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The predictive value of circulating microRNAs for venous thromboembolism diagnosis: A systematic review and diagnostic meta-analysis

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ABSTRACT

Objective: Venous thromboembolism (VTE) is a common cardiovascular disease, in which pulmonary embolism (PE) is potentially life-threatening. Accurate biological markers for the early diagnosis of VTE are needed. The purpose of this study was to analyze and validate the predictive value of microRNAs for the diagnosis of VTE.
Methods: A comprehensive literature review was conducted using the PubMed, Embase, and Cochrane Library databases and is current through Sep 27, 2018. The diagnostic value of microRNAs for VTE was analyzed by creating a summary receiver operating characteristic curve (SROC) and calculating the area under the curve (AUC).
Results: Our analysis included 12 articles assessing a total of 1057 individuals. The most frequently researched microRNA was miR-134, and the pooled results of the predictive ability of this miRNA with 95% confidence intervals (CIs) showed an average sensitivity of 0.82 (0.69–0.91) and an average specificity of 0.83 (0.68–0.92). The average AUC for the SROC curves was 0.89 (0.86–0.92). For other microRNAs, AUC values > 0.8 were considered as potential diagnostic indices. These microRNAs included miR-1233, miR-134, miR-145, miR-483-3p, miR-582, miR-532, and miR-195.
Conclusions: MicroRNAs may act as novel diagnostic biomarkers for VTE, and miR-1233, miR-134, miR-145, miR-483-3p, miR-582, miR-532, and miR-195 are prime candidates. Of these, research on miR-134 is the most extensive and reliable.

Key questions

What is already known about this subject?

Circulating microRNAs are stable in whole blood, serum, and plasma.

MicroRNAs might be potentially ideal biomarkers for venous thromboembolism, but the results are contradictory.

What does this study add?

The diagnostic value of microRNAs for VTE was analyzed.

MiR-1233, miR-134, miR-145, miR-483 - 3p, miR-582, miR-532, and miR-195 demonstrate the potential to be used as VTE biomarkers. Among these, research on miR-134 is the most extensive and reliable.

How might this impact on clinical practice?

Clinicians should consider circulating microRNAs as a biomarker for VTE, especially miR-134. More clinical research should be carried out.

1. Introduction

Venous thromboembolism (VTE) is a common cardiovascular disease wherein a blood clot occurs within a blood vessel [1]. This disease predominantly presents as either deep vein thrombosis (DVT) or pulmonary embolism (PE). PE can be further categorized as either acute pulmonary embolism (APE) or chronic thromboembolic pulmonary hypertension (CTEPH) [2].

DVT occurs in deep veins generally located in the lower extremities, and the thrombus is only loosely connected to the vascular wall and may easily detach. Once the thrombus releases from the venous wall, it will enter the blood stream and cause embolism of lungs. When a

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Table 1
Characters of included studies.

Author	Year	Location	Sample size	Average age#	Type of disease and control	microRNA source	miRNA laboratory methods
Qian Wang [12]	2017	China	148	61 ± 11.9	APE and normal volunteers	Plasma	Quantitative reverse transcription-PCR
Zhiyun Jiang [13]	2018	China	90	52.56 ± 15.42	DVT/PTS and healthy volunteers	Plasma	Quantitative real-time PCR
Yaping Wang [14]	2017	China	80	58.49 ± 11.68	APE and healthy controls	Plasma	Quantitative real-time PCR
Anita Sahu [15]	2017	India	40	31.5 ± NA	VTE and control	Plasma	Quantitative real-time PCR
Zi Li [16]	2017	China	85	53 ± 8.63	DVT and healthy controls	Whole blood cells	Quantitative reverse transcription-PCR
Xin Zhou [17]	2015	China	74	42 ± 11	PE and normal volunteers	Plasma	Quantitative reverse transcription-PCR
Xiao Wang [18]	2016	Sweden	238	59.8 ± 19.1	DVT and individuals without DVT	Plasma	Quantitative reverse transcription-PCR
Lingshang Kong [19]	2016	China	26	55.4 ± 11.6	DVT and healthy controls	EPC	Quantitative real-time PCR
Thorsten Kessler [20]	2016	Germany	72	62 ± 14	APE and healthy controls	Serum	Quantitative real-time PCR
Jizheng Qin [21]	2015	China	38	69.4 ± 8.1	DVT and healthy controls	Serum	Quantitative real-time PCR
Lijuan Guo [22]	2014	China	80	51.9 ± 11.6	CTEPH and healthy controls	Plasma	Quantitative real-time PCR
Juejie Xiao [23]	2011	China	86	54.78 ± 16.20	APE, non-APE (dyspnea, chest pain, or cough), and healthy controls	Plasma	Quantitative reverse transcription-PCR

Abbreviations: VTE: Venous thromboembolism; DVT: deep vein thrombosis; PE: pulmonary embolism; APE: acute pulmonary embolism; CTEPH: chronic thrombo embolic pulmonary hypertension; EPC: endothelial progenitor cells.
#Mean ± Standard deviation.

thrombus attaches to the lungs, it can cause APE, and the repeated abscission of small clot fragments can result in CTEPH [3]. To avoid serious or even fatal outcomes resulting from thromboembolisms such as APE, early diagnosis and preventative treatment should be a priority for high risk VTE patients. Due to the lack of specificity in clinical manifestations of VTE, the discovery of accurate biological markers is critical for early and accurate diagnosis.

Current VTE clinical diagnostic methods include the use of the subjective evaluation scale, such as the Wells score, D-dimer analysis, compression ultrasound, computed tomography pulmonary angiography, and others; however, each method has its limitations. For example, injection of contrast agents during computed tomography (CT) angiography may increase the risk of allergies or renal insufficiency, and although D-dimer analysis shows high sensitivity (96%), it lacks specificity (40%) [4].

MicroRNAs are endogenous non-coding RNAs that are approximately 20–25 nucleotides in length. As a newly discovered molecular marker, microRNAs play a powerful role in the post-transcriptional regulation of gene expression and are widely involved in cell differentiation, proliferation, apoptosis, and other physiological and pathological processes. Circulating microRNAs are stable in whole blood, serum, and plasma. These molecules are either located within microparticles or exocrine bodies, or they bind to protein complexes to avoid degradation [5]. microRNAs might be potentially ideal biomarkers with advantages such as strong stability and low limits of detection.

Previous meta-analyses studied the expression of circulating microRNA and various target genes in DVT patients. A total of 13 microRNAs and 149 target genes were included in this research [6]. Only the VEGF and PI3K-Akt signaling pathways were found to be associated with the thrombosis regulatory network; however, this study was not conducted as a diagnostic meta-analysis. Another study analyzed the diagnostic value of microRNAs in PE and concluded that microRNAs could be used as novel non-invasive diagnostic biomarkers [7]. This study, however, used the inappropriate and risky approach of combining four different microRNAs and was unable recommend specific microRNAs for further studies. Therefore, the potential for microRNA to be used as a suitable biomarker for clinical application requires further analysis. In this study, diagnostic meta-analysis and bioinformatics methods are used to analyze and validate the predictive value of microRNAs in the diagnosis of VTE.

2. Methods

This meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews extension for Diagnostic test Accuracy (PRISMA-DTA) guideline [8]. Ethical approval was not required for this study as the data were based on analysis of existing literature and individual patient data were not used.

2.1. Data sources and retrieval strategy

Literature research and review was independently conducted by two authors using the PubMed, Embase, and Cochrane library databases and is current through Sep 27, 2018. The terms used in the searches included "MicroRNA", "miRNA", "thrombus", "thrombosis", "clotting", "clot", "embolus", "embolism", "thromboembolism", and "thrombophilia". The detailed search strategy is provided in Supplementary Table 1. Authors also conducted manual searches from the reference lists of relevant review articles to identify additional eligible studies.

2.2. Selection criteria

Our meta-analysis included several studies. Specifically, we analyzed research on VTE patients in the context of DVT, APE, and CTEPH, and also study related to the diagnostic utility of circulating microRNAs in the context of VTE. We used data obtained from healthy individuals

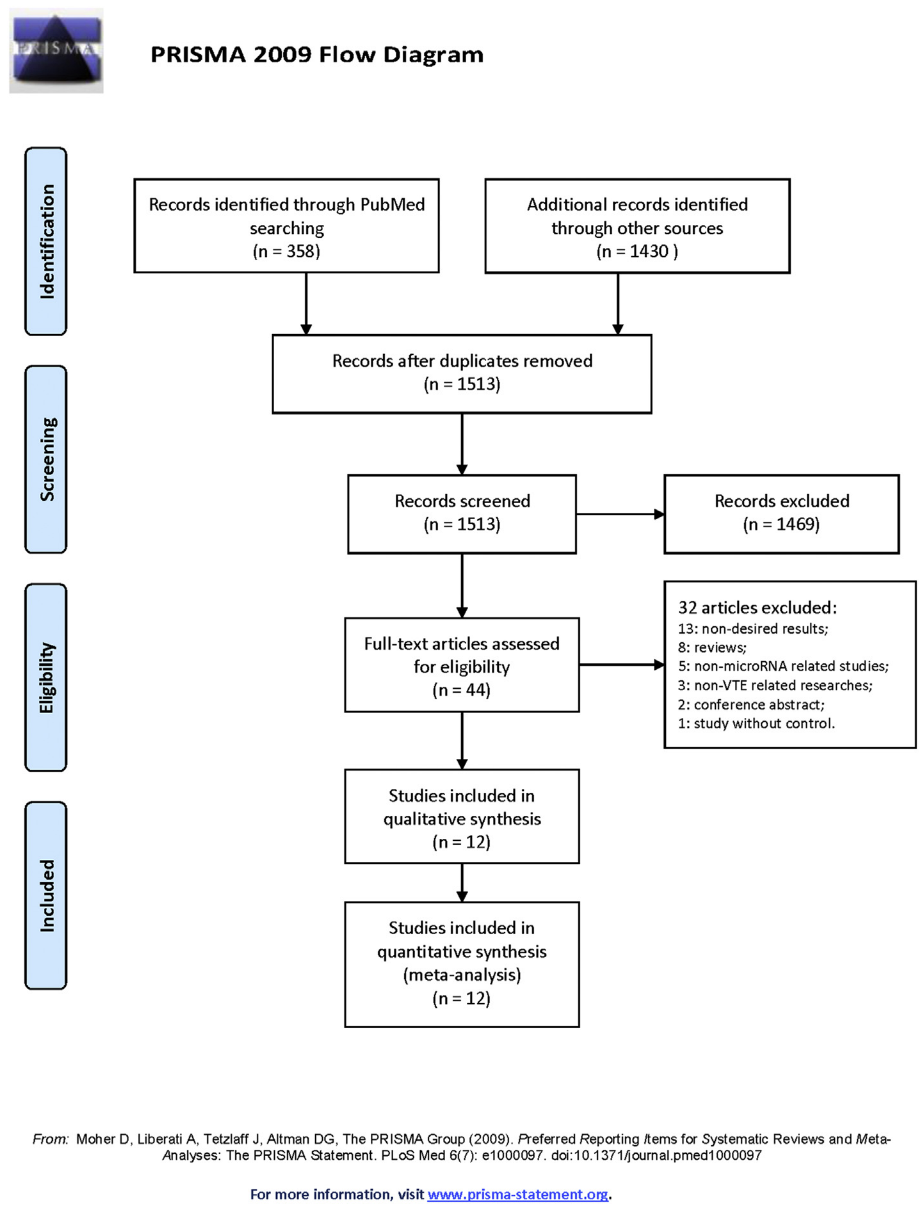

Fig. 1. Flow chart illustrates the study progress.

or individuals not diagnosed with thromboembolism, and the collected data included true-positive (TP), false-positive (FP), true-negative (TN), false-negative (FN) results and/or ROC curve based or deducible diagnosis. The studies excluded from the analysis included studies examining non-VTE patients, studies lacking separate VTE patient reports, non-microRNA related studies, studies where the control group population had been diagnosed with thromboembolism, and non-deducible studies lacking diagnostic results. Conference reports, reviews, commons, letters, and dissertations were also excluded from the analysis.

2.3. Data extraction and quality assessment

Two authors extracted the data independently; and all discrepancies were resolved by discussion. Information extracted from each eligible study included the first authors' name, the publication year, the sample size, the average age, the type of disease and control, the miRNA source, and miRNA laboratory methods. The outcome variables included cutoff value, true-positive (TP), false-positive (FP), false-negative (FN), and true-negative (TN), and these values were extracted or derived for each study. The quality of each included study was assessed through diagnostic accuracy studies-2 (QUADAS-2). These studies consist of four domains including patient selection, index test, reference standard, and flow and timing [9].

2.4. Statistical analysis

The I^2 statistic was used to estimate the degree of heterogeneity among the studies. If I^2 was $\geq 50\%$, the random-effects model was used. If not, then the fixed-effects model was used. We analyzed the diagnostic power of microRNAs in the diagnosis of VTE by pooling sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratios (DOR). Summary receiver operating characteristic (SROC) curves and the area under the curve (AUC) were calculated [10]. AUC values of > 0.8 indicate that a microRNA possesses potential diagnostic value. Sensitivity analyses were conducted to assess the potential source of between-study heterogeneity. The publication bias was also evaluated using Deeks' funnel plot [11]. STATA 14.0 software (midas command) and RevMan 5.3 were used to perform the analysis. A $p < 0.05$ was considered as statistic difference.

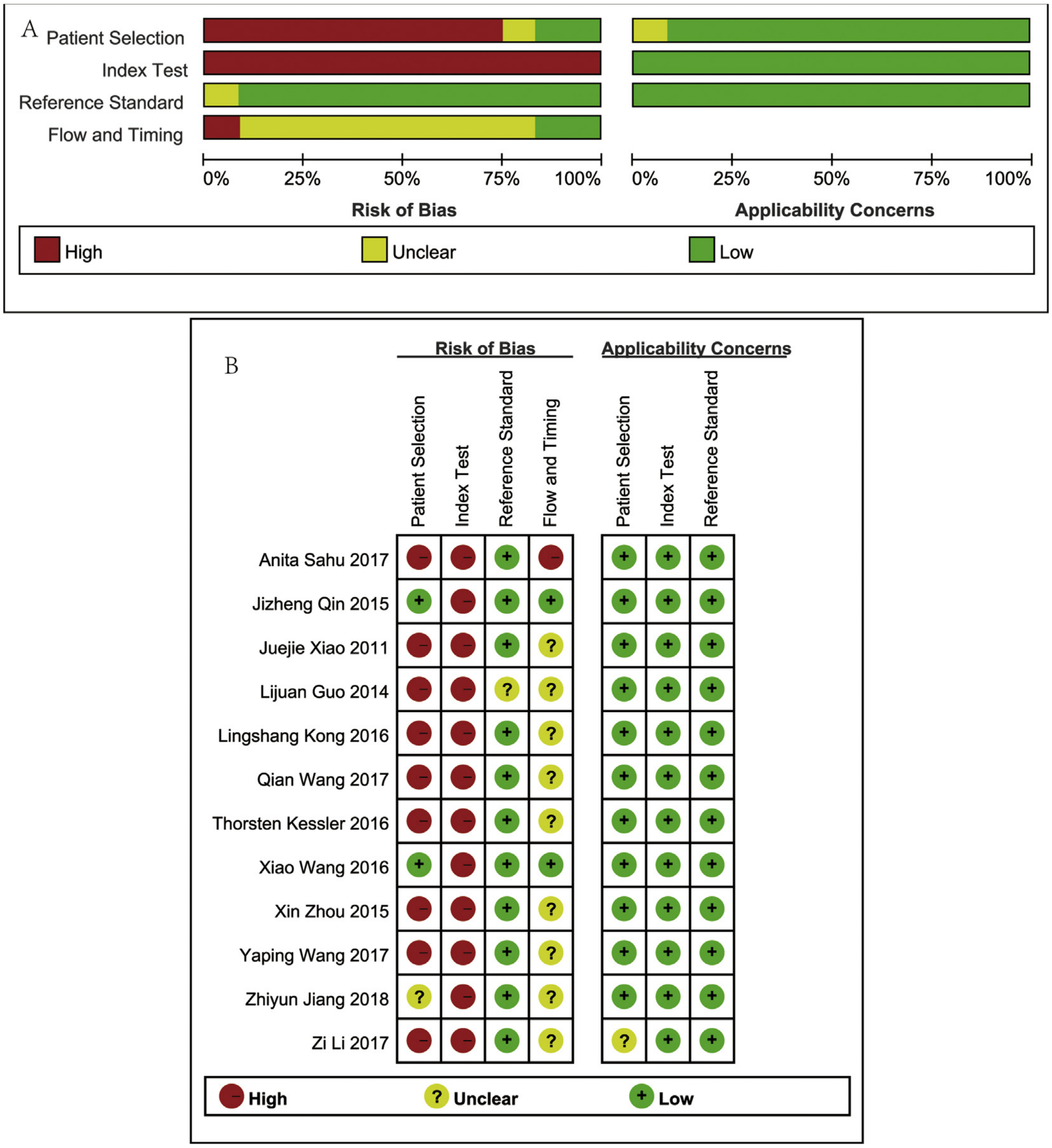

Fig. 2. Risk of bias analyses in included studies. A: Risk of bias and applicability concerns graph: review author judgements concerning each domain presented as percentages across included studies; B: Risk of bias and applicability concerns summary: review author judgements concerning each domain for each included study.

3. Results

Overall 1513 articles were identified through database analyses after the removal of duplicates. A total of 1469 articles were excluded after screening the titles and abstracts. The full text of the remaining 44 articles was assessed. Articles including non-desired results (13), reviews (8), non-microRNA related studies (5), non-VTE related studies (3), conference abstracts (2), and studies lacking controls (1) were removed. Ultimately, 12 articles assessing a total of 1057 patients were included in our analysis (Table 1, Fig. 1) [12–23].

The included studies were published from 2011 to 2018, and the populations of interest were mainly from China. The average age of patients was typically between 30 and 70 years, and one study did not specify patient age. The types of diseases include DVT and PE. MicroRNAs examined in this study include miR-27a, miR-27b, miR-1233, miR-320a, miR-320b, miR-134, miR-145, miR-1, miR-26, miR-28-3p, miR-136-5P, miR-424-5p, miR-483-3p, miR-582, miR-532, miR-195, let-7b, and miR-22. The microRNA sources included plasma, serum, and blood cells.

The quality of the analyzed studies is indicated in Fig. 2. The main factor affecting study quality was that the case-control design was not thorough for almost all studies. Groups were initially divided based on whether or not subjects were diagnosed with VTE, followed by the measurement of microRNA levels in each group. Additionally, although a majority of the control groups included healthy individuals, the studies did not confirm the presence or absence of VTE in control samples. The overall analytical quality is, therefore, acceptable (Fig. 2).

From among the studies reviewed, miR-134 was found to be the most frequently researched microRNA, having been shortlisted in 5 sets of data. The forest plot for the measurement of miR-134 diagnostic sensitivity and specificity for VTE is shown in Fig. 3. The pooled results showed sensitivity of 0.82 with a 95% confidence interval (95%, CI: 0.69–0.91), specificity of 0.83 (95%, CI: 0.68–0.92), PLR of 4.8 (95%, CI: 2.6–8.8), NLR of 0.21 (95%, CI: 0.12–0.38), and DOR of 22 (95%, CI: 10–50). The SROC curve is indicated in Fig. 4, and the AUC is 0.89 (95%, CI: 0.86–0.92).

The heterogeneity (I^2) of sensitivity was 74.40% ($p < 0.01$), and that of specificity was 73.58% ($p < 0.01$). It is generally believed that the threshold effect is mainly responsible for observed heterogeneity of diagnostic meta-results. In this study, the correlation between logits of

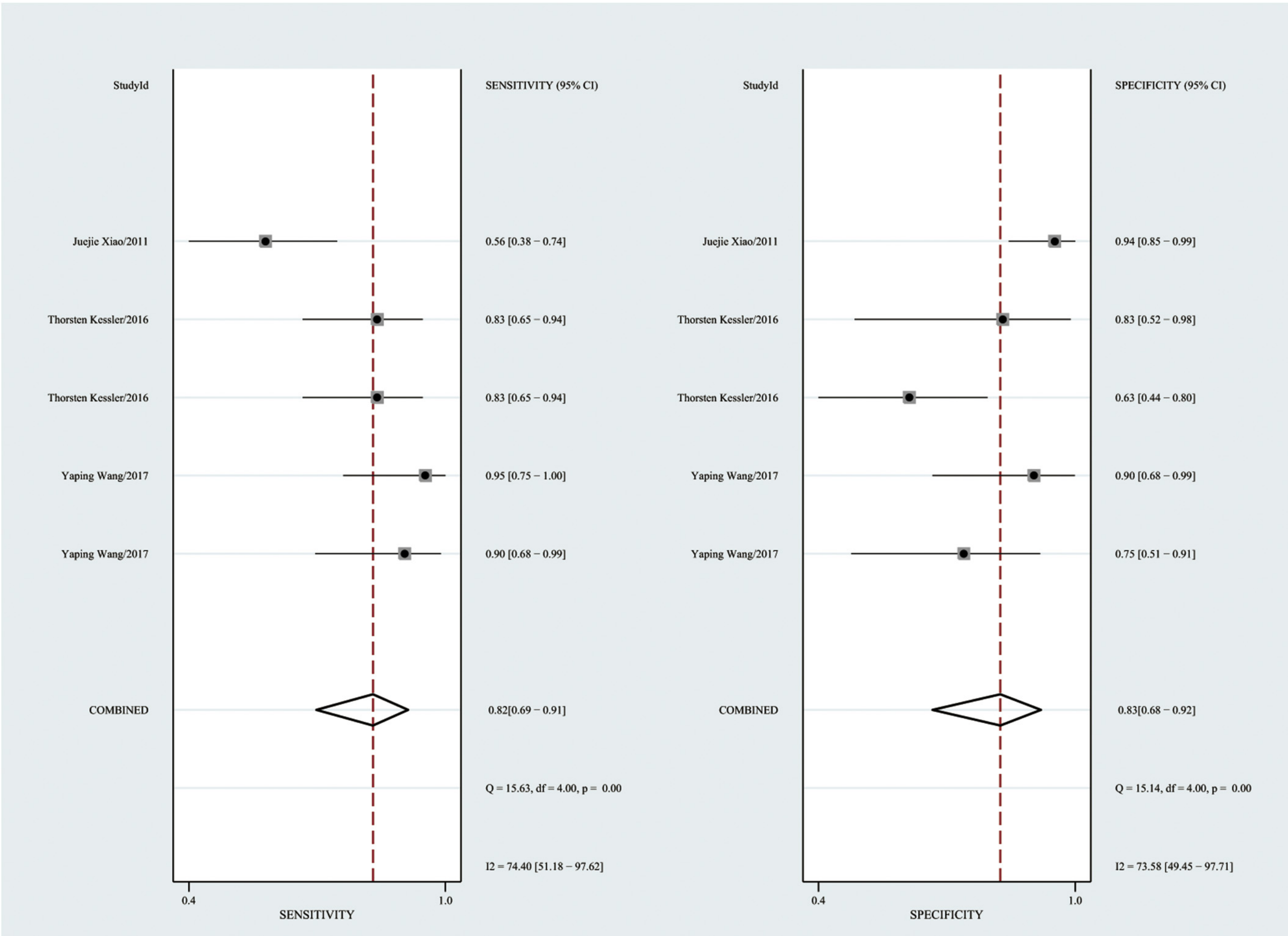

Fig. 3. Forest plots of the pooled sensitivity and specificity for miR-134.

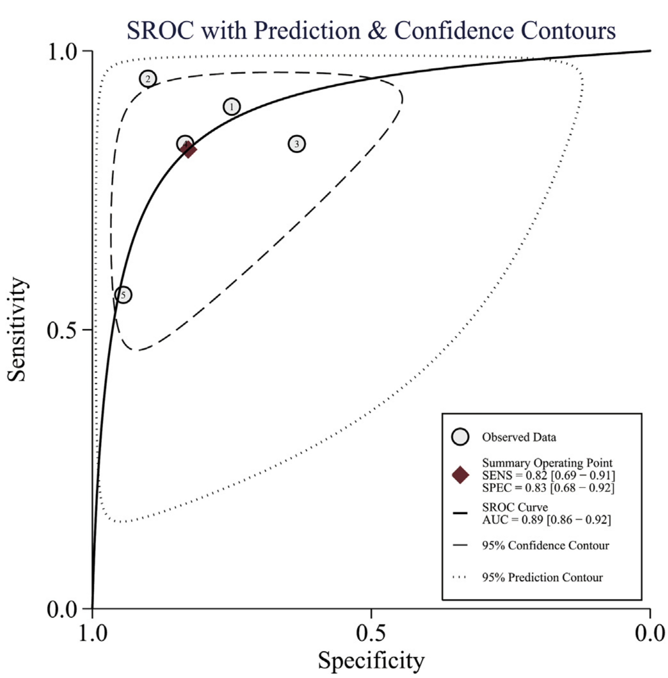

Fig. 4. Summary ROC curve for miR-134.

sensitivity and specificity was −0.91, and 83% of heterogeneity was likely a result of the threshold effect. Results obtained by Juejie Xiao were largely responsible for the heterogeneity of sensitivity results [23], wherein the control group consisted of healthy people and non-APE patients exhibiting suspected symptoms. Results obtained by Thorsten Kessler were largely responsible for the heterogeneity of specificity results [20], wherein comparisons were performed between the APE population and the non-ST-Elevation Myocardial Infarction (NSTEMI) population. This indicates that the heterogeneity of the study may be due to a combination of the threshold effect and the control population set. Deeks' funnel plot asymmetry test was used to determine publication bias, and the results indicated that there was no significant publication bias among the studies included in this analysis ($p = 0.43$, Fig. 5).

The sensitivity, specificity, and AUC of analyzed miRNAs are described in Table 2, and the details of abstracted data are presented in Supplemental Table 2. Scatter plots of these results for each miRNA are illustrated, and these plots are weighted according to the number of included patients (Fig. 6). The sensitivity results indicate that miR-1233, miR-134, miR-145, miR-483-3p, miR-532, miR-195, and let-7b demonstrate relatively high (> 0.8) sensitivity for VTE diagnosis. The specificity results demonstrate that miR-27a, miR-1233, miR-134, miRNA-320b, miR-145, miR-1, miR-26, miR-28-3p miR-483-3p, miR-582, miR-532, miR-195 possess relatively high (> 0.8) specificity for VTE diagnosis. These microRNAs include miR-1233, miR-134, miR-145, miR-483-3p, miR-582, miR-532, and miR-195. Of these, miR-134 was detected in the largest number of individuals included in our

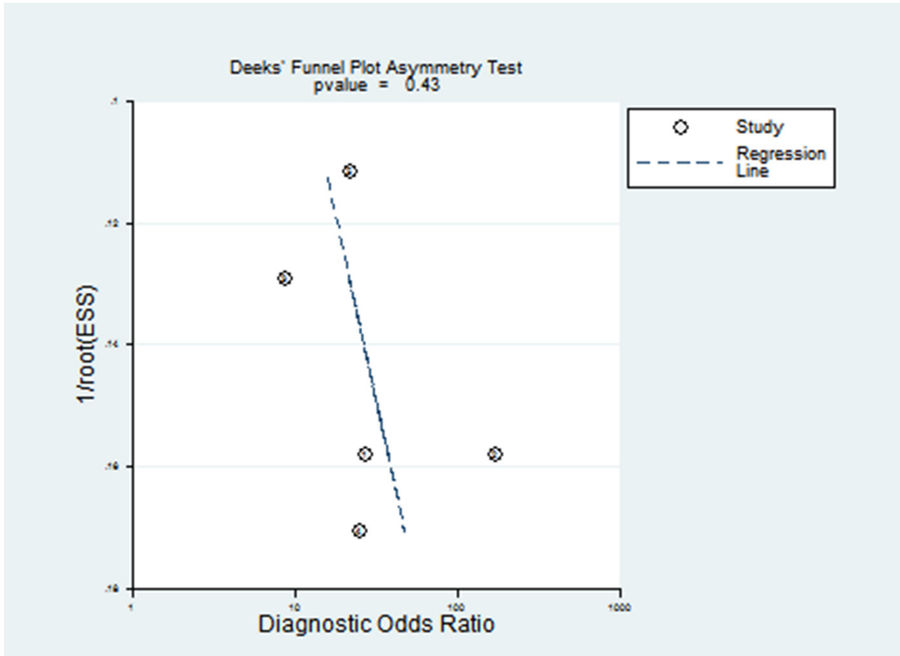

Fig. 5. Funnel plot for miR-134.

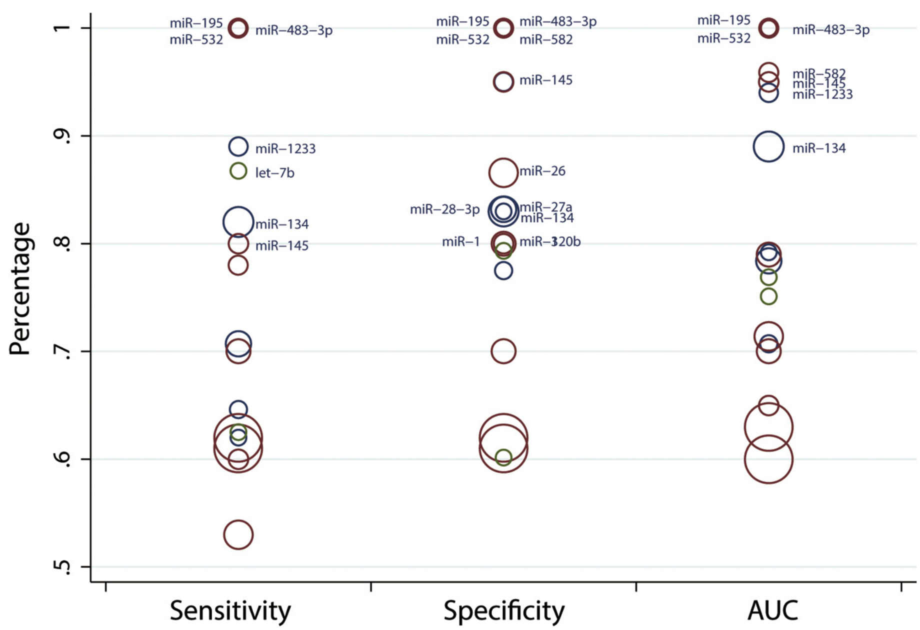

Fig. 6. Scatter plots for each microRNA, weighted according to the number of included patients.

analysis.

4. Discussion

This meta-analysis evaluated the diagnostic value of microRNA in the context of VTE disease, including DVT and PE. In this study, we concluded that miR-1233, miR-134, miR-145, miR-483 - 3p, miR-582, miR-532, and miR-195 demonstrate the potential to be used as VTE biomarkers. Among these, miR-134 research is the most extensive.

Two systematic review studies have previously analyzed the correlation between microRNAs and VTE diseases [6,7]. Unlike the previous two studies, our current study includes specific types of common VTE diseases, and we also use diagnostic meta-analysis to analyze our results. Given this, the conclusions of this study are more robust and meaningful than those of previous studies.

Although indicators such as miR-134 have the potential to diagnose VTE disease, the pooled results still demonstrate no obvious advantages over traditional indicators such as D-dimer analysis, particularly for sensitivity results. Therefore, in the preliminary clinical study multiple indicators were measured simultaneously to improve the sensitivity and specificity of VTE diagnosis. For example, in Qian et al., simultaneous measurements of miR-27a/b and D-dimer were used to improve the sensitivity and specificity of diagnosis [12]. A variety of simultaneous microRNA measurements can also be used to improve diagnostic accuracy [20]; however, currently such research is uncommon and has not been utilized for widely studies miRNA-related combinations.

The main biological function of microRNA is the post transcriptional regulation of gene expression. In this study, we concluded that miR-

Table 2
Sensitivity, specificity, and ROC AUC of all microRNAs analyzed.

microRNAs	Disease	Number of patients	Sensitivity	Specificity	AUC
miRNA 27a	APE	190	0.71	0.83	0.78
miRNA 27b	APE	88	0.65	0.78	0.71
miRNA 1233	APE	102	0.89	0.95	0.94
miRNA-320a	DVT	60	0.70	0.70	0.70
miRNA-320b	DVT	60	0.70	0.80	0.79
miRNA 134	APE	268	0.82	0.83	0.89
miRNA 145	VT	40	0.80	0.95	0.95
miRNA 1	VT	40	0.60	0.80	0.65
miRNA-26	DVT	85	0.53	0.87	0.71
miRNA 28-3p	PE	74	0.62	0.83	0.79
miR 136-5P	DVT	238	0.61	0.61	0.60
miR 424-5p	DVT	238	0.62	0.62	0.63
miRNA-483-3p	DVT	26	1.00	1.00	1.00
miRNA 582	DVT	38	0.78	1.00	0.96
miRNA 532	DVT	38	1.00	1.00	1.00
miRNA 195	DVT	38	1.00	1.00	1.00
let-7b	CTEPH	80	0.87	0.60	0.77
miR 22	CTEPH	80	0.63	0.79	0.75

Abbreviations: VTE: Venous thromboembolism; DVT: deep vein thrombosis; PE: pulmonary embolism; APE: acute pulmonary embolism; CTEPH: chronic thrombo embolic pulmonary hypertension; AUC: Area under curve.

1233, miR-134, miR-145, miR-483 - 3p, miR-582, miR-532, and miR-195 possess potential as diagnostic indicators of VTE. Target genes of the above microRNAs were screened through TargetScanHuman (www.targerscan.org) and selected when the cumulative weighted context ++ score was < -0.4. It was found that only miR-532-5p and miR-483-3p had the same target *NFATC2IP* gene (Supplementary Fig. 1). This gene is expressed in T-helper 2 (Th2) cells and regulates the transcription of cytokine genes, including IL-3, IL-4, IL-5, and IL-13. In PE patients, the expression of cytokines in peripheral blood mononuclear cells was abnormal, and cellular immune function was decreased [24]. These results reflect the correlation between VTE and cellular immune abnormalities.

Additionally, all target genes are annotated through the DAVID website (www.david.ncifcrf.gov). With the exception of the cancer-related pathways, the MAPK signaling pathway and the PI3K-Akt signaling pathway contained a relatively large number of target genes. These two pathways are involved in the inflammatory and oxidative stress response of macrophages [25], and they may also be involved in the apoptosis of endothelial cells [26]. After GO annotation, it was observed that the target genes were located mainly in cytoplasm and are involved in protein binding function, positive regulation of transcription at the RNA polymerase 2 promoter, cell proliferation, and apoptotic process. Other specific GO and KEGG classifications are listed in the Supplementary Table 3.

MicroRNAs are diverse and exert complex regulatory functions. The microRNA types included in this study are still limited, and more studies are required to confirm the potential diagnostic value of microRNAs and to obtain relatively stable thresholds values. The discovery of additional novel microRNAs is also required to promote the application of microRNAs in the context of VTE disease diagnosis. However, we also need to know the limitations in the miRNA laboratory methods, including the different miRNA source (such as plasma, serum or whole blood cells), plasma centrifugal speed and batch effects in detection [27]. If new technological platforms provide direct analysis of miRNAs without miRNA extraction and standardized miRNA measurement, they will significantly improve in clinical application [27].

This meta-analysis still has several limitations. First, this study is based on the study level and not the individual level. This study also mainly analyzed the diagnostic value of miR-134 in VTE, but the results exhibit heterogeneity. This heterogeneity may be due to differences in the control group and the threshold effect. Additionally, due to the lack of research there is no further confirmation of heterogeneity source. In this study, each of the microRNAs possessed a small sample size that could impact the effectiveness and robustness of the results. We could also not perform subgroup analysis of the sample source, patient type, or control group. Moreover, whether the predictive value of microRNAs on VTE could affect by disease status remains controversial due to the data from stratified by individuals' characteristics were not available. Furthermore, mostly included studies were conducted in China, which might induce potential selection bias, and the predictive value of microRNAs on VTE according to race should be evaluated in further study. Finally, as this study was a diagnostic meta-analysis, the case-control studies lacking diagnostic results were excluded from this study even though they showed significant differences in microRNAs between VTE patients and controls. The microRNAs in these studies do still have research value in terms of diagnostic accuracy, and with the new research and evidence, more microRNAs may be studied in the future. Therefore, this study was limited by the current availability of research.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2019.07.024.

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Declaration of Competing Interest

None declared.

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Not applicable.

References

[1] W. Hernandez, E.R. Gamazon, E. Smithberger, et al., Novel genetic predictors of venous thromboembolism risk in African Americans, Blood 127 (2016) 1923–1929.
[2] E.A. Nutescu, C. Crivera, J.R. Schein, et al., Incidence of hospital readmission in patients diagnosed with DVT and PE: clinical burden of recurrent events, Int. J. Clin. Pract. 69 (2015) 321–327.
[3] G. Piazza, S.Z. Goldhaber, Acute pulmonary embolism: part I: epidemiology and diagnosis, Circulation 114 (2006) e28–e32.
[4] C.E. Dempfle, W. Korte, M. Schwab, et al., Sensitivity and specificity of a quantitative point of care D-dimer assay using heparinized whole blood, in patients with clinically suspected deep vein thrombosis, Thromb. Haemost. 96 (2006) 79–83.
[5] Y. Cheng, N. Tan, J. Yang, et al., A translational study of circulating cell-free microRNA-1 in acute myocardial infarction, Clin. Sci. (Lond.) 119 (2010) 87–95.
[6] Z. Jiang, J. Ma, Q. Wang, et al., Circulating microRNA expression and their target genes in deep vein thrombosis: a systematic review and bioinformatics analysis, Medicine (Baltimore) 96 (2017) e9330.
[7] H.Y. Deng, G. Li, J. Luo, et al., MicroRNAs are novel non-invasive diagnostic biomarkers for pulmonary embolism: a meta-analysis, J. Thorac. Dis. 8 (2016) 3580–3587.
[8] M.D.F. McInnes, D. Moher, B.D. Thombs, et al., Preferred reporting items for a systematic review and meta-analysis of diagnostic test accuracy studies: the PRISMA-DTA statement, Jama 319 (2018) 388–396.
[9] P.F. Whiting, A.W. Rutjes, M.E. Westwood, et al., QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies, Ann. Intern. Med. 155 (2011) 529–536.
[10] S.H. Park, H.S. Lee, Assessing predictive validity of pressure ulcer risk scales- a systematic review and meta-analysis, Iran. J. Public Health 45 (2016) 122–133.
[11] Q. Liao, P. Han, Y. Huang, et al., Potential role of circulating microRNA-21 for hepatocellular carcinoma diagnosis: a meta-analysis, PLoS One 10 (2015) e0130677.
[12] Q. Wang, J. Ma, Z. Jiang, et al., Diagnostic value of circulating microRNA-27a/b in patients with acute pulmonary embolism, Int. Angiol. 37 (2018) 19–25.
[13] Z. Jiang, J. Ma, Q. Wang, et al., Combination of circulating miRNA-320a/b and D-

dimer improves diagnostic accuracy in deep vein thrombosis patients, Med. Sci. Monit. 24 (2018) 2031–2037.
[14] J. WYPZZZYQJXD, Plasma miRNA-134 is a diagnostic biomarker for acute pulmonary embolism in middle and high altitude areas, Biomed. Res. (Aligarh) 28 (2017) 5494–5498.
[15] A. Sahu, P.K. Jha, A. Prabhakar, et al., MicroRNA-145 impedes thrombus formation via targeting tissue factor in venous thrombosis, EBioMedicine 26 (2017) 175–186.
[16] Z. Li, J. Ni, Role of microRNA-26a in the diagnosis of lower extremity deep vein thrombosis in patients with bone trauma, Exp. Ther. Med. 14 (2017) 5069–5074.
[17] X. Zhou, W. Wen, X. Shan, et al., MiR-28-3p as a potential plasma marker in diagnosis of pulmonary embolism, Thromb. Res. 138 (2016) 91–95.
[18] X. Wang, K. Sundquist, J.L. Elf, et al., Diagnostic potential of plasma microRNA signatures in patients with deep-vein thrombosis, Thromb. Haemost. 116 (2016) 328–336.
[19] L. Kong, N. Hu, X. Du, et al., Upregulation of miR-483-3p contributes to endothelial progenitor cells dysfunction in deep vein thrombosis patients via SRF, J. Transl. Med. 14 (2016) 23.
[20] T. Kessler, J. Erdmann, B. Vilne, et al., Serum microRNA-1233 is a specific biomarker for diagnosing acute pulmonary embolism, J. Transl. Med. 14 (2016) 120.
[21] J. Qin, H. Liang, D. Shi, et al., A panel of microRNAs as a new biomarkers for the detection of deep vein thrombosis, J. Thromb. Thrombolysis 39 (2015) 215–221.
[22] L. Guo, Y. Yang, J. Liu, et al., Differentially expressed plasma microRNAs and the potential regulatory function of Let-7b in chronic thromboembolic pulmonary hypertension, PLoS One 9 (2014) e101055.
[23] J. Xiao, Z.C. Jing, P.T. Ellinor, et al., MicroRNA-134 as a potential plasma biomarker for the diagnosis of acute pulmonary embolism, J. Transl. Med. 9 (2011) 159.
[24] W. Lv, Q. Duan, L. Wang, et al., Gene expression levels of cytokines in peripheral blood mononuclear cells from patients with pulmonary embolism, Mol. Med. Rep. 7 (2013) 1245–1250.
[25] Y. Zhang, Y. He, Zong Y, et al. 17beta-estradiol attenuates homocysteine-induced oxidative stress and inflammatory response as well as MAPKs cascade via activating PI3-K/Akt signal transduction pathway in raw 264.7 cells, Acta Biochim. Biophys. Sin. (Shanghai) 47 (2015) 65–72.
[26] Lou ZL, X.; Zhang, F.; Dong, Z.; Wang, B.; Zhao, X. Effects of PI3 - K/Akt and MAPKs signal pathway in oxidative stress induced HUVECs apoptosis. Chin J. Crit. Care Med. 2016;36:78–82.
[27] Sunderland N, Skroblin P, Barwari T, et al. MicroRNA biomarkers and platelet reactivity: the clot thickens. Circ. Res. 2017 20;120:418–35.