

Review Article

Serum microRNA-195 as a potential diagnostic biomarker for breast cancer: a systematic review and meta-analysis

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Abstract: Serum microRNA-195 (miR-195) expression has been shown to be significantly up-regulated in breast cancer, which implies that it could be a useful biomarker in the early detection of breast cancer. Hence, we performed this meta-analysis to investigate the diagnostic value of miR-195 for breast cancer. Relevant articles were collected from PubMed, Scopus, Embase, the Cochrane Library, BioMed Central, ISI Web of Knowledge, China National Knowledge Infrastructure, Wan Fang Data and Technology of Chongqing databases, from inception to May 24, 2019, by two independent researchers. The diagnostic capacity of miR-195 for breast cancer was assessed using pooled sensitivity and specificity, diagnostic odds ratio (DOR), area under the summary receiver operating characteristic (AUC) and Fagan's nomogram. Meta-analysis and heterogeneity source investigation were completed using Meta-Disc statistical software and Stata SE (version 14.0). Six studies with a total of 464 patients and 287 healthy controls were included in our meta-analysis. The pooled results for sensitivity, specificity and DOR were 0.79 (95% CI: 0.75-0.83), 0.86 (95% CI: 0.82-0.90) and 32.05 (95% CI: 17.04-60.30), respectively; positive and negative likelihood ratios were 5.18 and 0.20, and AUC was 0.9197 (95% CI: 0.86-0.91). In addition, heterogeneity was clearly apparent but was not caused by the threshold effect. In summary, this meta-analysis revealed that miR-195 may be suitable as a potential biomarker for early diagnosis of breast cancer with high sensitivity and specificity, and further investigations are needed to demonstrate its clinical application value.

Keywords: Breast cancer, miR-195, diagnosis, meta-analysis

Introduction

Breast cancer (BC) is the most common malignant tumor in women all over the world. According to the latest data from the National Central Cancer Registry of China (NCCRC), from 2000 to 2013, the incidence rate of cancer increased at an annual rate of about 3.5%. Specifically, the incidence of breast cancer ranks the first in both developed and developing countries, the mortality rate among women ranks the second in developed countries, and 15th in developing countries [1]. Breast cancer, like all other malignant tumors, early diagnosis and treatment play vital roles in a good prognosis. At present, the main methods of breast cancer diagnosis include imaging examination, pathological examination, and serological marker examination. Mammography is the first

choice of early screening of breast cancer. It can clearly show the various layers of the breast and can be found in various benign and malignant breast tumors and structural disorders of the breast, especially in the early stage characterized by tiny calcifications. Breast cancer has a characteristic diagnostic significance [2]. However, the mammary gland target has weak penetrating power, and its detection rate of dense breast lesions is relatively low, which is easy to cause misdiagnosis or missed diagnosis. The ionizing radiation generated by it is an important cause of breast cancer. Too early or too frequent use of molybdenum targets brings many disadvantages in the implementation of molybdenum targets, which makes its use in the early screening of breast cancer in women and the diagnosis of breast diseases is strongly limited [3]. The pathological examination can

represent the gold standard for tumor diagnosis, which is widely used in clinical practice. For breast cancer, common pathological examination methods include needle aspiration cytology, needle aspiration biopsy, and biopsy [4]. However, they are all invasive tests and are not suitable for use as a screening tool for breast cancer. The method of screening for cancer by serological markers has the advantages of convenience, quickness and less damage, and thus has been favored by people. The currently accepted serological markers for breast cancer diagnosis include carcinoembryonic antigen (CEA), breast cancer-associated antigen CA-153, CA125 secreted by secretory epithelial cells, etc., but when these tumor markers are used in early breast cancer, their sensitivity and specificity of cancer diagnosis are low [5-8].

MicroRNAs (miRNAs) are small, endogenous non-coding RNAs that have gene regulation functions at the post-transcriptional level [9]. Increasing studies have demonstrated that miRNAs plays vital roles in the occurrence and development of tumors via oncogenic or tumor-suppressive properties, and its abnormal expression is closely related to the occurrence of some tumors [10, 11]. In recent years, a number of studies have shown that during the occurrence and development of breast cancer, there are abnormal expressions of miRNA in plasma, and miRNA has the potential to become biomarkers for early diagnosis of breast cancer [12, 13]. miR-195 is a member of the miR-15 family, and the gene is located in the 17p13.1 region and distributed in clusters with miR-497. It has been found that the dysregulation of miR-195 expression in tumor tissues is closely related to various tumors. Recent serum-based miRNA studies have shown that serum miR-195 expression in breast cancer patients is significantly higher than that in healthy controls [14-16]. Moreover, compared with prostate cancer, kidney cancer, and colon cancer, serum miR-195 is a relatively characteristic miRNA of breast cancer [17]. Therefore, miR-195 may become a potential marker for the early diagnosis of breast cancer. Similarly, studies have shown that the relative expression level of up-regulated miR-195 in serum is associated with clinical staging of breast cancer [18]. However, some other studies suggested that upregulation of plasma miR-195 was not significantly correlated with clinicopathological

features such as breast invasive ductal carcinoma's mass size, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (her-2) status and lymph node metastasis status [19]. Beyond the limitations of these individual studies, the relationship between miR-195 and the diagnosis of early breast cancer needs to be further clarified. To address this issue, we designed this systematic evaluation and meta-analysis to confirm whether miR-195 can be used as an early diagnostic marker for breast cancer.

Methods

Search strategy

Articles published up to May 24, 2019, which were associated with the diagnostic application of miR-195, were searched based on PubMed, Scopus, Embase, the Cochrane Library, BioMed Central, ISI Web of Knowledge, China National Knowledge Infrastructure, Wan Fang Data and Technology of Chongqing databases. The search terms used for literature retrieval and abstracts were as follows: ("miR-195" OR "microRNA-195" OR "miRNA-195") AND ("breast" OR "mammary") AND ("cancer" OR "cancers" OR "tumor" OR "neoplasm" OR "carcinoma"). Publication languages were limited to English or Chinese. Two investigators independently carried out the literature search.

Study selection and exclusion criteria

Study eligibility criteria included (1) all the patients with BC must have been confirmed by pathological examination; (2) healthy controls had no history of cancer; (3) the study included clear sensitivity, total number of cases included and described how they were derived; and (4) all blood samples were collected for miR-195 analysis before any treatment. Exclusion criteria: (1) duplicate publications; (2) studies with insufficient data; (3) meeting, review and meta-analysis articles; (4) animal and cell studies; (5) studies with fewer than 20 patients.

Data extraction and quality assessment

Two independents extracted the following information from full texts and supplemental materials: first author, year of publication, ethnicity and number of patients and controls, detection method, true positives (TP), false positives (FP), false negatives (FN) and true negatives (TN).

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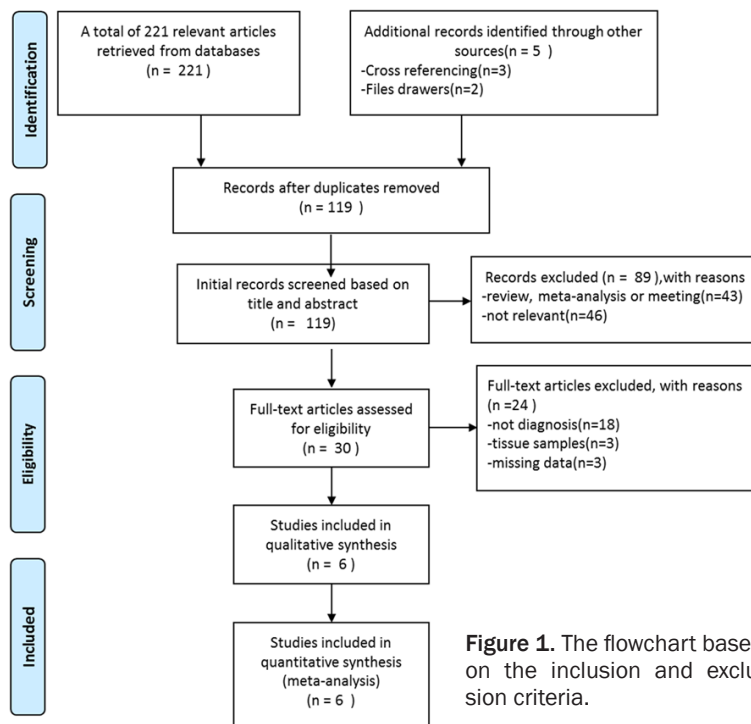


Figure 1. The flowchart based on the inclusion and exclusion criteria.

We contacted corresponding authors to obtain any missing information, and study whose author did not respond was excluded. The risk of bias was assessed independently by two reviewers using the Review Manager 5.3 software according to the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) [20]. All qualified publications are independently rated by two review authors for literature quality. Disagreements were resolved by a consensus process and in consultation with a third reviewer.

Statistical analysis

Revman was used to evaluate the quality of literature, meta-disc 1.4 [21] was used for meta-analysis and heterogeneity analysis of the obtained data. Heterogeneity test methods include Q qualitative test and I^2 quantitative test. $P > 0.05$ or $I^2 \leq 50\%$ indicates that there is little heterogeneity between the included studies, and the fixed effect model can be adopted. While $P \leq 0.05$ or $I^2 > 50\%$ indicates significant inter-study heterogeneity, the random effect model was used. The Spearman correlation coefficient and SROC curve between the sensitivity and specificity of all the included studies were calculated to determine whether the th-

reshold effect existed between the included studies. If Spearman correlation analysis showed that $P > 0.05$, it indicates that there is no threshold effect contribute to different qualitative. Thus, we performed meta-regression to explore sources of heterogeneity. After that, we conducted sensitivity analysis to explore sources of heterogeneity, through the exclusion of specific studies one by one and comparing the results. In summary, the following values were calculated: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic ratio (DOR), ROC curve and AUC. According to AUC, PLR, DOR, etc., the value of miR-195 in the diagnosis of breast cancer was

determined. Using Stata 14.0, Deek's funnel graph, Begg's test, and Egger's test were conducted to correct possible publication bias. Finally, Fagan's nomogram was used to describe the diagnosis value of miR-195 for BC.

Results

Selection results of studies and quality assessment

The initial search from the selected literature databases and other sources returned a total of 226 articles. 196 articles are excluded, of which 107 were duplicated, 43 were reviews, meta-analyses or meeting reports, and 46 were irrelevant. The remained 30 articles were assessed for eligibility as full text. We excluded 24 articles that failed to satisfy our inclusion criteria, of which 18 failed to meet our diagnostic criteria, 3 were based on tissue samples and 3 did not include complete data. Finally, 6 high-quality articles were used in this meta-analysis [14-16, 18, 19, 22]. Our flow diagram of each stage for inclusion and exclusion is presented in **Figure 1**.

The six studies used in our study included a total of 464 BC patients and 287 healthy controls, and all diagnoses were confirmed inde-

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Table 1. Summary of studies included in this meta-analysis

Author	Year	Racial	Patients (controls)	Detection method	TP	FP	FN	TN
Tingting Fan et al.	2018	China	49 (19)	BRCA	49	4	0	15
Yan Zhu et al.	2013	China	20 (10)	RT-PCR	17	3	3	7
Fulong Zhao et al.	2014	China	210 (102)	RT-PCR	145	11	65	91
Helen.M.H et al.	2010	Ireland	83 (63)	RQ-PCR	73	6	10	57
Jiangang Xu et al.	2014	China	48 (35)	RT-PCR	45	11	3	24
Wenzhao Zhang et al.	2016	China	54 (58)	RT-PCR	38	4	16	54

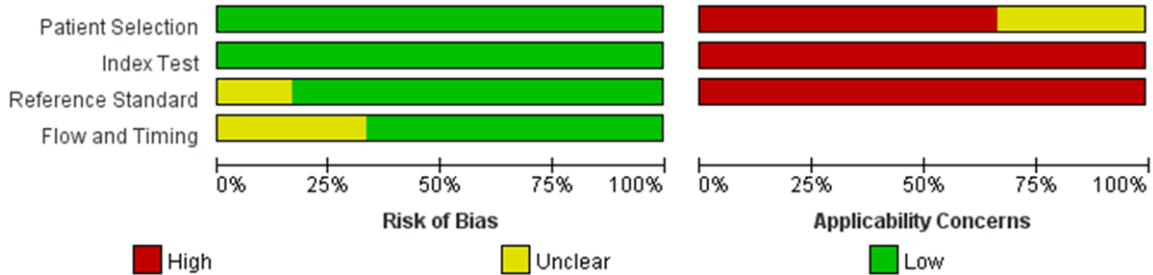


Figure 2. Risk assessment of bias and clinical applicability.

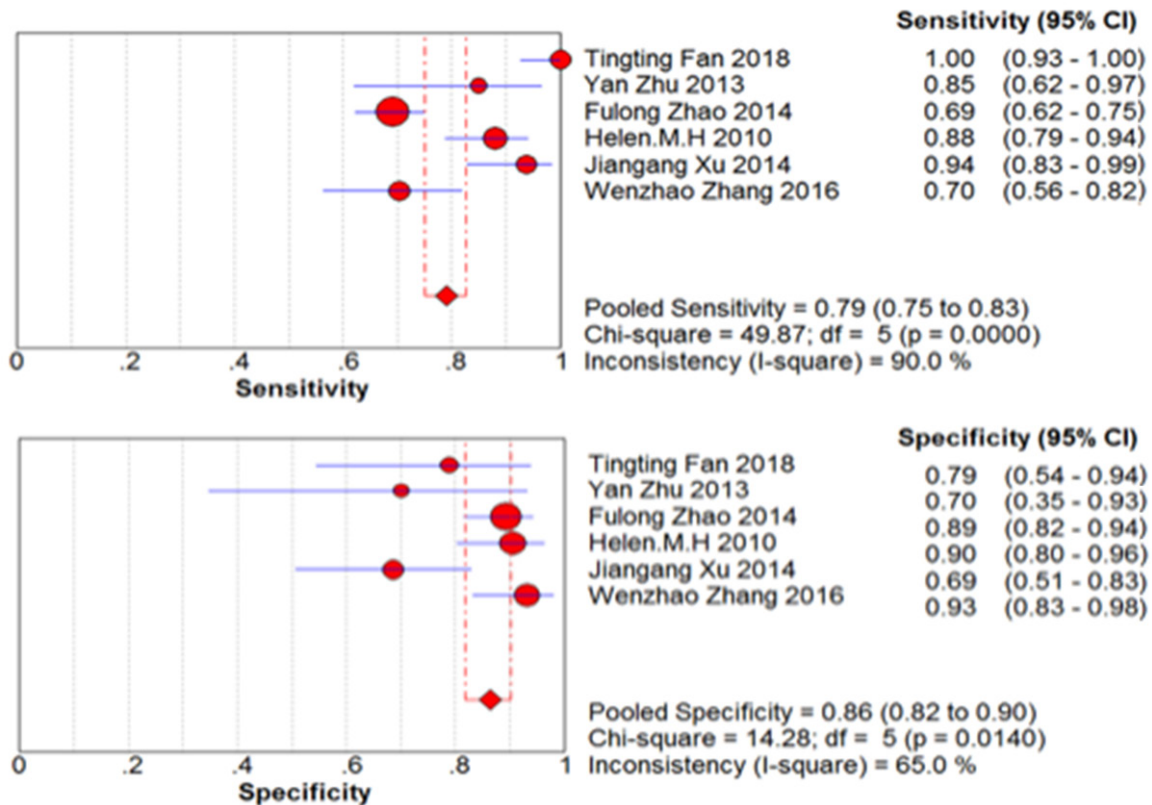


Figure 3. Sensitivity and specificity.

pendently by at least two pathologists. The characteristics of the included studies are sum-

marized in **Table 1**. Being dependent on the results of the QUADAS-2 (**Figure 2**), the risk of

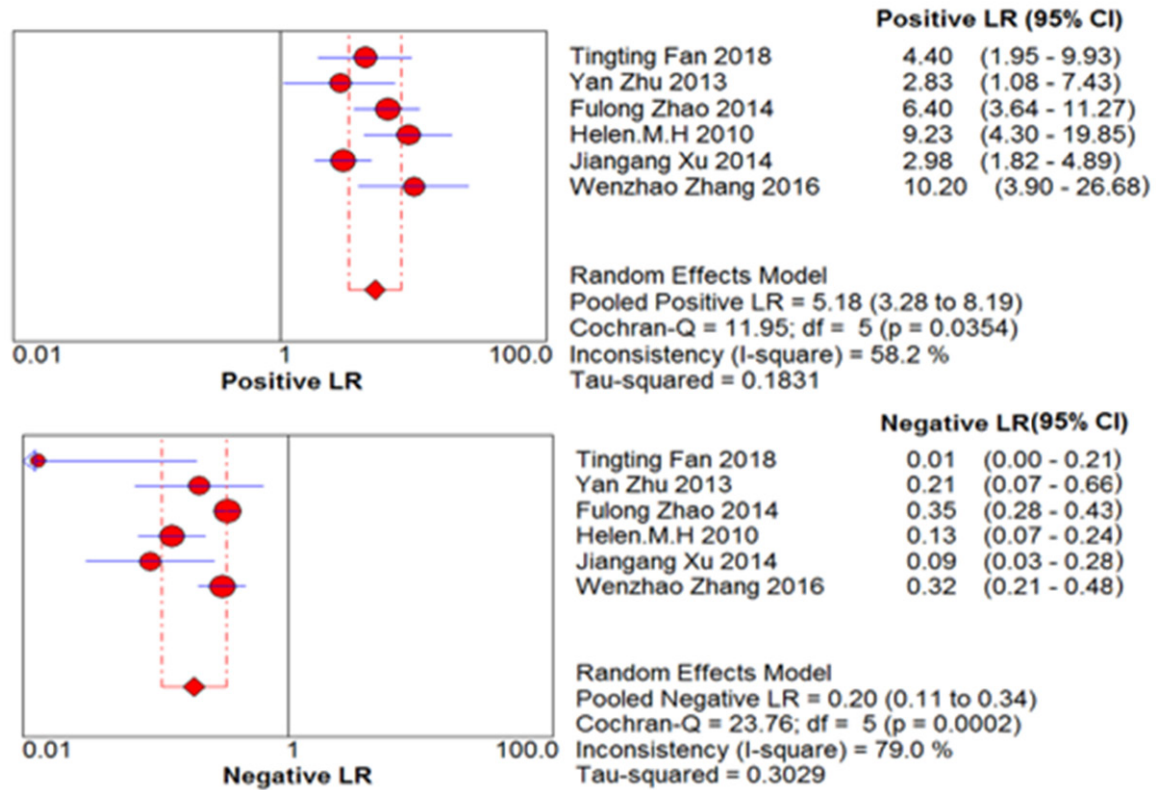


Figure 4. PLR and NLR.

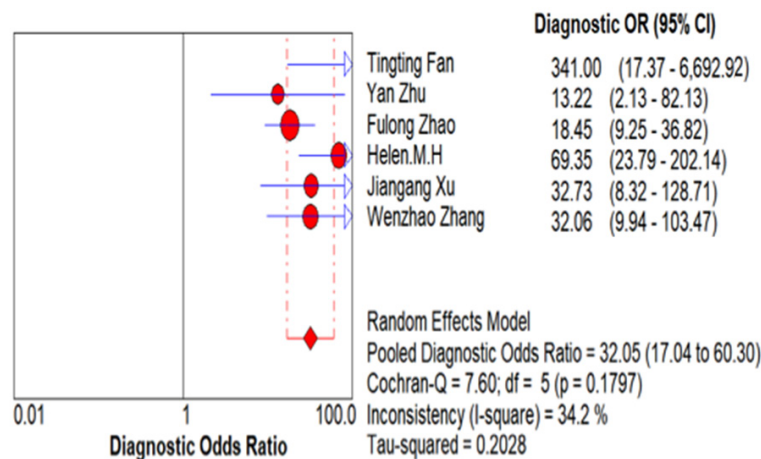


Figure 5. Diagnostic odds ratio.

bias in the study was low, and the clinical applicability was high.

Data analysis

Forest plots for the enrolled studies on the pooled sensitivity, pooled specificity, positive likelihood ratio (PLR) and negative likelihood

ratio (NLR) of miR-195 are shown in Figures 3 and 4. Pooled sensitivity, Pooled specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) were 0.79 (95% CI: 0.75-0.83), 0.86 (95% CI: 0.82-0.90), 5.18 (95% CI: 3.28-8.19) and 0.20 (95% CI: 0.11-0.34), respectively. The PLR is used to describe the chances of having a disease and not having a disease when the diagnosis is positive, which reveals the possibility of a positive diagnosis is accurate. The larger the PLR, the better. While NLR is used to

describe the chances of having a disease and not having a disease when the diagnosis is negative, which reveals the possibility of a negative diagnosis is wrong. The smaller the NLR, the better. Given a PLR of 5.18 (95% CI: 3.28-8.19) and a NLR of 0.20 (95% CI: 0.11-0.34), the conclusion could be fairly drawn that miR-195 is a great indicator for BC diagnosis. However, the I²

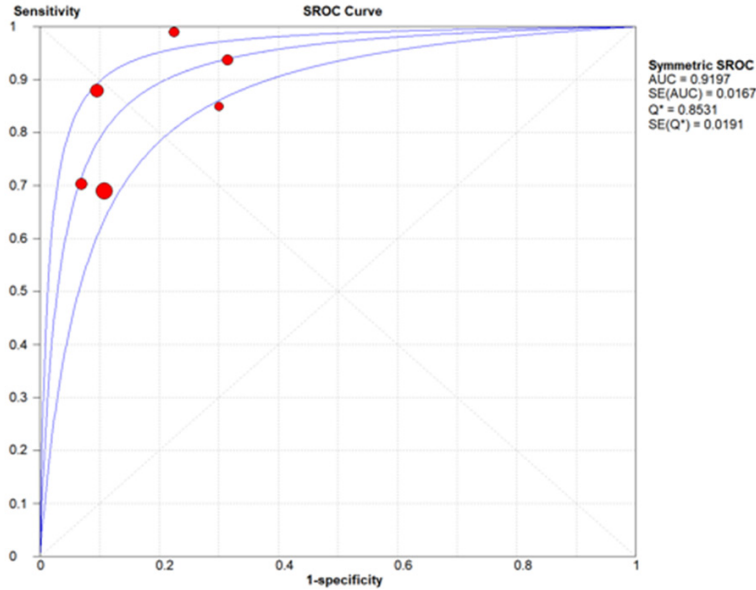


Figure 6. Summary ROC curves miR-21 diagnostic value in breast cancer.

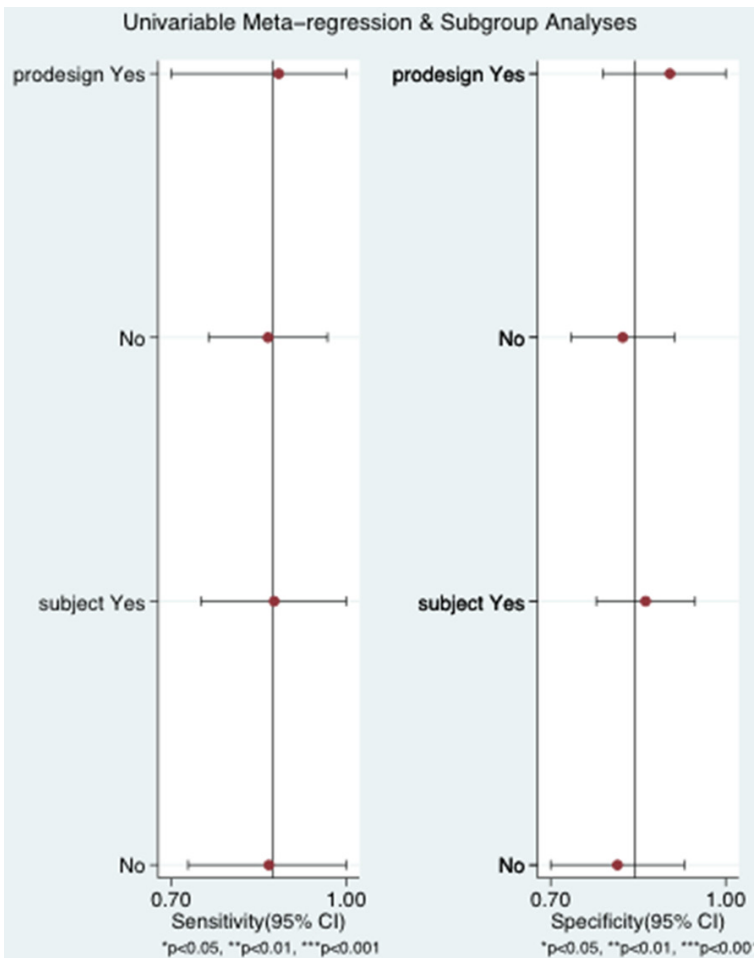


Figure 7. Univariable meta-regression.

value of sensitivity, specificity, PLR and NLR were 90.0%, 65.0%, 58.2% and 79.0%, respectively, indicating significant heterogeneity in our study. Thus, we selected the random effects model. Diagnostic accuracy was evaluated by the pooled DOR and the area under the curve (AUC), which were 32.05 (95% CI: 17.04-60.30; **Figure 5**) and 0.92 (**Figure 6**), respectively, indicating that miR-195 has high diagnostic accuracy for BC.

Threshold effect

Threshold effect is an important cause of heterogeneity in diagnostic tests, considered to result from the differences between sensitivity and specificity, which usually is indicated by a “shoulder-arm”-shaped distribution in the SROC curve. The SROC curve (**Figure 6**) showed no “shoulder-arm”-shaped distribution. The corresponding Spearman correlation coefficient was 0.486 (P = 0.329), suggesting that there was no heterogeneity from the threshold effects. In conclusion, the heterogeneity of the meta-analysis in this paper is caused by non-threshold effects.

Meta-regression analysis

The source of heterogeneity was completely examined by meta-regression analysis using study covariates such as predesign and subject. By associating each covariate with logit (sensitivity) and logit (specificity), we can examine the source of heterogeneity. However, the result showed no significant heterogeneity among these factors (**Figure 7**).

Table 2. Sensitivity analysis results

Elimination study	DOR	95% CI
Tingting Fan 2018	28.28	16.39~48.80
Yan Zhu 2013	35.86	17.85~72.01
Fulong Zhao 2014	41.62	20.86~83.03
Helen.M.H 2010	24.57	13.78~43.80
Jiangang Xu 2014	33.00	15.14~71.94
Wenzhao Zhang 2016	33.35	14.84~74.96

Sensitivity analysis

Sensitivity analysis showed that excluding Helen's article causes a significant drop in DOR, from 32.05 to 24.57. Given that all studies are from China except Helen, the conclusion can be made that the country is an important cause of inter-study heterogeneity. After removing the literature such as Fulong Zhao and Tingting Fan, the DOR also dropped significantly, indicating that the diagnostic criteria, detection methods, etc. of the three documents may differ considerably from the rest of the literature. Sensitive analysis results are shown in **Table 2**.

Publication bias

To evaluate publication bias, Deeks' funnel plot was used. The funnel plots exhibited no symmetry (**Figure 8**) and Deeks' test returned a *p* value of 0.47. Begg's test and Egger's test were also performed to estimate publication bias, and their results were 0.707 and 0.266. All results are suggesting that there is no significant publication bias between the literature included in the meta-analysis. However, considering the limited number of studies, publication bias still may exist in the present study.

Clinical utility and index test

Fagan's nomogram was used to describe the diagnosis value of miR-195 for BC (**Figure 9**). When the pretest probability was set to 20%, the data showed posttest probability increased to 59%, the PLR of 6 indicates that a person with BC is five times more likely to have a positive diagnosis than a healthy woman. Similarly, the probability would decrease to 4%, and the NLR was 0.15, suggesting that miR-195 could be a promising indicator for the diagnosis of BC.

Discussion

The incidence rate of breast cancer accounts for nearly 1/3 of female malignant tumors, which seriously affects the life safety of patients. In China, breast cancer has high incidence rate, low early diagnosis rate and high mortality rate, which is the key and difficult point in clinical diagnosis and treatment [23]. For breast cancer lesions with a diameter of more than 1.0 cm, conventional molybdenum target examination and b-mode ultrasound can be used to effectively distinguish them, but the sensitivity of early lesions with a diameter less than 5 mm is low [24]. In addition, routine serum protein detection has low sensitivity and specificity for early breast cancer, so it is increasingly urgent to search for biomarkers with high specificity and sensitivity for early breast cancer [25, 26].

In recent years, many miRNAs from tumor cells have been found to be associated with prognostic factors such as breast cancer TMN staging, vascular invasion, proliferation index, ER and/or PR status [27, 28]. Besides, miRNAs have also recently been found to be stable in serum and plasma [29]. A series of studies were conducted to investigate the use of circulating miRNAs as potential early tumor screening markers. Besides, studies have shown that they can inhibit the proliferation and invasion of tumor cells [30]. As one of the most researched miRNAs, miR-195 is closely related to the malignancy degree of breast cancer and may be considered good diagnostic biomarkers with for breast cancer. In this paper, we systematically reviewed clinical studies on the application of miR-195 in the diagnosis of breast cancer in recent years and performed meta-analysis to discuss the value of miR-195 in the diagnosis of breast cancer.

Our meta-analysis included a total of 6 articles according to the pre-established exclusion and inclusion criteria. As the result showed, the total sensitivity was 0.79 (95% CI 0.75-0.83), and the total specificity was 0.86 (95% CI 0.82-0.90), indicating its potential diagnostic ability. The area under the DOR and SROC (AUC) curves is used to represent the performance of diagnostic tests. DOR values range from 0 to infinity, and higher values indicate better test identification [31]. The ideal SROC curve is near the

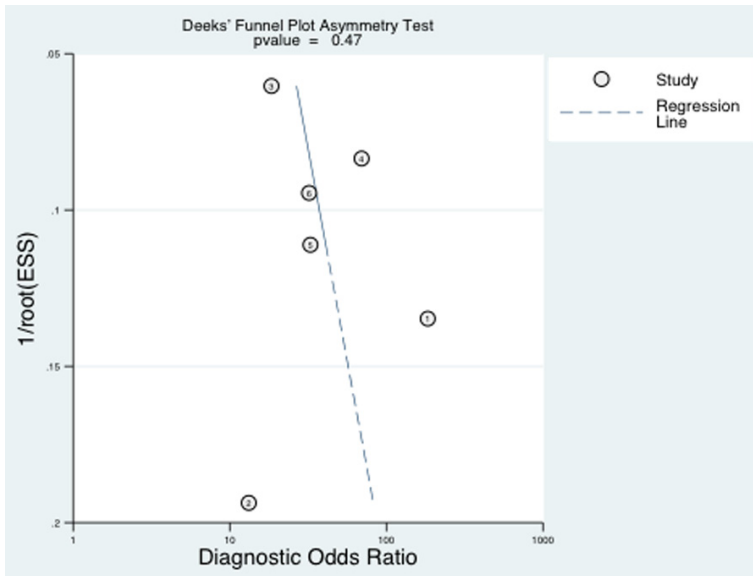


Figure 8. Deeks' funnel figure.

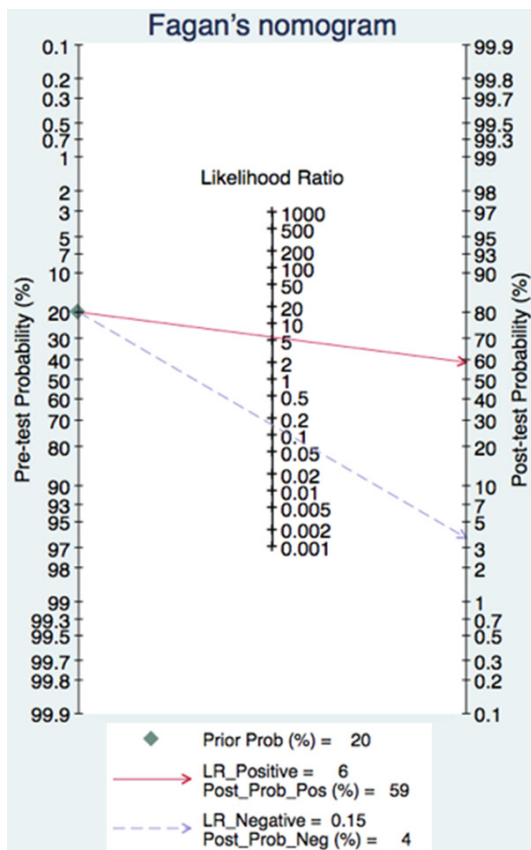


Figure 9. Fagan's nomogram of the miR-195 test for diagnosis of breast cancer.

top left corner, which indicates the best results [32]. The miR-195 presented DOR of 32.05

(95% CI 17.04-60.30) and AUC of 0.9197, indicating that miR-195 had excellent test performance. The I^2 value of sensitivity, specificity, PLR and NLR were 90.0%, 65.0%, 58.2% and 79.0% respectively, indicating significant heterogeneity in our study. Thus, we attempted to explore the sources of heterogeneity, which is critical to a meta-analysis. Threshold effect is a primary cause of heterogeneity in test accuracy studies [21], however, given Spearman correlation coefficient of 0.486 ($P = 0.329$), no heterogeneity due to the threshold effect was confirmed. We also performed meta-regression by using study

covariates such as predesign and subject to explore the source of heterogeneity, and the data showed no significant heterogeneity among these factors. Sensitivity analysis was next used to see if the heterogeneity came from any individual study. The result showed that the country may be the potential source contributing to heterogeneity in this study. In addition, the heterogeneity across studies was probably due to different baseline characteristics with regard to the distributions of age and gender, histological type, tumor stage, detection approach, and follow-up period. Unfortunately, we failed to find other sources.

However, there are also following limitations in our work: 1) The current clinical research is limited; 2) The literature included in the meta-analysis are mostly case-control studies, there may exist selection bias; 3) Although there is no obvious publication bias in methodological analysis, the retrieval languages are limited to Chinese and English, and thus language bias may exist. However, this article will bring positive reference to the research in many fields in the future: 1) This paper aims to explore the value of miR-195 for breast cancer diagnosis, and will promote the development of more clinical research; 2) For further bioinformatics, this study provides important reference value; 3) Bioinformatics studies can be performed on target genes and proteins regulated by miR-195, which may further clarify the pathogene-

sis of breast cancer and find potential therapeutic targets.

Conclusions

In summary, the use of circulating miR-195 to diagnose breast cancer has important potential value. Nonetheless, further large-scale clinical studies are warranted to provide more data support for miR-195 in early diagnosis and treatment of breast cancer.

Disclosure of conflict of interest

None.

Abbreviations

TP, true-positives; FP, false-positives; FN, false-negatives; TN, true-negatives; Fig, figure; DOR, diagnostic odds ratio; AUC, area under the summary receiver operating characteristic; SROC, summary receiver operating characteristic; CEA, carcinoembryonic antigen; ER, estrogen receptor; PR, progesterone receptor; miR-195, MicroRNA-195; BC, Breast cancer; predesign, Whether it is a prospective study; subject, Whether to describe the population to be evaluated in detail.

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