Systematic Review and Meta-Analysis

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miRNAs as biomarkers for diagnosis of heart failure

A systematic review and meta-analysis

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Abstract

Background: With the rapid development of molecular biology, the kind of mircoRNA (miRNA) has been introduced into emerging role both in cardiac development and pathological procedure. Thus, we conduct this meta-analysis to find out the role of circulating miRNA as a biomarker in detecting heart failure.

Methods: We searched PubMed, EMBASE, the Cochrane Central Register of Controlled Trials, and World Health Organization clinical trials registry center to identify relevant studies up to August 2016. We performed meta-analysis in a fixed/random-effect model using Meta-disc 1.4. We used STATA 14.0 to estimate the publication bias and meta-regression. Besides, we took use of SPSS 17.0 to evaluate variance between several groups. Information on true positive, false positive, false negative, and true negative, as well as the quality of research was extracted.

Results: We use results from 10 articles to analyze the pooled accuracy. The overall performance of total mixed miRNAs (TmiRs) detection was: pooled sensitivity, 0.74 (95% confidence interval [CI], 0.72 to 0.75); pooled specificity, 0.69 (95%CI, 0.67 to 0.71); and area under the summary receiver operating characteristic curves value (SROC), 0.7991. The miRNA-423-5p (miR-423-5p) detection was: pooled sensitivity, 0.81 (95%CI, 0.76 to 0.85); pooled specificity, 0.67 (95%CI, 0.61 to 0.73); and SROC, 0.8600. However, taken the same patients population, we extracted the data of BNP for detecting heart failure and performed meta-analysis with acceptable SROC as 0.9291. Among the variance analysis, the diagnostic performance of miR-423-5p claimed significant advantages of other pooled results. However, the combination of miRNAs and BNP could increase the accuracy of detecting of heart failure. Unfortunately, there was no dramatic advantage of miR-423-5p compared to BNP protocol.

Conclusion: Despite interstudy variability, the performance test of miRNA for detecting heart failure revealed that miR-423-5p demonstrated the potential to be a biomarker. However, other miRNAs were not able to provide enough evidence on promising diagnostic value for heart failure based on the current data. Moreover, the combination of miRNAs and BNP could work as a better method to detection. Unfortunately, BNP was still the most convinced biomarker for such disease.

Abbreviations: ANP = atrial natriuretic peptides, AUC = area under the curve (AUC), BNP = brain natriuretic peptides, DOR = diagnostic odds ratio, HF = heart failure, HFpEF = preserved ejection fraction, HFrEF = reduced ejection fraction, miR, miRNA = mircoRNAs, NT-proBNP = N-terminal pro-BNP (NT-proBNP), NYHA = New York Heart Association, QUADAS = Quality Assessment of Diagnostic Accuracy Studies, SROC = summary receiver operating characteristic curves value.

Keywords: biomarker, heart failure, meta-analysis, miRNA

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HY and FM contributed equally to this work.

Authors' contributions: HY, FM, and YL participated in research design and other authors collectively contributed to the data collection and analysis. YL wrote the manuscript.

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1. Introduction

Heart failure (HF) is a terminal stage of most types of cardiovascular diseases, which always leads to a negative prognosis.[1–3] Among most developed and developing countries, the increasing number of HF patients has already become a significant epidemic and a major cause of hospitalizations, morbidity, and mortality.[4] According to previous reports, the prevalence of HF is stable at approximately 1% to 2% of the general population, while this number sharply increases to 20% among those over 80 years old.[1] However, with significant improvement in emergency medical administration of acute coronary syndromes with transcatheter stent implantation, the management of HF remains a target of debate in the cardiovascular field.

Decades ago, the diagnosis of HF was mainly based on echocardiography, and the clinical manifestations were classified as the New York Heart Association (NYHA) classifications. With rapid development of molecular biology, circulating biomarkers have been identified, and their emerging roles in managing various kinds of diseases, including HF, have provided insight into the underlying pathophysiological mechanism of HF.[5] The atrial natriuretic peptides (ANPs) and brain natriuretic peptides (BNPs) have already been shown to have a role in detecting HF or some types of cardiac overload. Moreover, N-terminal pro-BNP (NT-proBNP) has been identified as a more powerful biomarker for diagnosing HF by several well-regarded clinical trials that provided further understanding of early and chronic stages of HF patient dynamics and pathological changes, which allowed improvement in medical management.

MicroRNAs (miRNA, miR) are a class of single-stranded and endogenously small noncoding RNAs that have a similar functional role as siRNA, which is thought to be exogenous double-stranded RNA. miRNAs can shift cardiac differentiation, proliferation, maturation, and pathological remodeling responses to stress, injury, and abnormal regulator expression.[6–9] Several miRNA arrays in human heart tissue have been reported, and a few have addressed plasma miRNAs profiles in HF.[10–12] Tijsen et al[13] suggested that miR-423-5p was a diagnostic marker for HF. Others revealed classes of miRNAs in detecting HF. In addition, many studies have identified miRNAs (miR-1, -133, -499, and -208) as markedly elevated in acute myocardial infarction (MI), and a series of meta-analyses have also been carried out to verify the role of miRNAs in MI.[14] However, although numerous reports have been published, the reported impacts of miRNAs in HF management are still varied. Therefore, in this study, we launched a meta-analysis to test the diagnostic performance of miRNAs for HF, and we aimed to verify the pioneering role of miR-423-5p among the total miRNAs. In addition, we further extracted the data on the diagnostic value of other biomarkers, such as BNP or troponin, only using the enrolled studies, which provided more consistent results based on the same sample population and pooling of the miRNAs. We therefore aimed to gather evidence on how the diagnostic performance of miRNAs, especially miR-423-5p, distinguished HF, and we rated the capability of miR-423-5p in comparison with BNPs.

2. Materials and methods

2.1. Study protocol

This analysis was conducted in accordance with a predetermined protocol following the recommendations of Deeks.[15] And there is no existed protocol. The data collection and reporting were in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. As this is a literature systematic research, so the ethical approval was not necessary.

2.2. Search strategy

PubMed, EMBASE, the Cochrane Central Register of Controlled Trials, and World Health Organization clinical trials registry center were searched using a highly sensitive and highly specific search strategy, which was "(heart failure [MeSH Terms] OR heart failure OR heart dysfunction) AND (miRNA [MeSH Terms] OR miRNA OR miR OR mircoRNA)." Search was updated to June 2012.

2.3. Study selection

Citations initially selected by systematic search were first retrieved as title and/or abstract and preliminarily screened. Potentially relevant reports were then retrieved as complete manuscripts and assessed for compliance to inclusion and exclusion criteria.

The inclusion criteria were as followings: the patients were measure specific miRNA expression level during HF; diagnostic test; HF was diagnosed with echocardiography or clinical features, especially for the NYHA classification; contained the date of true positive, false positive, false negative, and true negative; or the sensitivity, specificity, and essential sample size; the sample should be collected within the first 24 hours after appeared in hospital; all the RNA should be extracted along with whole genomic RNA; qPCR, real-time PCR, micro array, and miRNA sequence are acceptable methods to evaluate the expressions of miRNAs.

The exclusion criteria were as followings: the patients had received medications before harvested the blood or serum samples; the same cohort had been studied in other study; unable to construct 2×2 table; conferences articles; and not focused on HF.

2.4. Data collection and assessment of study quality

Two investigators (HY and FM) independently assessed eligibility of reports at the title and/or at abstract level, with a third reviewer (YL) determining the divergences together; studies that met the inclusion criteria were selected for further analysis.

The 14-item Quality Assessment of Diagnostic Accuracy Studies (QUADAS) list[16] has been used to evaluate the qualities of all the enrolled studies. Answer should be provided as yes, no, or unclear to evaluate each case. As the assessment of quality is basically related to manuscript reporting, so that a well-conducted study might score poorly once related parts were missing among the methods and results. Therefore, all the assessments were reported in descriptive forms only.

2.5. Evaluation indicators

The enrolled test performances of different types of miRNAs detection for HF were measured for the following indicators: sensitivity, specificity, diagnostic odds ratio (DOR), and area under the summary receiver operating characteristic curves value (SROC). Sensitivity was represented by the proportion of patients with HF that was correctly identified by the positive results of miRNAs expression. Specificity was represented by the non-HF cases that were correctly identified by the negative results of miRNAs. Moreover, it was more reliable to define the summary

of test performance using DOR than simply pooling sensitivity and specificity together across the studies. DOR was an independent indicator ranging from 0 to infinity, which represented how much greater the odds of having HF were for patient with a positive detecting result than for patient with a negative measurement result. The higher the DOR, the better the discriminatory ability of the test was.[17] The SROC was plotted based on the combination of sensitivity and specificity, and the area under the curve (AUC) value was then calculated as a global measurement of test performance. The closer the AUC was to 1, the better the test performance.[18]

2.6. Publication bias

Stata statistical software (STATA, version 14.0) were used to obtain quantitative analysis of all the publication bias according to funnel plots and the Deek's test. Once an asymmetric distribution of data points appears in the funnel plot companied with a quantified result of $P<.05$, it will indicate the presence of potential publication bias.[19]

2.7. Heterogeneity

The χ^2 test was used to examine heterogeneity in pooling sensitivity and specificity. The Cochran Q test was used to examine heterogeneity in pooling DOR. Heterogeneity was considered to be statistically significant when $P<.05$ in these qualitative tests. The I^2 test was also conducted in every pooling analysis to quantitatively estimate the proportion of total variation across studies that was attributable to heterogeneity rather than chance. The I^2 value would range from 0 to 100%, with a value over 50% indicating significant heterogeneity. The existence of a threshold effect would manifest as a curvilinear shape in the SROCs.

2.8. Meta-regression and sensitivity analysis

The meta-regression was carried out to detect where the potential factor for heterogeneities origin from. To determine whether any single study was incurring undue weight in the analysis, 1 set of study data were systematically removed, and the pooled results for the remaining studies were rechecked whether the results had a significant change. The sensitivity analysis was conducted for every study.

2.9. Statistical analysis

Data were analyzed using Meta-Disc Version 1.4.[20] Besides, publication bias and meta-regression analysis were conducted by STATA version 14.0. And the Z tests for evaluating the AUC among different pooled miRNAs detecting methods were performed using Statistical Package for the Social Sciences (SPSS) 22.0. Because of potential heterogeneity between studies, effect sizes were pooled by random-effect models of DerSimonian and Laird in Meta Disc.[21] Empty cells were handled using a 0.5 continuity correction.

3. Results

3.1. Study evaluation

A total of 671 citations were retrieved by the method aforementioned. After reading titles and abstracts, 641 citations were excluded according to the selection criteria, and we identified 30 articles initially.[13,22–50] Among them, 20 articles were excluded after reading the complete article as we were unable to construct a 2×2 table for 15 articles, 2 articles included patients who received medication before the samples were collected, 1 article did not use include a gold-standard treatment, 1 article mixed HF and myocardial infarction cases, and 1 article only provided miRNA array data, which could not be pooled. No articles were added through manual retrospective research after reading the related publications. Finally, 10 articles[13,22,27,29,32,34,42,45,47,50] with diagnostic test studies for HF diagnosis were enrolled in this meta-analysis (Fig. 1). Among them, 33 individual diagnostic tests were extracted for total mixed miRNA (TmiR) evaluation, 4 individual diagnostic tests were extracted for miRNA-423-5p (miR-423–5p) evaluation, 29 individual diagnostic tests were enrolled for total mixed miRNA knock out miR-423-5p (TmiRs-KO-423-5p) evaluation, 11 individual tests were enrolled for mixed miRNA combined with BNP (TmiRs+BNP) evaluation, and only 3 individual tests were selected for BNP evaluation within the identified articles. The basic characteristics of the included studies are shown in Table 1.

3.2. Study quality

The QUADAS list of questions was used to review the test quality of the included studies. Most of the studies satisfied a majority of the items on the QUADAS list. The most common missing items in the studies included in this analysis were reports of uninterruptible test results and withdrawn cases. In addition, almost all of the studies failed to mention blinded interpretations between the miRNA testing results and classifications of NYHA or echocardiography performances (Table 2).

3.3. Publication bias

Funnel plots were used to evaluate the publication bias of the included studies. Each dot represents a study, and the distance between each dot and the vertical line suggests bias in each study. The absence of any asymmetric distribution suggested that there was no publication bias. An asymmetric distribution indicated that publication bias existed. Deeks' test revealed the possibility of significant publication bias among the included reports of TmiRs+BNP ($P=.02$, 95%CI, 28.86 to 314.63) evaluation pooled results. The funnel plots in Fig. 2D present a certain degree of asymmetry, indicating the potential for publication bias among the studies included in this analysis. Otherwise, there were no significant publication biases among the included reports of TmiRs ($P=.08$, 95%CI, –34.79 to 2.24), TmiRs-KO-423-5p ($P=.09$, 95%CI, –38.06 to 3.05), miR-423-5p ($P=.85$, 95%CI, –97.81 to 88.67), and BNP ($P=.82$, 95%CI, –2535.48 to 2419.99) among the evaluation pooled results. The funnel plots in Fig. 2A–C and E present a certain degree of symmetry, indicating there was no potential for publication bias among the studies included in this analysis.

3.4. Overall diagnostic performance of miRNAs and related biomarkers

3.4.1. Total mixed miRNAs. The overall diagnostic measurement in detecting HF of TmiRs has been summarized in Fig. 3. The summary sensitivity was 0.74 (95%CI, 0.72 to 0.75), and the summary specificity was 0.69 (95%CI, 0.67 to 0.71). Both pooled estimations showed significant heterogeneity in Fig. 3A and B (sensitivity: $P=.0000$, $\chi^2=116.94$, $I^2=72.6\%$; specificity: $P=.0000$, $\chi^2=418.66$, $I^2=92.4\%$). Figure 3C and D showed the

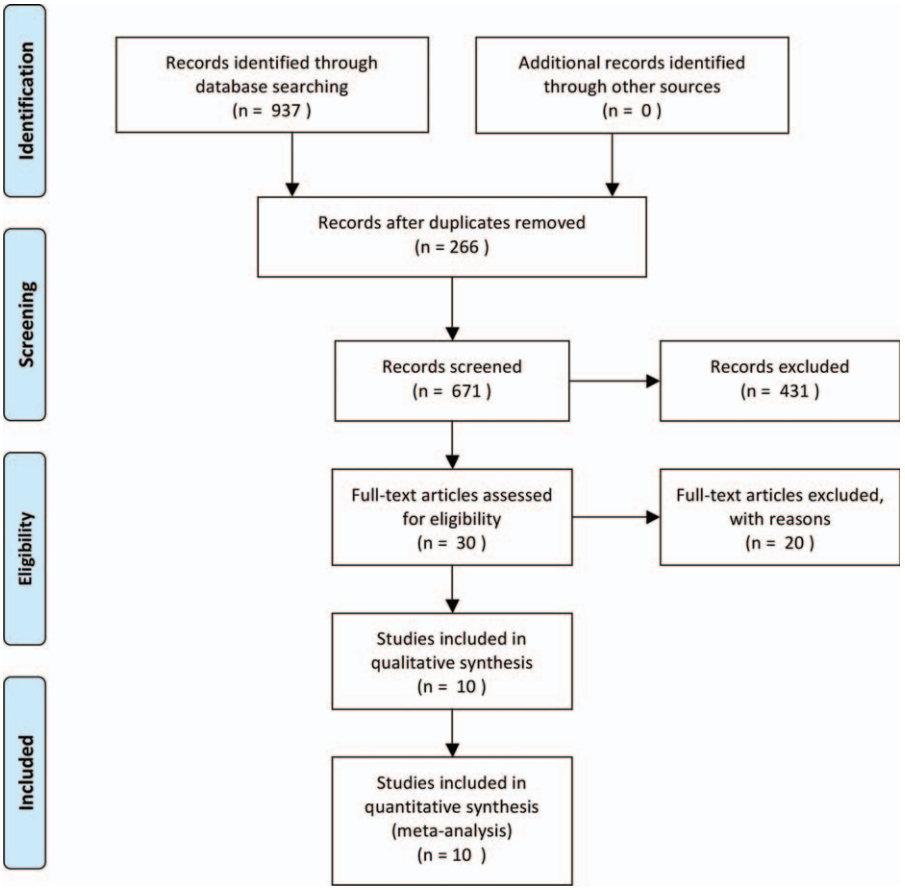

Figure 1. Flow diagram of study selection process.

pooled DOR and the SROCs. The pooled DOR was 7.41 (95% CI, 5.35 to 10.27) with a noticeable heterogeneity ($P=.0000$, Cochran-Q=131.13, $I^2=75.6\%$). The calculated AUC value was 0.7991 ± 0.0164. The absence of a curvilinear shape in the SROC suggested no potential presence of a threshold effect.

3.4.2. miRNA-423-5p. The overall diagnostic performance in detecting HF of miR-423-5p has been demonstrated in Fig. 4. The summary sensitivity was 0.81 (95%CI, 0.76 to 0.85), and the summary specificity was 0.67 (95%CI, 0.67 to 0.73). Both pooled estimations showed some heterogeneities (sensitivity: $P=.6269$, $\chi^2=1.75$, $I^2=0.0\%$; specificity: $P=.0000$, $\chi^2=26.42$, $I^2=88.6\%$). The pooled DOR was 9.91 (95%CI, 4.47 to 22.00) with a noticeable heterogeneity ($P=.0289$, Cochran-Q=9.03, $I^2=66.8\%$). It revealed that the AUC value was 0.8600 ± 0.0275. The absence of a curvilinear shape in the SROC suggested no potential presence of a threshold effect.

3.4.3. Total mixed miRNAs with miR-423-5p knock out. The overall diagnostic performance of TmiRs-KO-423-5p (Supplemental Fig. 1, http://links.lww.com/MD/B710) showed the potential diagnostic capability of miRNAs for HF without the impact from miR-423-5p. The summary sensitivity was 0.72 (95%CI, 0.70 to 0.74), and the summary specificity was 0.70 (95%CI, 0.68 to 0.72). Both pooled estimations showed significant heterogeneity (sensitivity: $P=.0000$, $\chi^2=103.55$, $I^2=73.0\%$; specificity: $P=.0000$, $\chi^2=391.72$, $I^2=92.9\%$). The pooled DOR and the SROCs based on summary sensitivity and specificity across all datasets are shown in Supplemental Fig. 1, http://links.lww.com/MD/B710. The pooled DOR was 7.13 (95%CI, 4.99 to 10.20). The results of DOR showed consistency across the included reports, with noticeable heterogeneity ($P=.0000$, Cochran-Q=119.86, $I^2=76.6\%$). The point size in the SROC represents the proportional study weight. The AUC value was 0.7916 ± 0.0183. The absence of a curvilinear shape in the SROC suggested no potential presence of a threshold effect.

3.4.4. miRNAs combine BNP. The overall diagnostic performance of miR+BNP (Supplemental Fig. 2, http://links.lww.com/MD/B710) showed the elevated diagnostic capability of miR +BNP in detecting HF. The summary sensitivity was 0.85 (95% CI, 0.82 to 0.88), and the summary specificity was 0.81 (95% CI, 0.78 to 0.83), with individual specificities ranging from 0.98 to 1.00. Both pooled estimations showed some heterogeneities (sensitivity: $P=.1054$, $\chi^2=15.80$, $I^2=36.7\%$; specificity: $P=.0000$, $\chi^2=57.72$, $I^2=82.7\%$). The pooled DOR curve and the SROC based on summary sensitivity and specificity across all datasets are shown in Supplemental Fig. 2, http://links.lww.com/MD/B710. The pooled DOR was 28.91 (95%CI, 16.99 to 49.18). The results of DOR showed no consistency across the included reports, with noticeable heterogeneity ($P=.0027$, Cochran-Q=26.88, $I^2=62.8\%$). The point size in the SROC represents the proportional study weight. The AUC value was 0.9146 ± 0.0155. The absence of a curvilinear shape in the SROC suggested no potential presence of a threshold effect.

Table 1

Characteristics of included studies.

No.	Author	Year	Journal	Design	Countries	Enrolled patients	Enrolled control	Selected miRNAs	Comparison with BNP	Comparison with troponin	Samples source	Golden standard
1	Tijsen et al[13]	2010	*Circ Res*	Prospective and consecutive among multiple centers	Netherland	30 HF patients; age: 68.2 ±2.5 y; gender (m/f): 16/14	39 healthy controls; age: 55.7 ±0.7 y; gender (m/f): 15/24	miR-18b, miR-423-5p, miR-675	None	None	Plasma	Met the Framingham criteria and NT-proBNP >1000 ng/L
2	Ellis et al[27]	2013	*Eur J Heart Fail*	Prospective and consecutive	New Zealand	44 patients with HF; age: 75.0±13.2 y; Gender (m/f): 31/13	32 COPD and 59 other breathless; age: 66.7± 13.1 y; gender (m/f):52/39	miR-103, miR-142-3p, miR-199a-3p, miR-23a, miR-27b, miR-30b, miR-324-5p, miR-342-3p, miR-598, miR-423-5p	NT-proBNP	hs-troponin	Plasma	Transthoracic echocardiography LVEF <45%
3	Fan et al[28]	2013	*Indian Heart J*	Prospective	China	45 HF patients with DCM; age: 47.76±12.28 y; gender (m/f): 32/13	39 healthy age-and gender-matched people; age: 47.59±11.85 y; gender (m/f): 25/14	miR-423-5p↑	NT-proBNP	None	Plasma	NYHA and echocardiography LVEF <45%
4	Akat et al[22]	2014	*Proc Natl Acad Sci USA*	Prospective among multiple centers	USA	24 advanced HF patients; age: 66 (33–78) y; gender (m/f): 22/2	13 healthy people; age: 60 (32–70) y; gender (m/f): 9/4	miR-208b	BNP	cTnI	Plasma	Details not provided
5	Olivieri et al[42]	2013	*Int J Cardiol*	Prospective	Italy	81 patients had acute HF; age: 81.3±6.8 y; gender (m/f): 34/47	99 healthy control subjects; age: 79.5±5.4 y; gender (m/f): 40/59	miR-499-5p	None	cTnT	Plasma	Echocardiography
6	Gupta et al[34]	2013	*J Mol Cell Cardiol*	Prospective and consecutive	USA	44 HF patients with DCM; age: 51.9±8.6 y; gender (m/f): 24/20	48 nonfailing controls; age: 55.2±9.0 y; gender (m/f): 25/23	miRNA-548c	BNP	None	Peripheral mononuclear cells	Echocardiography LVEF < 40%
7	Vogel et al[47]	2013	*Eur Heart J*	Prospective and consecutive	Germany	53 HF patients; age: 60 ±16 y; gender (m/f): 44/9	39 controls with no cardiovascular diseases, age: 63±13 y; gender (m/f): 23/16	miR-122, miR-1228, miR-1231, miR-200b, miR-519e, miR-520d-5p, miR-558, miR-622	NT-proBNP	None	Serum	Echocardiography LVEF < 50% and NYHA
8	Goren et al[32]	2012	*Eur J Heart Fail*	Prospective	Israel	41 patients with chronic, stable, stage C, systolic HF; age: 61.0±11.0 y; gender (m/f): 35/6	35 healthy volunteers; age: 65.7±10.2 y; gender (m/f): 32/3	miR-423-5p+ miR-320a+ miR-22+ miR-92b	BNP	None	Peripheral blood, serum	Echocardiography LVEF < 40%
9	Zhao et al[50]	2013	*Cardiovasc Pathol*	Prospective	China	22 patients with chronic HF; age: 59.1±11.1 y; gender (m/f): 17/5	18 volunteers without HF; age: 51.3±14.4 y; gender (m/f): 7/11	miR-210, miR-30a	None	None	Serum	Framingham standards, NYHA, and pro-BNP ≥1000 ng/L
10	Seronde et al[45]	2015	*Plos One*	Prospective	France	236 patients with acute HF; age: 76 (65.5–84.5) y; gender (m/f): 93/143	58 nonacute HF people with dyspnea; age: 72.5 (62–79.75) y; gender (m/f): 27/31	miR-1, miR-21, miR-23, miR-126, miR-423-5p	None	None	Plasma	Details not provided

BNP = brain natriuretic peptide, COPD = chronic obstructive pulmonary disease, cTnI = cardiac troponin I, cTnT = cardiac troponin T, DCM = dilated cardiomyopathy, HF = heart failure, hs-troponin = high-sensitivity troponin, LVEF = left ventricular ejection fraction, miR = miRNA, NYHA = New York Heart Association, NT = N-terminal, TmiR = total mixed miRNAs.

Table 2

QUADAS criteria of included studies.

No.	Spectrum composition	Selection criteria	Reference standard	Disease progression bias	Partial verification	Differential verification	Incorporation bias	Index test execution	Reference standard execution	Test review bias	Reference standard review bias	Clinical review bias	Uninterruptible test results	Withdrawals
1	+	+	+	+	+	+	+	+	+	+	?	+	+	–
2	+	+	+	+	+	+	+	+	+	+	+	+	+	–
3	+	+	+	+	+	+	+	+	+	+	?	+	+	–
4	+	+	+	?	–	+	+	+	–	+	?	+	+	–
5	+	+	+	+	+	+	+	+	+	+	?	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	–
7	+	+	+	+	+	+	+	+	+	+	?	+	+	+
8	+	+	+	+	+	+	+	+	+	+	?	+	+	–
9	+	+	+	+	+	+	+	+	+	+	?	+	+	–
10	+	+	+	?	–	+	+	+	+	+	–	+	+	+

QUADAS = Quality Assessment of Diagnostic Accuracy Studies.

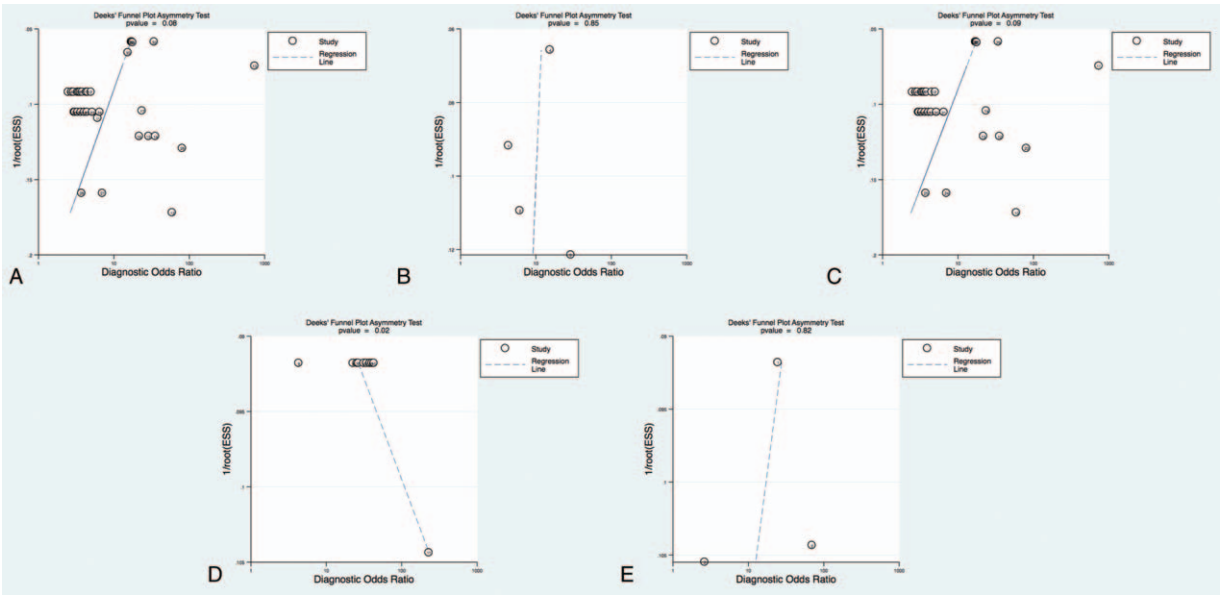

Figure 2. Funnel plot for the assessment of potential publication bias. The funnel graphs plot the square root of the effective sample size (1/ESS1/2) against the DOR. Each circle represents each study in the meta-analysis. Asymmetry of the circle distribution between regression lines indicates potential publication bias. (A) TmiRs pooled result, (B) miR-423-5p pooled result, (C) TmiRs-KO-423-5p pooled results, (D) miR+BNP pooled result, and (E) BNP pooled result. This funnel plot indicates no publication bias with a P value >.05. DOR = diagnostic odds ratio, ESS = effective sample size, miR = mircoRNA.

3.4.5. BNP. The overall diagnostic performance of BNP (Supplemental Fig. 3, http://links.lww.com/MD/B710) shows the capability of BNP in detecting HF among the same patient population in comparison with the miRNA diagnostic value evaluation. The summary sensitivity of BNP was 0.70 (95%CI, 0.62 to 0.78), and the summary specificity was 0.80 (95%CI, 0.74 to 0.85) with some heterogeneities (sensitivity: $P=.0000$, $\chi^2=22.68$, $I^2=91.2\%$; specificity: $P=.0000$, $\chi^2=61.75$, $I^2=96.8\%$). The pooled DOR of BNP was 49.50 (95%CI, 3.82 to 641.81). The point size in the SROC represents the proportional study weight. The AUC value of BNP was 0.9291 ± 0.0697. The absence of a curvilinear shape in both SROCs of BNP suggested no potential presence of a threshold effect.

3.5. Meta-regression and sensitivity analysis

The meta-regression analysis was carried out to identify the potential factors that might cause the heterogeneities. For the meta-regression, we took all the potential factors that were extracted from the baseline measurement and original testing procedures into consideration. The meta-regression could determine the correlation between the potential factors and the existing heterogeneities. When a significant difference was discovered, the factor should have a dramatic impact on the homogeneity of the enrolled studies. After reviewing the baseline data and the original data producing procedure, types of miRNAs, individual studies, gold-standard selection, and the sample sources were taken into account in the meta-regression to detect the origins of heterogeneities. According to the results (Fig. 5), the types of miRNAs did not make a contribution to the significant heterogeneities above; thus, the type of protocol pooled with the mixed miRNA type seemed be acceptable, and the results from mixed pooled analysis were consistent. In addition, the individual studies for each diagnostic test were not responsible for the existing heterogeneities, while the different gold-standard methods also made no contribution to the heterogeneities. Among the enrolled studies, there were 3 different types of sample collection protocols, which were from serum, plasma, and mononuclear cells. The meta-regression analysis, which should have a significant impact on the pooled results in measuring the sensitivity among included studies,

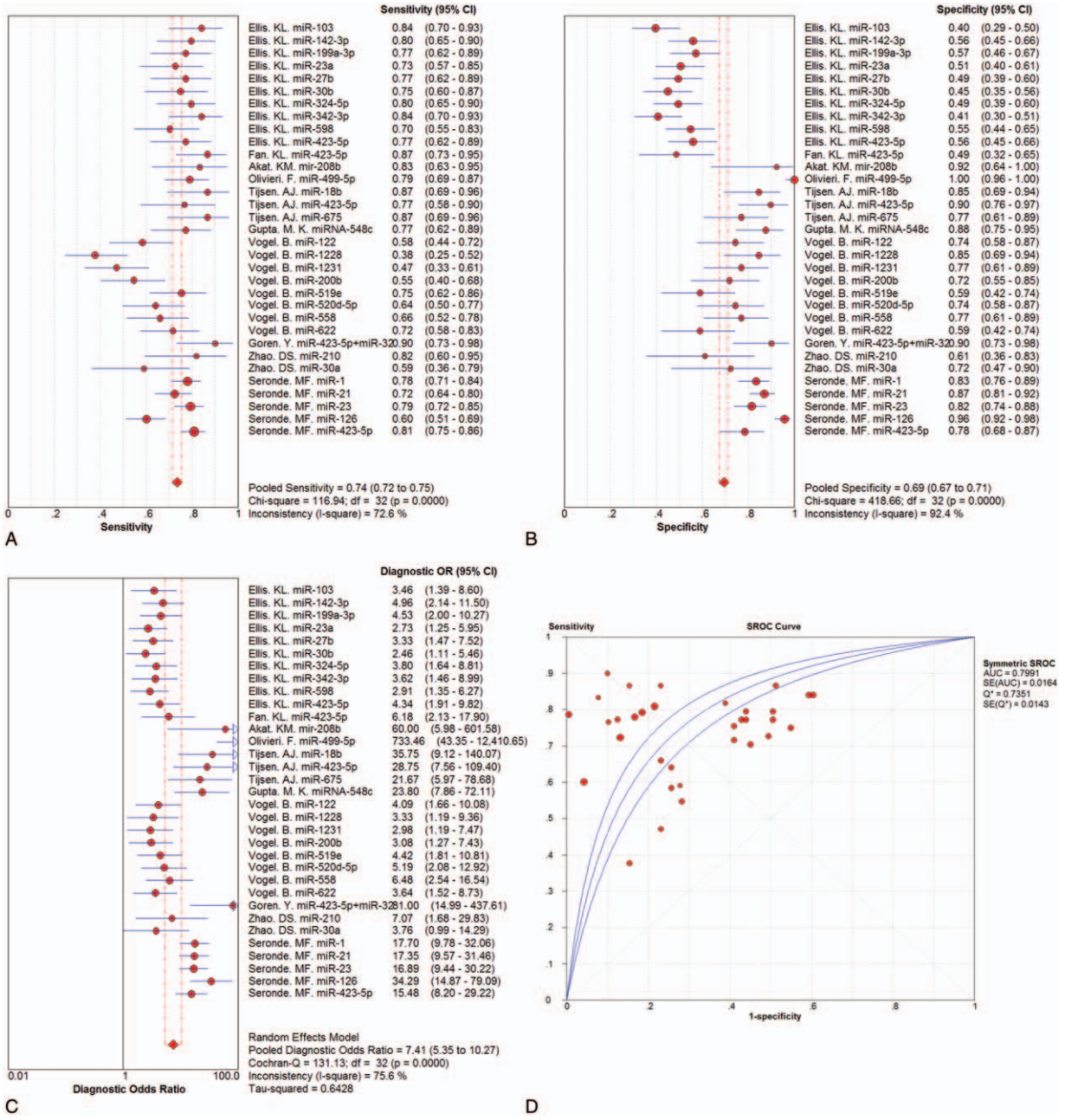

Figure 3. Performance of TmiRs detection for the diagnosis of HF. (A) Pooled sensitivity. (B) Pooled specificity. (C) Overall DOR. (D) The SROCs for all datasets. The point estimates from each study are shown as solid squares. The pooled estimates are shown as a solid diamond. Effect sizes were pooled by random-effect models. Each square in the SROC represents 1 study. Sample size is indicated by the size of the square. Error bars represent 95% CIs. CI = confidence interval, DOR = diagnostic odds ratio, HF = heart failure, miR = mircoRNA, OR = odds ratio, SROC = summary receiver operating characteristic curves value.

suggested that the sample sources were responsible for the heterogeneities in sensitivities evaluation. However, there was no contribution of the sample sources that had an impact on the calculated results of the specificity assignment, which indicated that the expression of miRNAs would be variable among some sample sources. Moreover, the meta-analysis indicated that the subject numbers would not shift the heterogeneities; thus, this result could cause the sensitivity analysis to be negative. Consistent with the regression results, we also systematically removed 1 dataset at a time and recalculated the DOR and AUC values for the remaining studies as a sensitivity analysis. These results indicated that no single dataset carried enough weight to significantly influence the pooled test performance reported for the ability of each type of miRNA detecting protocol to identify cases of HF. Finally, sensitivity analysis was carried out using a larger sample size subgroup analysis that included more than 5 studies, and every analysis confirmed the findings of the overall analysis in both directions and the magnitudes of statistical significance.

3.6. Analysis of variance

The comparison of AUC values of SROC among different types of echocardiography was performed with a Z test. Among the 6 groups, the SROCs were not all the same for the pooled results.

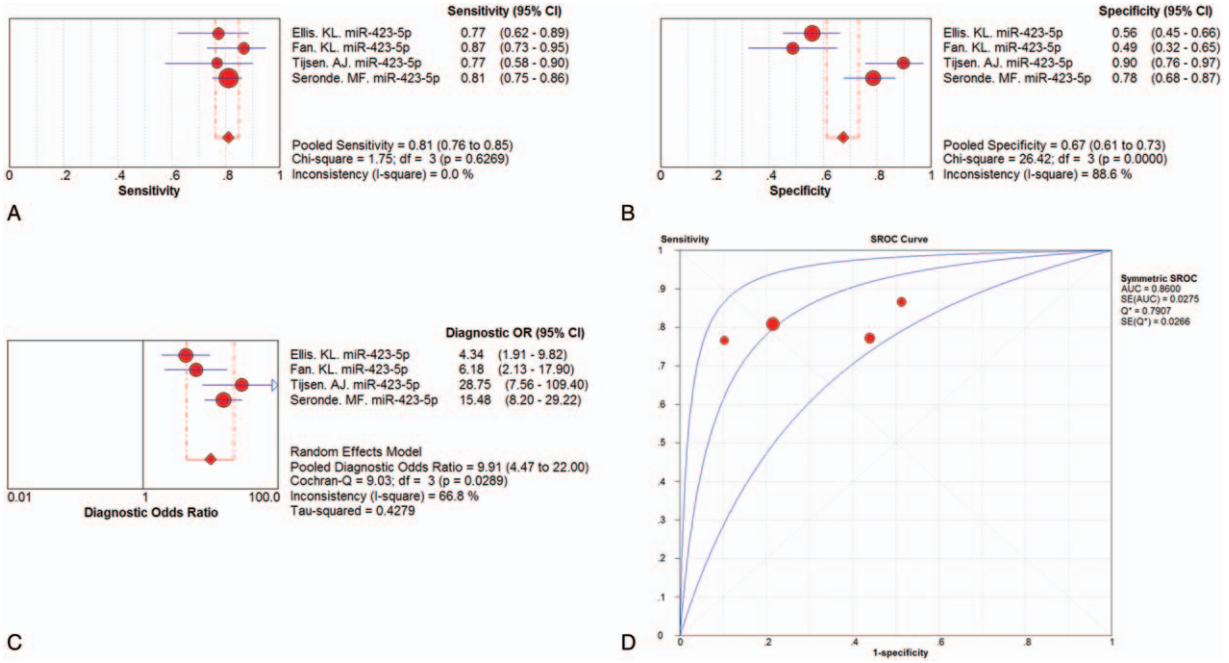

Figure 4. Performance of miR-423-5p detection for the diagnosis of HF. (A) Pooled sensitivity. (B) Pooled specificity. (C) Overall DOR. (D) The SROCs for all datasets. The point estimates from each study are shown as solid squares. The pooled estimates are shown as a solid diamond. Effect sizes were pooled by random-effect models. Each square in the SROC represents 1 study. Sample size is indicated by the size of the square. Error bars represent 95% CIs. CI = confidence interval, DOR = diagnostic odds ratio, HF = heart failure, miR = mircoRNA, OR = odds ratio, SROC = summary receiver operating characteristic curves value.

Moreover, the AUC values of miR-423-5p showed a significantly better diagnostic performance than TmiRs and TmiR-KO-423-5p, while there were no differences between TmiRs and TmiR-KO-423-5p. However, the AUC values of miRs+BNP and BNP demonstrated significant differences compared with miR-423-5p, indicating better detecting capabilities. There also were no dramatic differences among the 3 groups (Table 3).

4. Discussion

This meta-analysis was restricted to the characteristics and accuracy of different protocols with miRNAs in detecting HF. Since the introduction of miRNAs almost a decade ago, studies that focused on miRNA have remained of interest across several fields of study. A series of publications have demonstrated the emerging role of miRNAs in the regulation of heart development, maturation, proliferation, differentiation, and the pathological mechanisms of heart disease with varying origins.[31,42,51–53] miRNAs have been shown to play a great role in the regulation of cardiac function, and investigations had been carried out to assess whether miRNAs have sufficient capability to be used as biomarkers for HF. Moreover, as miRNAs could contribute to the progression of heart disease from the very beginning of pathological initialization, it is important to determine whether there is a significant value in using miRNAs for the detection of these kinds of diseases, even in early stages, which would be helpful in the management of related conditions.[54] Currently, HF is always defined using echocardiography and assessment of the clinical manifestations. Although these methods detect HF, the results can be quite diverse as the results of echocardiography are affected by the actions of the operators and HF clinical

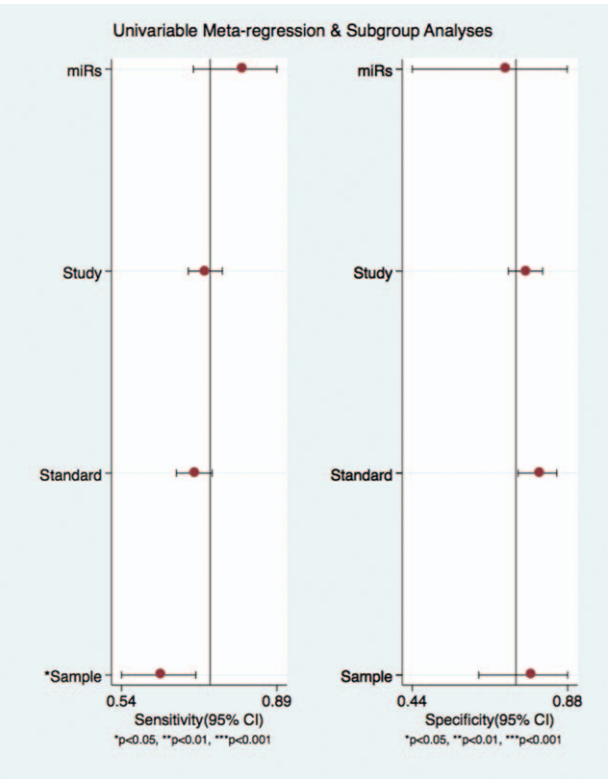

Figure 5. The meta-regression of the enrolled studies. CI = confidence interval, miR = mircoRNA.

Table 3

Analysis of variance.

	miR-423-5p	Pooled miRs knock out 423-5p	miRs+BNP	Troponin	BNP
Pooled miRs	<0.05	>0.05	<0.05	<0.05	<0.05
miR-423-5p		<0.05	<0.05	<0.05	<0.05
Pooled miRs knock out 423-5p			<0.05	<0.05	<0.05
miRs+BNP				>0.05	>0.05
Troponin					>0.05

BNP = brain natriuretic peptide, miR = miRNA.

manifestations differ among individuals. Moreover, with further understanding of HF with a preserved ejection fraction (HFpEF) carrying equal morbidity and mortality risks as HF with a reduced ejection fraction (HFrEF), the previous diagnostic protocols were challenged.[55] Regarding this point, it is therefore important to make a definite scientific diagnosis with specific biomarkers that could assess all the conditions of cardiac dysfunction. To our knowledge, this is the first meta-analysis that focused on the accuracy of miRNAs in detecting HF using several different protocols that were then compared. Therefore, the level of evidence for the accuracy of miRNAs in detecting HF has been elevated, and the effectiveness of different views has been evaluated. Cheng et al[14] performed a systematic review of miRNAs as biomarkers to analyze myocardial infarction, and their role in cardiac diseases was confirmed.[14] This is the first meta-analysis of miRNAs and HF. As there are not enough studies to analyze specific individual miRNAs, the pooled results of mixed total miRNAs as the first look at miRNAs in detecting HF seems reasonable. We used a similar analysis protocol as in the previous meta-analysis on miRNAs by Cheng et al.[14] We enrolled studies using mixed miRNAs first and then analyzed specific miRNAs for further evaluation. This meta-analysis was a pioneer study to evaluate the potential for miRNAs to be used in the diagnosis of HF. We concentrated on the published protocols and provided current insight into this issue.

In this meta-analysis, we included 10 relevant studies.[13,22,27,29,32,34,42,45,47,50] Although the results were not consistent across the different pooled results, the overall TmiRs diagnostic performance in detecting HF showed pooled sensitivities of 0.74 (95%CI, 0.72 to 0.75) and a summary specificity of 0.69 (95%CI, 0.67 to 0.71), as well as an AUC value of 0.7991 ± 0.0164. Reviewing the previous studies on miRNAs in detecting other diseases, the pooled sensitivities and the specificities were always approximately 0.70, which suggested the potential capacity for miRNAs to be used in the diagnosis of HF. However, compared to other approaches to assess HF, the accuracy of miRNAs still remains low and should not be considered acceptable for clinical practice.

In the next step, we examined single individual miRNAs among the total miRNA library. Unfortunately, only miR-423-5p was repeatedly used in individual studies. Based on this, we enrolled 4 studies on miR-423-5p and found a summary sensitivity of 0.81 (95%CI, 0.76 to 0.85) and summary specificity of 0.67 (95%CI, 0.67 to 0.73). In addition, the AUC value reached 0.8600 ± 0.0275. After conducting a comparison between the TmiR and miR-423-5p detecting protocols, we revealed that the AUC value of miR-423-5p was significantly higher than that of TmiR, indicating the miR-423-5p was superior. Moreover, for further confirmation, we also pooled the results of TmiRs after excluding the 4 studies on miR-423-5p. Consistent with the previous results, the AUC value dropped slightly to 0.7916 ± 0.0183 compared to that of TmiR, which confirmed the advantages of miR-423-5p in detecting HF. miR-423-5p is a novel miRNA that had already been analyzed in a few studies. miR-423-5p was usually found to target the key regulators of the metabolism of cardiomyocytes, such as O-GlcNAc transferase, which could impair cardiac function.[36] Moreover, several studies also demonstrated that there was a positive correlation of miR-423-5p and BNP among HF patients.[31,56] This provided more convincing evidence that miR-423-5p should be chosen from the total miRNAs to analyze HF.

Although miR-423-5p is suggested as key among the total miRNAs, whether it has the same diagnostic value or better of the current biomarkers for HF remains to be determined. To evaluate this, we analyzed BNP results only from the included studies of this meta-analysis to take advantage of the same population and avoid significant bias. Surprisingly, the results of TmiRs+BNP showed a large shift to an AUC value of 0.9146 ± 0.0155. Moreover, the diagnostic capability of BNP alone also reached a perfect level with an AUC value of 0.9291 ± 0.0697. Furthermore, to investigate potential variables of AUC values among such protocols, a Z test analysis was conducted to provide clues about methodological indications. The Z test analysis showed that once the BNP was enrolled for diagnosis, there was great elevation in the accuracy of detecting HF. Considering this, miRNAs still have remarkable limitations in distinguishing HF compared with the current clinical test of BNP. However, several studies aimed to determine whether a specific miRNA could be useful to detect details or types of heart dysfunctions or act as a supplementary method to existing methods. It has been claimed that miRNAs could not be used as an independent biomarker for HF diagnosis.

The limitation of this meta-analysis is some pooled results showed large heterogeneities. The potential influencing factors should be the sample origins. However, subgroup analyses were not performed due to the limited number of included studies, which might produce unconvincing results for few studies.

5. Conclusion

In conclusion, despite interstudy variability, the analysis of miRNA performance in detecting HF revealed that miR-423-5p has the potential to be a biomarker for HF diagnosis. However, other miRNAs were not shown to have promising diagnostic value for HF based on the current data. Moreover, a combination of miRNAs and BNP could work as a better method for detection. BNP was still the most convincing biomarker for these diseases; therefore, more work needs to be done to launch the application of miRNAs as biomarkers for HF detection in the clinic.

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