy, and the others originated from India, Turkey, Croatia, Brazil, Spain.

The percentage of number of male ranged from 51.34% to 70.69%. The range of the eligible studies' mean age was 10.0 to 23.0. The mean observed years of eligible studies ranged from 2.1 years to 10.7 years, but 8 studies did not report the mean observed years. The positive rate of serium LDH ranges from 5.88% to 62.65% in all studies.

The location of the most tumor cells includedextraosseous, central, extremity, pelvis, femur, extremities, axial skeleton, distal, trunk. One study did not report the location of the most tumor cells. The distant metastasis rate of cancer was reported in 11 studies ranging from 12.26% to 66.67%.

A total of eleven articles with 2162 patients provided the prognostic data on OS [8, 11, 19-25, 27, 28]. Two articles with 87 patients provided prognostic data on EFS [19, 20] and 3 articles with 342 patients on DFS [26, 28, 29].

Table 1. Main characteristics of the including studies relating sLDH to patients' prognosis

First author (years)	Country	Study design	N (Male %)	Mean age	Observed years (mean)	Distant metastasis (%)	Serum LDH high (%)	Tumor location (most)	Analysis	Cut-off value	NOS
Biswas (2014)	India	Retro.	60 (70.00)	15.1	2003-2011 (2.1 yr)	66.67	41.86	Extraosseous	OS, EFS	> 458 U/L	6
Tural (2012)	Turkey	Retro.	27 (66.67)	23.0	1997-2010 (2.7 yr)	14.81	44.44	Central	OS, EFS	> 240 U/L	7
Patrick (2009)	America	Retro.	262 (60.69)	14.2	1988-1994 (NR)	45.80	62.65	Extremity	OS	> 250 U/L	6
Gaetano (2007)	Italy	Retro.	888 (62.73)	17.8	1983-2006 (NR)	12.26	37.47	Pelvis, femur	OS	> 460 U/L	8
Ilic (2004)	Croatia	Retro.	34 (58.82)	11.0	1988-1999 (3.96 yr)	38.24	5.88	Extremities	OS	> 500 U/L	7
Da Costa (2003)	Brazil	NR	105 (51.43)	10.0	1984-1996 (NR)	32.38	23.61	Pelvis and femur	OS	> 370 U/L	5
Ferrari (2000)	Italy	Retro.	482 (63.28)	NR	1972-1997 (NR)	28.22	32.57	Extremity	OS	> 460 U/L	7
Roberto (1999)	Italy	Retro.	73 (65.75)	12.5	1974-1998 (NR)	20.00	54.69	Pelvis	OS	> 460 U/L	7
Aparicio (1998)	Spain	Retro.	116 (70.69)	14.0	1970-1993 (10.7 yr)	17.24	32.22	Axial skeleton	DFS	> 300 U/L	8
Kinsella (1991)	America	Retro.	109 (58.72)	15.9	1968-1990 (NR)	25.23	23.86	Central	OS, DFS	> 350 U/L	7
Farley (1987)	America	Retro.	56 (NR)	NR	1973-1986 (4 yr)	NR	28.89	NR	OS	> 230 U/L	7
Glaubiger (1980)	America	Retro.	117 (NR)	12.0	1964-1980 (NR)	32.48	40.79	Distal	DFS	> 200 U/L	7
Pomeroy (1975)	America	Retro.	66 (63.64)	NR	NR	NR	56.52	Trunk	OS	> 170 U/L	6

Abbreviations: NR: not reported; TN: total number; Retro: retrospective study; OS: overall survival; DFS: disease free survival; EFS: event free survival; NOS: Newcastle-Ottawa Scale.

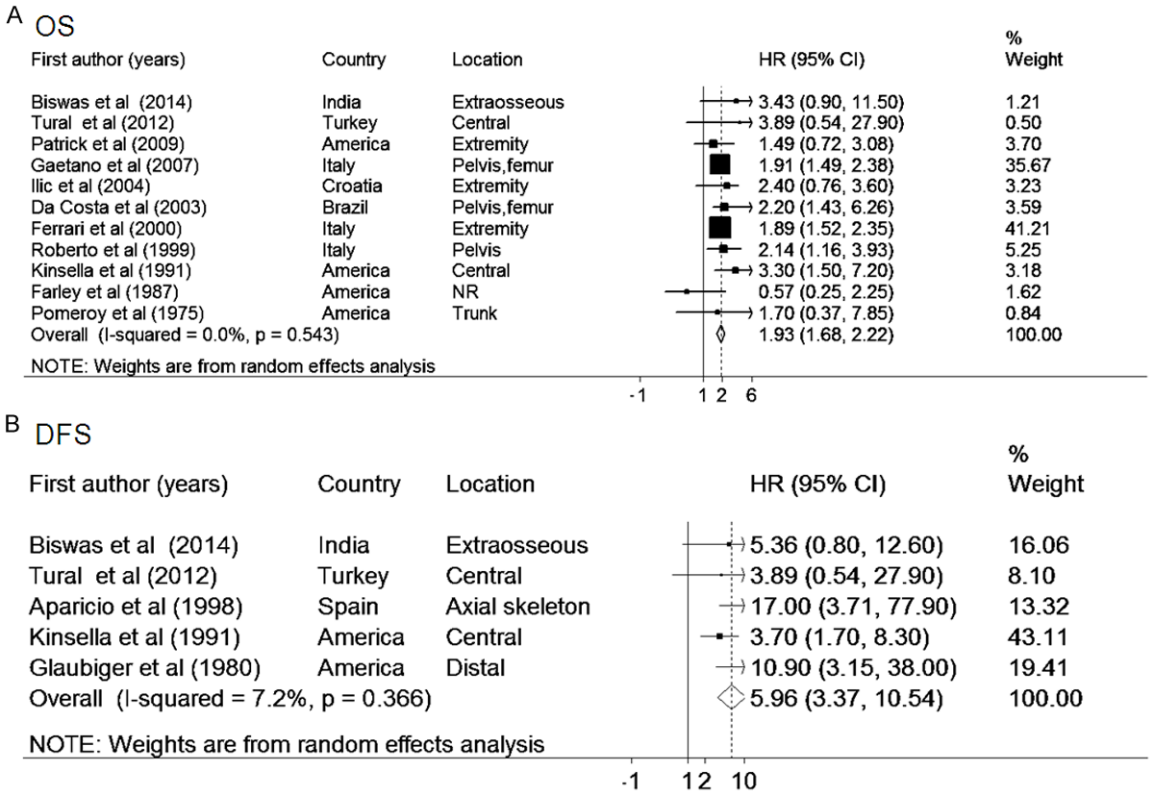


Figure 2. Forrest plots in Studies of sLDH in Patients with ES by HR estimation. survival data are reported as (A) Overall survival (OS), (B) disease free survival (DFS).

All the LDH value was measured in blood serum of patients. The cut-off value used for assessing LDH positivity was ranged from 170 U/L to 500 U/L. As known by us and according to the Cochrane Collaboration, NOS was used to assess the quality of the included studies in our meta-analysis. The score was ranged from 5 to 8 and with a mean point of 6.77.

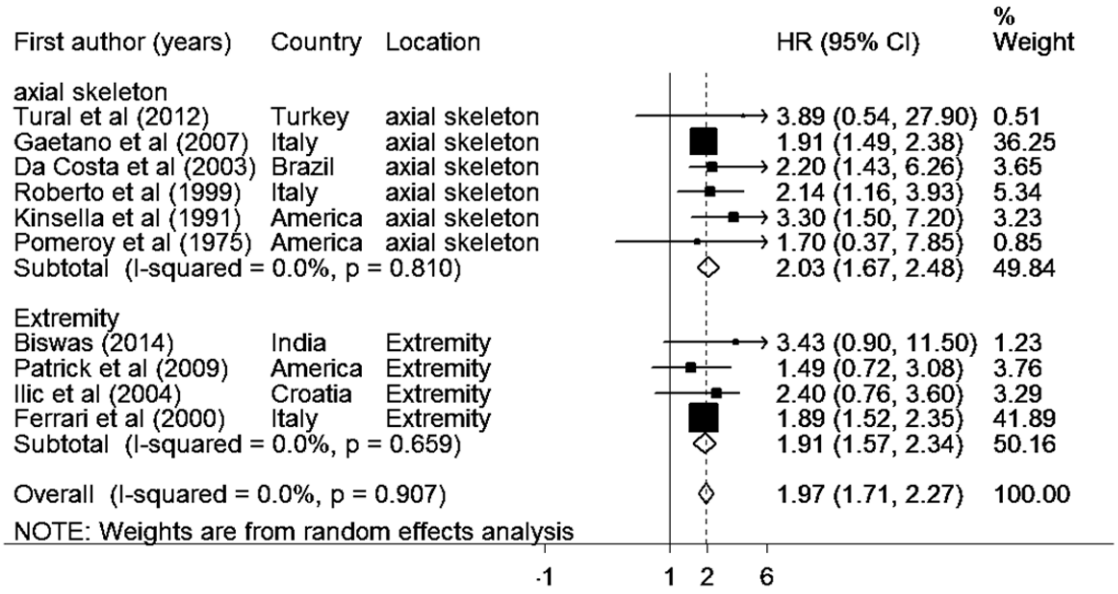
Meta-analysis

In our meta-analysis of the effect of LDH expression on overall survival, there was no significant heterogeneity among those 11 studies [8, 11, 19-25, 27, 28] (I squared = 0%), so the fixed effect model was used to calculate the HRs and 95% CIs. The pooled data suggested that compared with cancer patients with low or negative LDH expression, high concentration of LDH was associated with a bad prognosis on OS (HR = 1.93, 95% CI 1.68-2.22) (**Figure 2**). For DFS in overall population, the fixed effect model was also used among those five studies because of the lack of heterogeneity (I squared = 7.2%) and an extremely worse prognosis (HR = 5.96, 95% CI 3.37-10.54) was observed among patients considered LDH positive.

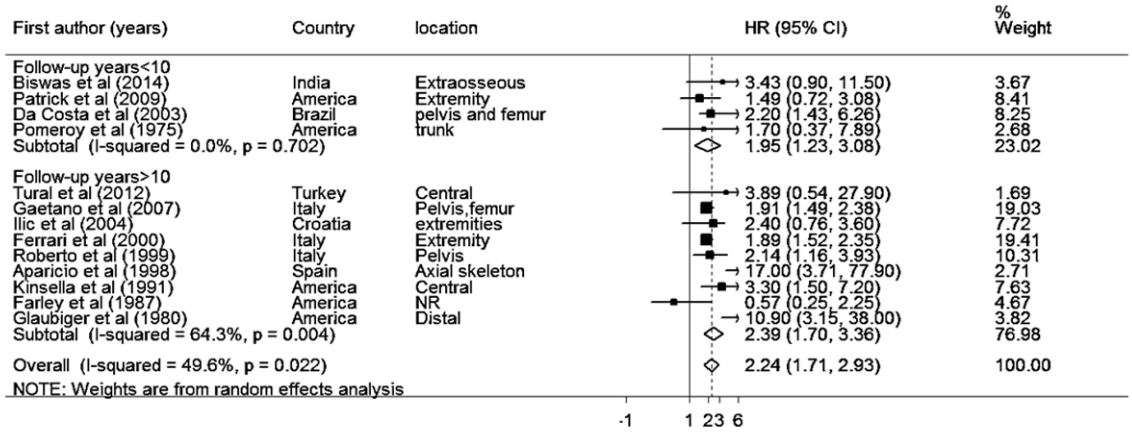
The first subgroup analysis was assessed by us according to the location of the ES (**Figure 3A**). Theoretically, ES can occur and progress in systemic bones or soft tissues [30, 31]. It is common in axial skeleton and limb bones, especially the femur [32]. In this meta-analysis, six studies reported that compared to low or negative LDH expression, high concentration of LDH was significantly related to poor OS in patients with ES in axial skeleton (HR = 2.03, 95% CI 1.67-2.48) [20, 21, 23, 25, 27, 28]. And in the remaining four studies [8, 19, 22, 24], LDH density was positively correlated with extended survival in patients with ES in extremity (HR = 1.91, 95% CI 1.67-2.34).

With respect to follow-up time, the effect of LDH concentration in patients with ES was further analyzed and described. There were also four studies whose follow-up years were less than ten years [8, 19, 23, 27] and indicated a bad prognosis (HR = 1.95, 95% CI 1.23-3.08) (**Figure 3B**). When follow-up years were more than ten years in nine articles [11, 20-22, 24-26, 28, 29], an statistically significant HR of 2.24 (95% CI 1.71-2.93) was shown in **Figure**

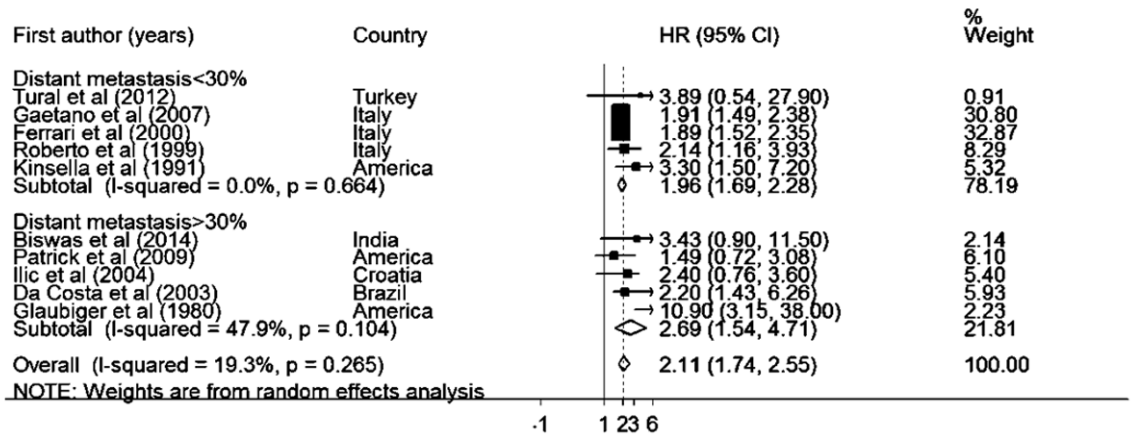
A Location



B Follow-up time



C Distant metastasis



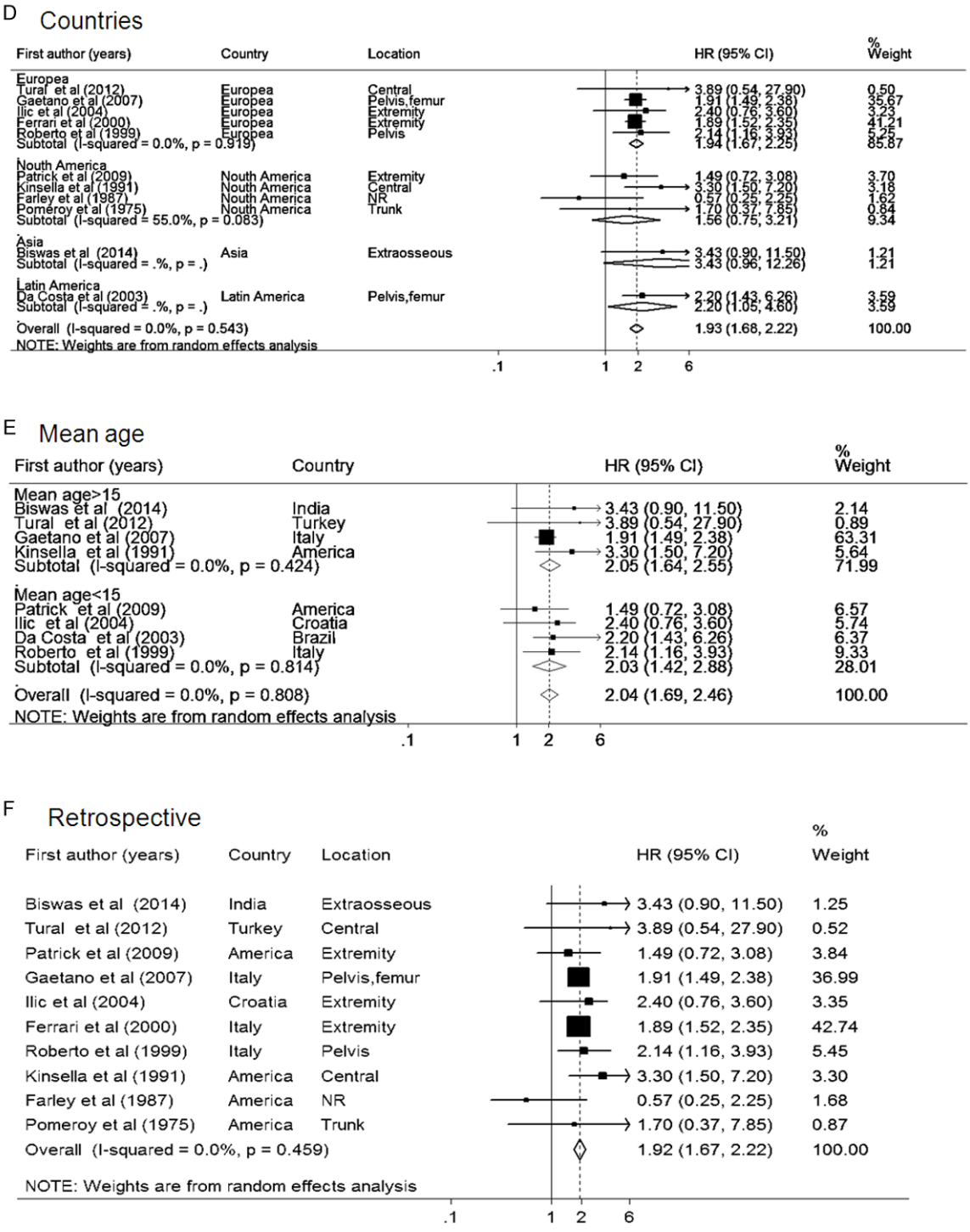


Figure 3. Forrest plots in Studies of sLDH Expression in Patients with ES by HR estimation for OS in Subgroups. Survival data are reported as (A) Location, (B) Follow-up time, (C) Distant metastasis, (D) Countries, (E) Mean age and (F) Retrospective study.

3B. As we all know, the efficacy of therapy appears to be closely dependent on the stage of the disease. However, TNM staging is connected with the prognosis of tumors closely [33, 34] and distant metastasis is particularly important index for the TNM staging in patients with ES. to analyze sLDH's prognostic value on different percents of distant metastasis, **Figure**

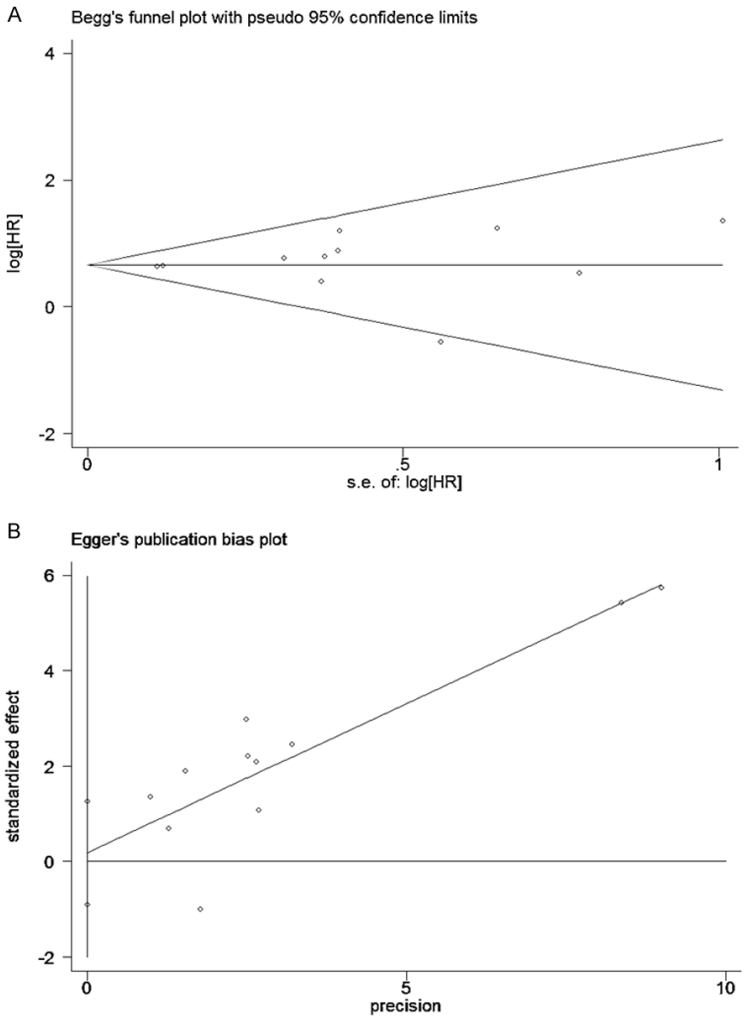


Figure 4. Funnel Graph of Begg's test (A) and Egger's test (B) in Studies of sLDH Expression in Patients with ES by HR estimation for the Assessment of Potential Publication Bias in **Figure 2A**. No indication of publication was shown in Begg's test (P = 0.186) and Egger's test (P = 0.713) of OS.

3C showed an HR of 1.96 (95% CI 1.69-2.28) by distant metastasis percents < 30% in five studies [20, 21, 24, 25, 28], and it was even worse (HR = 2.11, 95% CI 1.74-2.55) when distant metastasis percents > 30%. There was also significant difference in the summary estimate of sLDH on overall survival when cut-off value was in line with the concentration of sLDH (HR = 1.93, 95% CI 1.68-2.22), especially when the cut-off value was during 300-400 U/L (HR = 2.66, 95% CI 1.55-4.56) and more than 500 U/L (HR = 2.40, 95% CI 1.10-5.22).

At last, subgroup analysis was performed according to countries (**Figure 3D**). European countries, with 5 studies evaluable [20-22, 24, 25], showed a significant HR of 1.94 (95% CI 1.67-2.25). North American countries showed an HR of 1.56 (95% CI 0.75-3.21) in 4 included studies [8, 11, 27, 28]. Only one study reported that the sLDH density was negatively correlated with extended survival in patients with ES in Asian country (HR = 3.43, 95% CI 0.96-12.26) [19] and Latin America (HR = 2.20, 95% CI 1.05-4.60) [23]. In our meta analysis, age was not a clear prognosis index for patients with ES on OS. **Figure 3E** showed an HR of 2.05 (95% CI 1.64-2.55) by mean age less than 15, and an HR of 2.03 (95% CI 1.42-2.88) when mean age more than 15.

Evaluation of publication bias

Visual assessment of Egger's test and Begg's funnel plots was used by us to evaluate the possibility of publication bias [12] on the outcomes in all studies evaluating OS and DFS separately, and assessment was also performed in subgroup analysis. Begg's funnel plot did not find any evidence of asymmetry in overall meta analysis of OS (P = 0.186) and DFS (P = 0.624). In addition, no indication of publication was shown in Egger's test of OS (P = 0.713) and DFS (P = 0.376) (**Figure 4**). For those sub-groups in our meta analysis, there were also no significant evaluation of publication bias shown from Egger's or Begg's funnel test.

Discussion

At present, plenty of original articles and reviews around world have discussed the prognostic value of LDH in patients with ES and put forward the importance of LDH on its survival [20, 35, 36], which made it indispensable to perform a quantitative aggregation of the survival results. According to the literatures we found by searching the EMBASE and PubMed (MEDLINE) on 12/4/2015, this is the first study

performed by meta-analysis to clarify the prognostic value of LDH for OS, staging and DFS about ES. Our meta-analysis showed that compared with low or negative level of sLDH, the high LDH in patients with ES is a worse prognostic indicator with statistical significance for OS (HR = 1.93, 95% CI 1.68-2.22), which suggests a 1.93-fold higher OS for ES patients with overexpression detection of sLDH. This final result about OS by Stata is consistent with 10 of 11 included studies with a HR above 1. Furthermore, an extremely worse prognosis effect (HR = 5.96, 95% CI 3.37-10.54) of DFS was observed among ES patients considered LDH positive. Using Egger's, Begg's tests and the funnel plot, we regard an absent publication bias in our analysis also. Therefore, the findings from our meta-analysis of OS and DFS suggest that sLDH can be an effective biomarker of prognosis in patients with ES.

As we all know, compared to normal tissues, one of the principal important characteristics of malignant cells is higher glycolytic metabolism switch from oxidative phosphorylation, even under hypoxic conditions, and is called the Warburg effect [37, 38]. However, LDH can catalyze conversion of pyruvate to lactate and is considered as a key checkpoint of anaerobic glycolysis. The reliance and importance of tumor cells on LDH has been demonstrated in mouse models in many reports [39-41]. Fantin. in 2006 showed that tumor cells rely on the activity of LDH, whereas that non-malignant cells (normal cell) rely on OXPHOS, by demonstrating that the growth of LDHA-deficient cancer cells was severely reduced in rat Neu4145 mammary gland tumor cells even under hypoxic (0.5% oxygen) conditions [39]. On the other hand, LDH level is elevated in many types of cancers such as lung cancer, rectal cancer, pancreatic cancer and has been always linked to tumor growth, maintenance, invasion and metastasis. Anyway, LDH knockdown could inhibit tumorigenesis in vivo [42] and cell growth and migration in vitro [43]. It was suggested that silencing LDH expression activates apoptotic pathways and inhibits cell growth, which was showed by downregulating cyclin D1 and activation of AKT and increasing cleavage of poly-ADP-Ribose-Polymerase (PARP) and caspase 8 [43, 44]. One previous study on human hepatocellular carcinoma by Miao agree with this proposition by showing LDH knockdown in human hepatocellular carcinoma cells could induce apoptosis. In conclusion, these observations confirm that LDH is central to tumor happen, proliferation and malignant growth, and that high LDH level is a strong prognostic indicator of tumors [45].

Therefore, the inhibition of LDH may restrict the energy supply in tumors and thereby can reduce the metastatic and invasive potential of malignant cells. LDH enzyme is and will be receiving a great deal of attention as a predictive prognosis biomarker for many types of cancer especially for ES and as a therapeutic target for new anticancer treatments. We can detect the value of sLDH of ES to refine the neoadjuvant chemotherapy measures. For those high sLDH patients with ES, who are determined as bad prognosis, can adjust the chemotherapy and surgery program pertinently and expect to have a better recurrence rate and improvement of long-term living standards. Our results may provide further basis for the development of new tumor indicating marker and suggest that inhibition of lactate dehydrogenase activity can be as an approach to cancer therapy. Furthermore, these results can also improve the treatment strategy in patients with ES and have a better recurrence rate and improvement of long-term living standards.

The first subgroup analysis was conducted by us according to the location of the primary ES (**Figure 3A**). As we all know, it existed differences of pronosis in different part of tumors. In our meta-analysis, we divided the tumor site into two sites: six studies reported that high concentration of LDH was significantly related to poor OS in patients with ES in axial skeleton (HR = 2.03, 95% CI 1.67-2.48). And in the remaining studies, LDH density is also positively correlated with extended survival in extremity (HR = 1.91, 95% CI 1.67-2.34). Our results showed the prognosis of extremity group was better than axial skeleton group, which were also confirmed by Jie Z in 2010. In univariate analysis of his study showed that the 5-year overall survival rates of extremity and axial skeleton group was 38.8% and 18.5%, which meaned a worse prognosis of axis group. And the multivariate analysis showed that the location was an independent risk factor of prognosis.

It is known by all of us that the efficacy of therapy and the OS appears to be closely dependent on the stage of the disease. While TNM staging is connected with the prognosis of

tumors closely and the rate of distant metastasis is particularly important index for the TNM staging in patients with ES. Our analysis suggest that it is even worse prognosis (HR = 2.11, 95% CI 1.74-2.55) when distant metastasis percents > 30% than distant metastasis percents < 30% (HR = 1.96, 95% CI 1.69-2.28), which is agreed by lots of authors. Therefore, In order to obtain a better therapeutic effect and longer survival time, the earlier and the quicker we resect the tumor, the better.

The results of meta-analysis are confirmed as gold standards by authors globally [46-48], however, several limitations exist and need to be discussed in our meta-analysis and that may put forward a potential source of variability of meta-analysis. First of all, the main limitation in our meta-analysis was the item of primary outcome: different specimen from tissue or plasma, different survival rate, different analysis methods and especially no standard of cut-off value brings variability for LDH positive and negative. These differences may cause the obvious between-study heterogeneity among those studies in our meta-analysis of the effect of LDH expression. Thus, to provide further evidence for the prognostic role of LDH expression in patients with ES, more studies that are well designed by authors and having the same items of primary outcome are needed. Second, we included the literatures only published in English from three databases, which probably lead to a lack of valuable data published in other language like Japanese Chinese etc. Therefore, the prognostic significance of LDH could be overestimated by us because of a phenomenon called "file drawer problem", which was described by Earleywine that studies with positive results would be easier to be accepted and published by English magazines while most negative results are often published in native languages or even not received by the journal [49-52].

Conclusions

In conclusion, the results of our meta-analysis revealed that high level sLDH would correlate with poor OS and DFS in ES, can be regarded as a detrimental factor for ES and may represent as an important new therapeutic targets. In future, to achieve a more definitive conclusion enabling the clinical use of LDH in ES, more high-quality interventional original studies were needed following agreed research approach or standard.

Disclosure of conflict of interest

None.

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