IVYSPRING INTERNATIONAL PUBLISHER

Journal of Cancer

2018; 9(16): 2876-2884. doi: 10.7150/jca.25351

Research Paper

Diagnostic and Prognostic Value of Circulating MicroRNAs for Esophageal Squamous Cell Carcinoma: a Systematic Review and Meta-analysis

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Received: 2018.02.04; Accepted: 2018.06.11; Published: 2018.07.30

Abstract

Background and Aim: MicroRNAs, dysregulated in the circulation of esophageal squamous cell carcinoma (ESCC) patient, have been assumed to be with great potential in the diagnosis and prognosis of esophageal cancer. We aimed to review previous articles on ESCC.

Methods: A search of electronic databases was performed before Nov 12, 2017. We summarized the identification of microRNA imbalance in the blood of ESCC compared with the healthy controls, with the objective to evaluate the efficiency of microRNAs in diagnosing and forecasting ESCC.

Results: A total of 35 studies investigating plasma or serum microRNAs were included in the meta-analysis. Based on the consequences of the quality assessment of each study, the articles involved were appropriate for quantitative synthesis. For diagnostic meta-analysis. The overall pooled sensitivity, specificity, and area under the curve of circulating microRNA is 0.794 (95% CI: 0.765 - 0.820), 0.779 (95%CI: 0.746 - 0.808), 0.86 (95%CI: 0.82 - 0.88). The diagnostic value of each microRNA was calculated respectively. For prognostic meta-analysis, the overall pooled hazard ratios of higher microRNA expression in circulation was 1.34 (95% CI: 1.14-1.58), which could significantly predict poorer survival in ESCC.

Conclusions: Circulating microRNAs distinguish patients with ESCC from healthy controls with high sensitivity and specificity, compared to other invasive currently used screening methods. Simultaneously, there was prognostic value for the prognosis of ESCC.

Key words: Esophageal neoplasms; microRNA; diagnosis; prognosis; systematic review; meta-analysis

Introduction

Cancer is currently the secondary lethal cause in the world, only inferior to cardiovascular disease [1,2]. Esophageal squamous cell carcinoma (ESCC), with the tenth highest cancer morbidity and the sixth highest mortality rate, gradually gained worldwide attention [3]. Although with effective and positive treatment, there are still serious challenges waiting to be resolved about the diagnosis and prognosis of esophageal cancer [4]. Nowadays, endoscopy examination and pathological biopsy are still the golden standard methods for detecting ESCC, while imaging examination lacks a certain timeliness due to its insensitivity to small lesions [5,6]. Nevertheless, patients tend to be reluctant to carry out endoscopic examination because of its intrusiveness and discomfortableness. Conventional biomarkers, such as carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9) and squamous cell carcinoma antigen (SCC), are short of sensitivity and specificity to accelerate the early detection of cancer [7-9].

There aren't highly effective prognostic molecular markers that can forecast the clinical outcome and hereafter furnish guidance for treatment. An aberrant exaltation of the serum SCC antigen level is an effective predictor of advanced esophageal cancer correlative to poor survival after esophagostomy. Serum CEA levels are assumed to be significant in predicting clinically unapparent distant metastasis [7]. Hence, there is an enormous requirement to probe fresh and efficient means for ESCC prognosis.

MicroRNAs, non-protein-coding RNA molecules, play an important role in cell differentiation, cell-cycle progression, apoptosis, and tumorigenesis [10,11]. Substantial researches have been performed on the appliance of microRNA expression to distinguish between ESCC patients and healthy controls, suggesting the great capacity of microRNA as a novel biomarker in screening ESCC. In the meantime, based on considerable evidences, microRNAs are deemed to be an effective predictor of the clinical outcome owing to its expression level is significantly related to the prognosis of ESCC patients. Therefore, it is essential to summarize the diagnostic efficiencies of these microRNAs via a systematic review.

However, one of the meta-analyses investigated the value of diagnosis and prognosis of single microRNA [12]. Moreover, several researchers combined various microRNAs to get conclusions about the value of all microRNAs in ESCC, but overlooked the heterogeneity in diverse microRNAs from inconsistent sample sources [13-16]. Taking into consideration the drawbacks of previous publications, a more integrative meta-analysis of microRNA for ESCC, on the basis of all relevant prior studies, was conducted to acquire a better understanding of the diagnostic and prognostic efficiency of microRNA in ESCC.

Methods

Search strategy

An electronic search of PubMed, Embase and the Chinese Biomedical Literature Database (CBM) was performed for relevant articles published until Nov 12, 2017. The search strategy was (miRNA OR microRNA OR miR) AND ("esophageal neoplasms"[Mesh] OR "esophageal squamous cell carcinoma" OR "esophageal carcinoma" OR "esophageal adenocarcinoma") AND (blood OR serum OR plasma OR circulating) AND (diagnosis OR diagnostic OR diagnose OR prognosis OR prognoses OR prognose OR predict OR prognostic). No language restrictions were set. Duplicates were removed. By screening the title and abstract, eligible manuscripts were obtained for full-text review. The flow-process diagram for the literature is presented in Fig. 1.

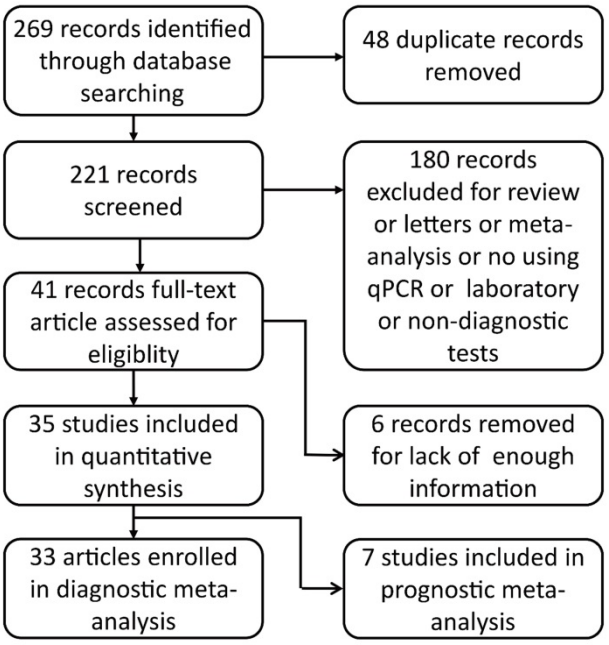

Fig. 1. Flow-process diagram.

Inclusion criteria and exclusion criteria

For eligible studies to be enrolled, the following criteria had to be fulfilled [17]: (1) studies were conducted comparing ESCC patients versus healthy controls; (2) samples were restricted to serum or plasma; and (3) methods had to include quantitative real-time PCR techniques. Articles were excluded based on the following criteria: (1) review articles, letters or meta-analysis, (2) studies with duplicate data reported in other studies, (3) laboratory studies.

Quality assessment

Quality of all studies included in meta-analysis are systematically assessed based on the criteria as proposed by the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) [18]. For prognostic meta-analysis, information and data were analyzed as previously described.

Data extraction

Each study for diagnosis and prognosis was retrieved and assessed independently by two investigators (CY and HNL). Any discrepancies were resolved by consensus. The extracted data and information included as following: the first author, year of publication, country of origin, ethnicity, characteristics of cases, characteristics of controls, type of blood-based fluid (serum or plasma), reference microRNA and clinical outcomes.

Statistical methods

Diagnostic meta-analysis was performed to determined sensitivity, specificity and area under the curve (AUC) of the summary receiver operating curve (SROC) of all microRNAs identified by previous diagnostic tests [19]. For prognostic meta-analysis, hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. Forest plots were used to estimate the effect of microRNA expression on overall survival (OS) and progression free survival (PFS). The percentage of Higgins I-squared statistic (I^2) was used to quantify the heterogeneity. A random effect model would be used if $I^2 > 50\%$, otherwise the fixed effect model was applied. Publication bias of diagnostic tests was considered by Deeks' funnel plot asymmetry test [20], while that of prognostic tests was estimated by Egger's test [21] and Begg's test [22], respectively. Level of significance was set at $p < 0.05$. All data analyses were computed with the Stata 12.0 (StataCorp LP, College Station, TX, USA) and Revman 5.2 (The Nordic Cochrane Centre, Copenhagen, Denmark).

Results

Literature search

269 records from the database search were initially identified. We removed 48 duplicates, 180 irrelevant studies and six articles that failed to provide necessary statistical data. 35 published studies were finally included in this systematic review and meta-analysis [12,23-56]. In these studies, 33 articles provided diagnostic information and seven were included in prognostic meta-analysis. 3156 ESCC patients and 2304 healthy controls were included in diagnostic meta-analysis, whereas there were 984 patients in prognostic meta-analysis. The main characteristics of the included articles were listed in Table 1.

Table 1. Characteristics of the included studies.

Article ID	First Author	Published Year	Country	Ethnicity	ESCC group			Control group			Specimen	MicroRNA	Reference RNA
					Sample size	Mean age	Gender	Sample size	Mean age	Gender			
1	Zhang C	2010	China	Asian	149	61.3	116/33	100	60.0	74/26	Serum	10a, 22, 100, 127-3p, 133a, 148b, 223	U6
2	Xu H	2015	China	Asian	50	60.0	27/23	50	60.0	30/20	Serum	10b, 29c, 205	U6
3	Xie ZJ	2013	China	Asian	29	61.3	25/4	16	57.5	13/3	Serum	10b	miR-16
4	Li JL	2017	China	Asian	106	62.3	70/36	106	61.8	68/38	Serum	15a	U6
5	Li BX	2015	China	Asian	38	-	-	19	-	-	Plasma	16, 21, 185, 375	miR-1228
6	Zhou X	2017	China	Asian	137	-	-	155	-	-	Plasma	18a, 20b, 106a, 223-3p, 486-5p, 584	miR-1228
7	Hirajima S	2013	Japan	Asian	106	-	87/19	54	-	-	Serum	18a	U6
8	Bai YY	2017	China	Asian	89	58.0	69/20	125	-	-	Plasma	19a	miR-39
9	He FC	2015	China	Asian	70	60.5	46/24	40	61.7	26/14	Plasma	20a	let-7a
10	Lv HB	2016	China	Asian	126	59.3	60/66	80	58.6	44/36	Serum	21,375	U6
11	Ye MH	2014	China	Asian	100	-	-	50	-	-	Serum	21	miR-16
12	Komatsu S	2011	Japan	Asian	50	-	-	20	-	-	Serum	21,375	U6
13	Li W	2015	China	Asian	112	54.2	65/47	100	54.2	52/48	Serum	21	miR-16
14	Guo WT	2016	China	Asian	60	52.6	37/23	60	53.1	36/24	Serum	21	U6
15	Dong W	2015	China	Asian	105	-	69/36	30	-	-	Serum	24	cel-miR-39
16	Komatsu S	2014	Japan	Asian	20	-	-	50	-	-	Plasma	25	U6
17	Wu CY	2014	China	Asian	28	-	-	28	-	-	Serum	25, 100, 193a-3p, 194, 223, 337-5p, 483-5p	U6
18	Wu CH	2014	China	Asian	194	61.4	79/115	98	-	-	Serum	25,223,375	U6, miR-16
19	Zhang TF	2011	China	Asian	120	56.3	79/41	121	58.3	76/45	Serum	31	miR-16
20	She XY	2016	China	Asian	40	56.8	24/16	50	55.0	28/22	Serum	100	U6
21	Zheng SY	2017	China	Asian	128	60.0	76/52	40	-	-	Serum	138	U6
22	Wang C	2015	China	Asian	154	-	-	154	-	-	Serum	146a	miR-16
23	Jing RR	2015	China	Asian	28	-	-	35	-	-	Plasma	185	U6
24	Tanaka K	2013	Japan	Asian	144	-	-	-	-	-	Serum	200c	cel-miR-39
25	Dong SL	2016	China	Asian	120	-	79/41	51	-	-	Plasma	216a, 216b	miR-16
26	Jiang ZJ	2015	China	Asian	106	62.0	69/37	60	-	-	Serum	218	miR-16
27	Hui BN	2015	China	Asian	78	58.6	57/21	23	-	-	Serum	365	miR-1228
28	Sun JT	2016	China	Asian	35	61.5	29/6	-	-	-	Serum	367	U6
29	Yang Y	2017	China	Asian	50	68.0	30/20	20	66.0	-	Serum	451	miR-2911
30	Li SP	2016	China	Asian	110	59.2	55/45	40	-	-	Plasma	506	U6
31	Guan SH	2015	China	Asian	75	65.0	43/32	75	-	-	Serum	613	U6
32	Sun L	2016	China	Asian	120	63.0	79/41	51	-	-	Plasma	718	miR-16
33	Takeshita N	2013	Japan	Asian	101	65.0	89/12	46	-	-	Serum	1246	miR-16
34	Wang C	2013	China	Asian	156	-	81/75	156	-	-	Serum	1297	U6, miR-16
35	Zhang TF	2012	China	Asian	201	58.2	128/73	201	55.7	118/83	Serum	1322	U6

"-" represents unclear.

Abbreviations: ESCC, esophageal squamous cell carcinoma.

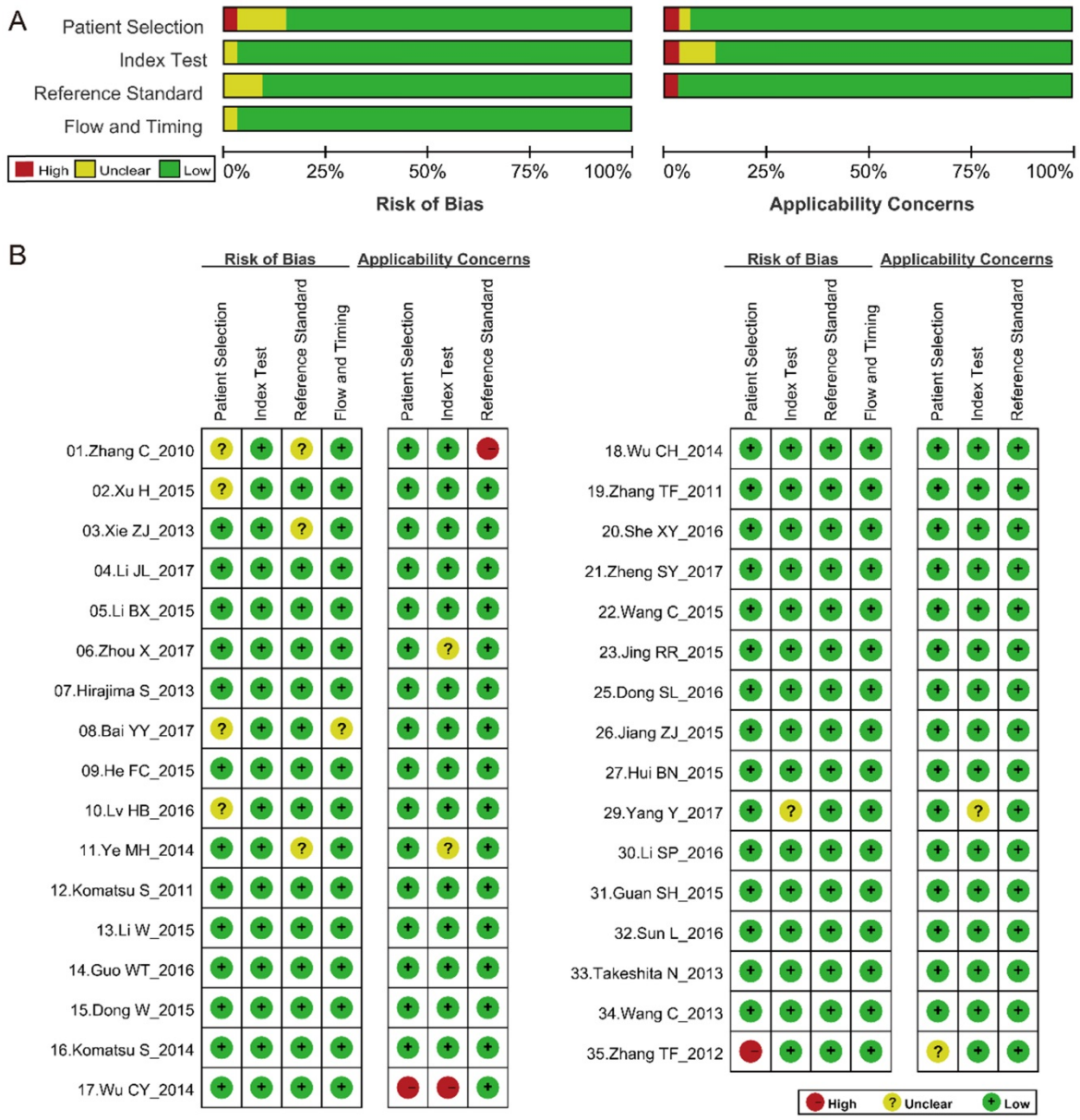

Fig. 2. QUADAS-2 quality assessment. Investigators' assessment regarding each domain for included studies. Risk of bias and applicability concerns' (A) graph and (B) summary. The article ID is same as that in Table 1.

Quality assessment

We assessed the quality of the 33 studies according to the QUADAS-2 assessment tool (Fig. 2). Of the articles included, three were considered to be deficient in patient selection and reference standards [48,55,56], while other articles were considered to have higher quality.

Diagnostic accuracy of circulating microRNAs

Studies that reported dysregulated microRNAs in ESCC cases compared with healthy controls, together with the corresponding AUC and sensitivity and specificity for the very microRNA, were enrolled in the meta-analysis. With these criteria, 43 individual microRNAs were included in 33 studies. The diagnostic accuracies for each microRNA are presented in Table 2.

The overall pooled sensitivity, specificity and AUC of all the 43 microRNAs in the diagnosis of ESCC were 0.794 (95%CI: 0.765 - 0.820), 0.779 (95%CI: 0.746 - 0.808) and 0.86 (95%CI: 0.82 - 0.88), respectively. These outcomes indicate excellent distinguishing ability for microRNAs as biomarkers to detect ESCC. The diagnostic odds ratios (DOR) value was 13.518 (95%CI: 10.772 - 16.964). Likelihood ratios are considered with higher clinically value than sensitivity and specificity. The overall positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were 3.584 (95%CI: 3.122 - 4.113) and 0.265 (95%CI: 0.232 - 0.303).

Table 2. Diagnostic accuracies of the microRNAs mentioned in the literature.

MicroRNA	Expression	ESCC sample size	Control sample size	Sensitivity (%)	Specificity (%)	AUC	Number of included articles
miR-10a	U	149	100	81.2	80.0	0.886	1
miR-10b	U & D	79	66	79.1	91.8	0.860	2
miR-15a	D	106	106	86.4	100.0	0.951	1
miR-16	U	38	19	94.5	55.8	0.643	1
miR-18a	U	243	209	87.2	97.4	0.870	2
miR-19a	U	89	125	66.3	66.4	0.712	1
miR-20a	U	70	40	64.3	75.0	0.767	1
miR-20b	U	137	155	56.6	67.5	0.627	1
miR-21	U	586	379	88.5	72.0	0.840	7
miR-22	U	149	100	88.6	86.0	0.949	1
miR-24	U	105	30	81.9	83.3	0.866	1
miR-25	U	262	176	72.4	77.0	0.800	3
miR-29c	D	50	50	68.0	68.0	0.720	1
miR-31	U	201	202	86.6	82.2	0.910	2
miR-100	U	217	178	65.5	82.5	0.700	3
miR-106a	U	137	155	77.0	47.4	0.639	1
miR-127-3p	U	149	100	78.5	87.0	0.899	1
miR-129	U	78	23	78.8	73.3	0.792	1
miR-133a	U	149	100	65.1	83.0	0.830	1
miR-138	D	128	40	87.5	69.5	0.871	1
miR-146a	D	154	154	83.9	76.7	0.870	2
miR-148b	U	149	100	66.4	87.0	0.855	1
miR-185	U & D	66	54	96.9	57.5	0.590	2
miR-193a-3p	U	28	28	75.1	87.1	0.851	1
miR-194	U	28	28	65.3	90.1	0.809	1
miR-205	D	50	50	70.0	64.0	0.720	1
miR-216a	D	120	51	80.0	90.2	0.877	1
miR-216b	D	120	51	55.8	90.2	0.756	1
miR-218	D	106	60	71.7	76.7	0.833	1
miR-223	U & D	508	381	73.1	77.1	0.810	4
miR-337-5p	U	28	28	79.1	84.0	0.848	1
miR-365	U	78	23	80.6	86.7	0.831	1
miR-375	U & D	408	2017	85.1	65.3	0.840	4
miR-451	U	128	43	84.4	81.4	0.900	2
miR-483-5p	U	28	28	70.8	74.5	0.739	1
miR-486-5p	U	137	155	55.3	75.8	0.688	1
miR-506	U	110	40	81.2	87.3	0.835	1
miR-584	U	137	155	65.4	63.0	0.659	1
miR-613	D	75	75	81.3	62.7	0.728	1
miR-718	D	120	51	69.2	66.7	0.715	1
miR-1246	U	101	46	71.3	73.9	0.754	1
miR-1297	D	156	156	82.7	84.0	0.900	2
miR-1322	U	201	201	82.1	80.6	0.880	2

Abbreviations: ESCC, esophageal squamous cell carcinoma; AUC, area under the curve; U, up-regulated expression; D, down-regulated expression.

Prognostic accuracy of circulating microRNAs

Nine microRNAs were enrolled in OS and three were in PFS. The prognostic accuracy of each microRNA was shown in Fig. 3. The pooled HR of OS was 1.56 (95% CI: 0.96 – 2.51, P = 0.070) with 87.5% of the I^2, whereas that of PFS was 1.82 (95% CI: 0.56 – 5.96, P = 0.321) with 93.0% of the I^2. Too high heterogeneity indicated synthesis of the data was inappropriate. Two studies reported the OS of miR-375, and the pooled HR was 0.62 (95% CI: 0.44 – 0.87, P = 0.005) with 0.0% of the I^2.

Publication bias

Publication bias for the diagnostic and prognostic meta-analysis was displayed in Fig. 4. The P value of the Deeks', Egger's and Begger's test were 0.120, 0.210 and 0.276, respectively, demonstrating that the meta-analysis was without publication bias.

Discussion

Circulation biomarkers are often used to diagnose early stages of ESCC. Invasive tests, by contrast, are inconvenience to monitor the progress of the ESCC, while are often rejected by patients because of their discomfort. The development of suitable biomarkers is crucial for diagnosing cancer and predicting the outcome of patients. Traditional biomarkers lack the sensitivity and specificity of early minimal lesions [7-9]. Currently, microRNAs are emerging as promising biomarkers to fill the gaps in early diagnosis and prognosis [57].

In circulation, microRNAs are stable by virtue of being bound to Argonaute proteins, and avoid the degradation by RNases [58]. As were reported in previous researches, various microRNAs were found to be abnormally expressed in malignant tumors of esophagus, stomach, large intestine, liver, pancreas,

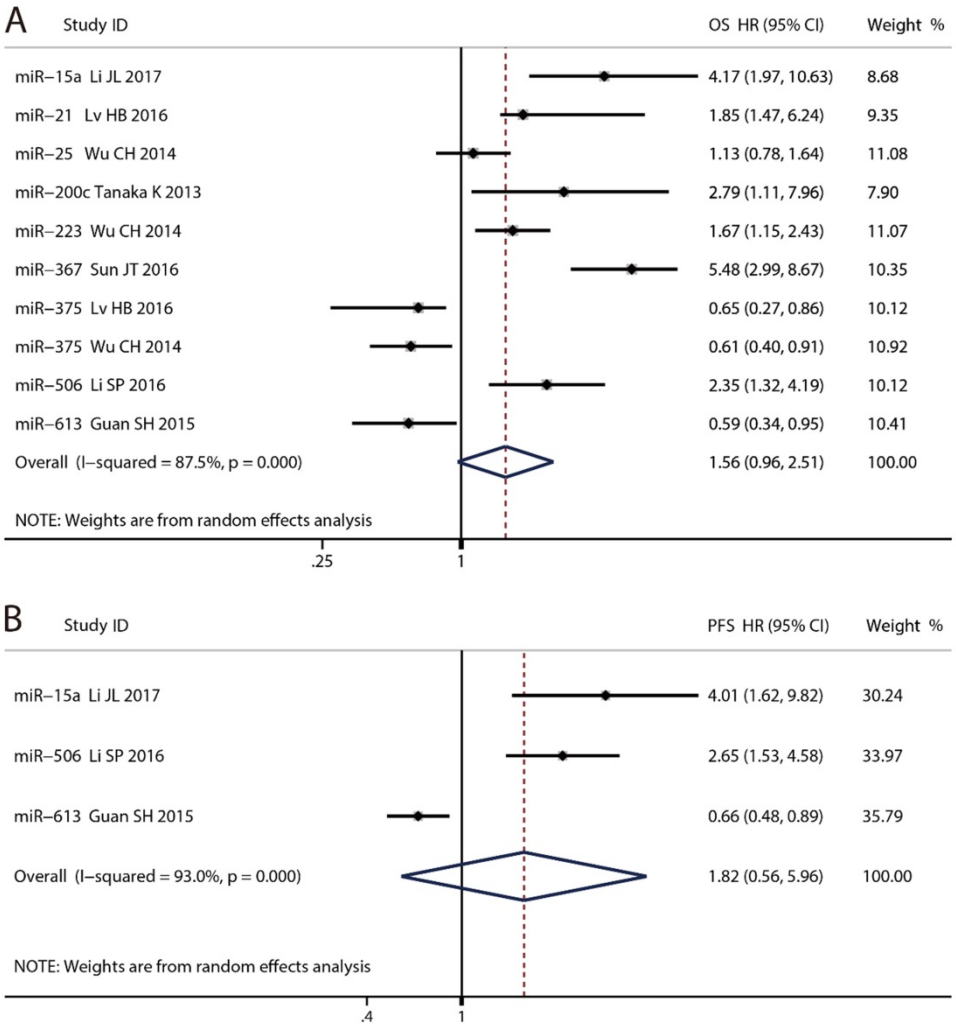

Fig. 3. Forest plots for the prognostic meta-analysis. (A) Hazard ratio (HR) for Overall survival (OS). (B) HR for progression free survival (PFS).

lung and ovary [59,60]. This suggests that these microRNAs can be used as biomarkers for cancers. Thousands of microRNAs have been discovered, some of which have significant diagnostic and prognostic value in ESCC [16]. To comprehensively analyze the value of microRNAs as biomarkers, we performed a meta-analysis of each microRNA, while more literatures than previous meta-analyses were included.

Our meta-analysis reviewed 35 articles and included over five thousand people. There were 28 up-regulated microRNAs and 11 down-regulated microRNAs, while four microRNAs were reported up-regulated and down-regulated in different studies. It is generally believed that the AUC value is greater than 0.9 with high diagnostic value, and the value is between 0.7 and 0.9 with moderate value. If it is less than 0.7, diagnostic value is usually low. In this study, five microRNAs, which were miR-15a, miR-22, miR-31, miR-451 and miR-1297, had high diagnostic value. 32 microRNAs had moderate diagnostic value, and six had low value. Circulation microRNAs achieved a pooled sensitivity of 0.794, specificity of 0.779, AUC of 0.86, and the DOR value was 13.518. The likelihood ratio (PLR and NLR), which are not affected by the prevalence rate, is more clinically meaningful for our diagnostic accuracy. A PLR value of 3.584 and an NLR value of only 0.265 implied that circulation microRNAs were clearly able to discriminate ESCC patients from healthy people.

For prognostic biomarkers, the heterogeneity was too high to merge the HR of all of the microRNAs. It was more appropriate to concentrate the prognostic efficiency of each single microRNA. Higher expression of six microRNAs meant a significantly poorer OS, and that of three microRNAs meant a significantly better OS. PFS was reported in only three microRNAs. The patients with high expression of miR-15a and miR-506 tended to have poorer PFS significantly, while miR-613 was just the opposite.

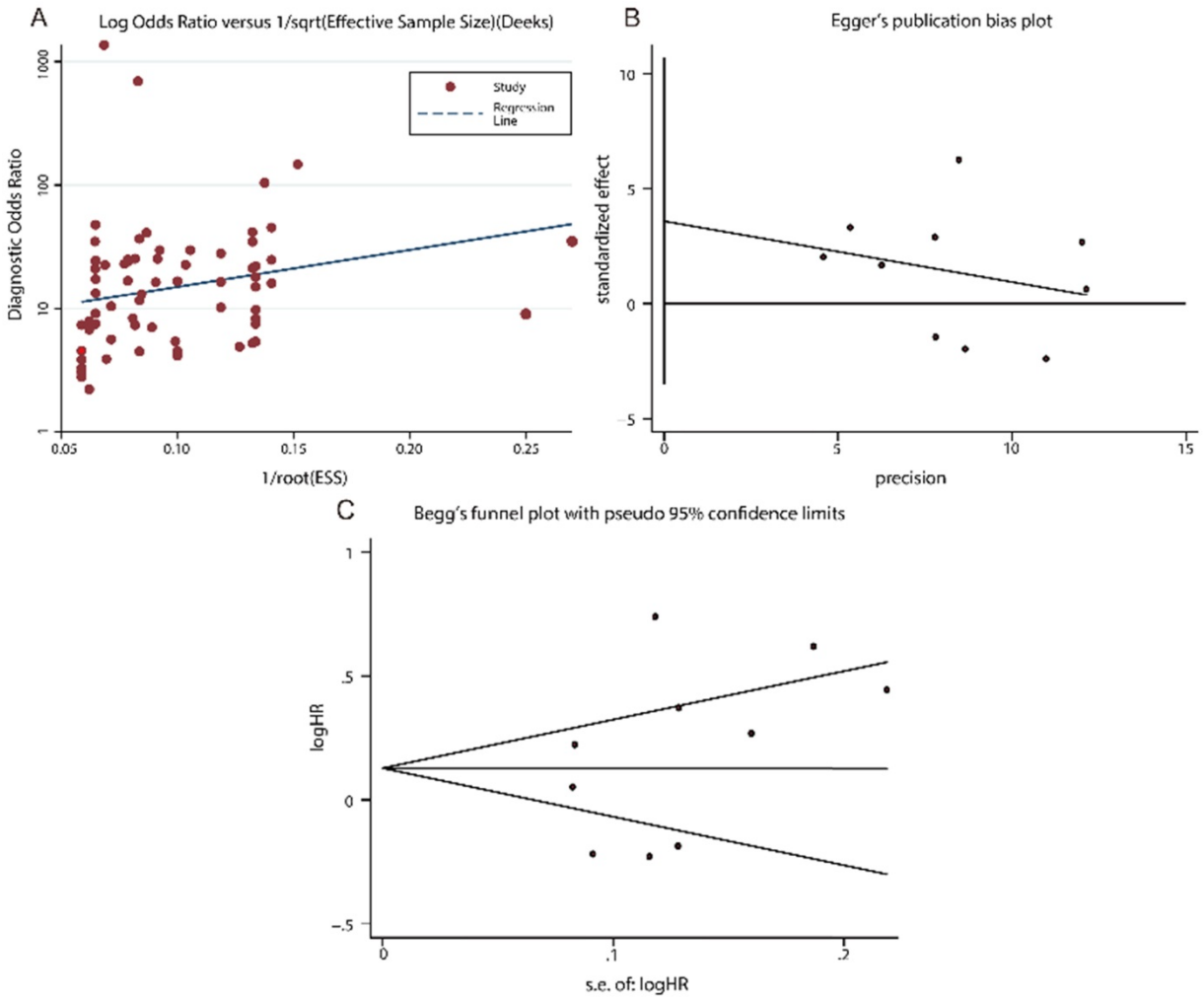

Fig. 4. Funnel plots of publication bias. (A) Deeks' funnel plot for diagnostic tests. (B) Egger's funnel plot for prognostic tests. (C) Begg's funnel plot for prognostic tests.

MicroRNAs have its superiority and weaknesses. Compared to endoscopic biopsies, microRNAs are more readily accepted by patients because of the invasiveness. MicroRNAs are more sensitive and specific than traditional serum markers [7]. In addition, microRNA is still stable after the treatment including boiling, freeze-thaw cycles, acid and alkali treatment [61]. The combination of microRNAs would make further efforts to increase the diagnostic efficiencies [24,28,31,38,48]. Novel and high-performance diagnostic panels of varies combinations of microRNAs should be developed in follow-up studies. However, the cost of microRNA detection is higher than the traditional means, which may affect the wide range of applications of this measure. There were several noteworthy limitations in the present research that should not be overlooked. The study population came from different sources, which suggests that inclusion criteria may be different. Additionally, the ethnicities of the study were restricted to Asians, which may affect the representativeness of the results. The incidence rate in East Asia is the highest, which may be attributed to genetic factors partially [62].

Up-regulation or down-regulation of microRNAs may be related to the different sources of microRNAs in different tumors. As has been reported, blood-based microRNAs are not only synthesized from blood cells but also from the tissues cell, subject to a variety of internal environment and mechanisms such as the appearance and progression of the tumor [61,63], implying that the dysregulated microRNA plasma levels in ESCC patients could be secreted actively or passively by tumor cells. This may explain why different microRNAs are up-regulated or down-regulated. In our study, four microRNAs were reported up-regulated and down-regulated in different studies simultaneously, which could be caused by the choice of reference RNA, the dosage of reagents, the operating process without standardization and the heterogeneity of patients [19].

Now the relationships between circulating microRNAs and ESCC seemingly have been clearly understood. The source of microRNAs is derived

from blood cells or tissue cells with different possible mechanisms, whatever the generating mechanism of the microRNAs might be, their circulating levels can be monitored and used for diagnostic and prognostic indicators. In the future, microRNAs may be used as relatively non-invasive circulating biomarkers of ESCC.

Abbreviations

ESCC: esophageal squamous cell carcinoma; CBM: Chinese Biomedical Literature Database; QUADAS-2: Quality Assessment of Diagnostic Accuracy Studies 2; AUC: area under the curve; SROC: summary receiver operating curve; HR: hazard ratio; CI: confidence interval; OS: overall survival; PFS: progression free survival; DOR: diagnostic odds ratio; PLR: positive likelihood ratio; NLR: negative likelihood ratio.

Acknowledgements

The authors would like to thank the members of Prof. Xi-Zhong Shen's laboratory for helpful discussions and critical reading of the manuscript. This study was supported by the National Natural Science Foundation of China (No. 81000968; No. 81101540; No. 81101637; No. 81172273; No. 81272388; No. 81301820; No. 81472673), the Doctoral Fund of Ministry of Education of China (20120071110058), and the National Clinical Key Special Subject of China.

Competing Interests

The authors have declared that no competing interest exists.

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