ESC European Society of Cardiology

European Journal of Heart Failure (2022) **24**, 2212–2225
doi:10.1002/ejhf.2700

RESEARCH ARTICLE

Diagnostic performance of microRNAs in the detection of heart failure with reduced or preserved ejection fraction: a systematic review and meta-analysis

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Received 26 May 2022; revised 18 September 2022; accepted 19 September 2022; online publish-ahead-of-print 27 October 2022

Aim Chronic heart failure (CHF) can be classified as heart failure with preserved ejection fraction (HFpEF) or with reduced ejection fraction (HFrEF). Currently, there is an unmet need for a minimally invasive diagnostic tool for different forms of CHF. We aimed to investigate the diagnostic potential of circulating microRNAs (miRNAs) for the detection of different CHF forms via a systematic review and meta-analysis approach.

Methods and results Comprehensive search on Medline, Web of Science, Scopus, and EMBASE identified 45 relevant studies which were used for qualitative assessment. Out of these, 29 studies were used for qualitative and quantitative assessment and allowed to identify a miRNA panel able to detect HFrEF and HFpEF with areas under the curve (AUC) of 0.86 and 0.79, respectively. A panel of eight miRNAs (hsa-miR-18b-3p, hsa-miR-21-5p, hsa-miR-22-3p, hsa-miR-92b-3p, hsa-miR-129-5p, hsa-miR-320a-5p, hsa-miR-423-5p, and hsa-miR-675-5p) detected HFrEF cases with a sensitivity of 0.85, specificity of 0.88 and AUC of 0.91. A panel of seven miRNAs (hsa-miR-19b-3p, hsa-miR-30c-5p, hsa-miR-206, hsa-miR-221-3p, hsa-miR-328-5p, hsa-miR-375-3p, and hsa-miR-424-5p) identified HFpEF cases with a sensitivity of 0.82 and a specificity of 0.61.

Conclusions Although conventional biomarkers (N-terminal pro-B-type natriuretic peptide and B-type natriuretic peptide) presented a better performance in detecting CHF patients, the results presented here pointed towards specific miRNA panels with potential additive values to circulating natriuretic peptides in the diagnosis of different classes of CHF. Equally important, miRNAs alone showed a reasonable capacity for 'ruling out' patients with HFrEF or HFpEF. Additional studies with large populations are required to confirm the diagnostic potential of miRNAs for sub-classes of CHF.

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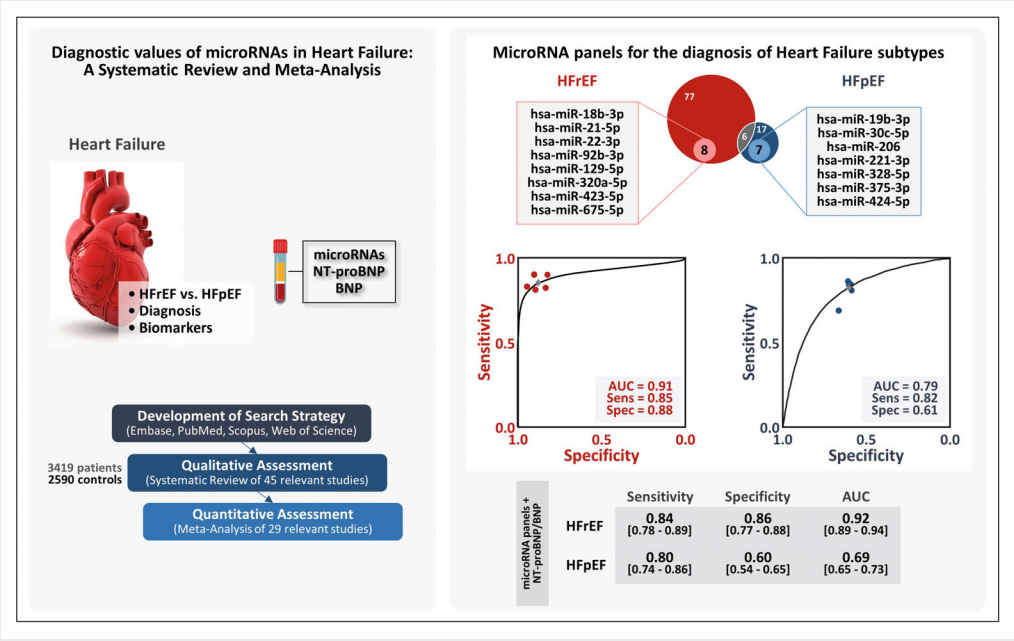
Graphical Abstract



MicroRNA-driven diagnostic biomarkers for heart failure with reduced or preserved ejection fraction. AUC, area under the receiver operating characteristic curve; BNP, B-type natriuretic peptide; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; Sens, sensitivity; Spec, specificity.

Keywords microRNA • Chronic heart failure • Biomarker • HFpEF • Meta-analysis

Introduction

Heart failure (HF) is among the most common causes of hospitalization and death worldwide.[1] In western countries, 1–9 cases per 1000 persons are diagnosed with HF every year,[2] and its prevalence is about 1–2% of the adult population.[3] HF is defined as a clinical syndrome consisting of cardinal symptoms and/or signs due to structural and/or functional abnormalities of the heart resulting in elevated intracardiac pressures and/or inadequate cardiac output at rest and/or during exercise.[3] Based on the European Society of Cardiology (ESC) guidelines,[3] HF is diagnosed using physical examination, natriuretic peptide (NP) level in plasma, and echocardiography examination. Based on ejection fraction (EF) as a critical echocardiographic parameter, HF can be categorized into three subgroups: reduced EF (HFrEF, EF <40%), mid-range EF (HFmrEF, 40% ≤ EF <50%), or preserved EF (HFpEF, EF ≥50%).[3,4] Currently, B-type natriuretic peptide (BNP) and especially the N-terminal (NT)-prohormone BNP (NT-proBNP) are the most accepted and extensively characterized biomarkers for the diagnosis of HF.[5] However, the diagnostic utility of NT-proBNP for HFpEF remains debatable. Ideally, novel biomarkers may help refine the diagnosis of the different subtypes of HF and be of additional value for the diagnosis of HFpEF.

Investigations started more than a decade ago suggested that small RNA molecules, called microRNAs (miRNAs), detected in the circulation, could constitute a family of novel disease biomarkers. miRNAs are small non-coding RNAs of ~22 nucleotides and are the main players in post-transcriptional regulation.[6] Their binding to the 3′ untranslated region (UTR) of messenger RNA (mRNA) molecules leads to translation inhibition or degradation of mRNA.[7] Importantly, miRNAs are detected in a wide range of body fluids (e.g. serum, plasma, whole blood, cerebrospinal fluid, urine, and saliva), with concentrations changing from physiological to pathological conditions.[8] Despite the presence of ribonucleases in circulation, circulating miRNAs are remarkably protected from degradation via binding to high-density lipoprotein, or packaging into extracellular vesicles.[9] The detectability of miRNAs in the bloodstream, their regulation upon organ dysfunction, as well as their functional association with disease progression has led to a plethora of studies investigating the diagnostic value of miRNAs in diverse disease conditions.[9] It is believed that miRNA signatures may provide an accurate molecular fingerprint of patient phenotypes and capture levels of information that could complement

traditional markers. In acute myocardial infarction patients, circulating miRNAs (let-7 g-5p, miR-106a-5p, miR-144-3p, miR-424-5p, and miR-660-5p) predicted cardiovascular disease-related mortality.[10] Importantly, these panels of miRNAs enhanced the performance of the Framingham risk score for assessing cardiovascular disease risk.[10]

To provide a state-of-the-art knowledge of the potential diagnostic value of circulatory miRNAs for chronic HF (CHF), we conducted a systematic review and meta-analysis. We focused our attention on the sensitivity and specificity of miRNAs for the diagnosis of subcategories of HF, i.e. HFrEF and HFpEF. Selected miRNAs were also compared to NT-proBNP, and their specific added value in combination with this conventional biomarker has been investigated. Furthermore, we also used bioinformatics to identify miRNA target genes and downstream signalling pathways affected during HF progression.

Methods

Protocol and registration

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA).[11] The protocol has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) on 16.12.2021 with the CRD42021286660 identification number.

Search strategy

We developed a comprehensive search strategy to retrieve all available studies from Medline (PubMed), Web of Science, Scopus, and EMBASE (Elsevier) for original articles in English from its inception up to September 2021. We used Boolean logic operators for the development of the search strategy. We used all retrieved keywords from the MeSH database and Emtree for 'heart failure' and 'non-coding RNAs'. We also reviewed the references of all relevant studies to avoid missing any publication (manual search). After removing duplicate studies, two independent reviewers (RP and MH) performed a screening based on the titles and abstracts of the articles. Any disagreement between reviewers was discussed and conflicts were resolved by a third researcher (GJJS). The search strategies are reported in the online supplemental material.

Study selection and eligibility criteria

Articles were included in this study when the following criteria were met: (i) case-control or cohort study, retrospective or prospective, cross-sectional study; (ii) patients diagnosed with HF based on guidelines and/or with an expert cardiologist; (iii) controls without any symptoms of HF according to guidelines; (iv) studies evaluating at least the level of one miRNA in serum/plasma; (v) studies restricted to English language. All reviews, systematic reviews, book chapters, and conference papers were excluded.

Data extraction

The elements of information were extracted from each article in two parts: general items (first author's name, publication year, country, age, gender, and study population) and specific items (patient recruitment guideline, experimental method, source of samples, area under the curve [AUC], true positive [TP], true negative [TN], false positive [FP], false negative [FN], sensitivity, specificity). Then, two reviewers (RP and MH) independently collected the data from each study. Subsequently, two of the authors (RP and GJJS) reviewed and discussed the articles to ensure the authenticity of the items extracted. In the final stage, the selected articles were summarized, and data were extracted. Disagreements between the two reviewers were mediated by two other reviewers (GJJS and YM) to achieve consensus.

Quality assessment

To evaluate the methodological quality of included studies, two independent reviewers (RP and MH) performed a risk of bias evaluation of the studies according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) recommended by the Cochrane collaboration.[12,13] QUADAS-2 scale is based on four domains: patients' selection, index test, reference test, and flow and timing. This is based on the reporting data from each article with 'yes' or 'no' questions. Consequently, we categorized each domain to low, intermediate, high, and unclear. Any disagreements were resolved by consensus discussion (YM).

miRNA expression across human tissues

We assessed a web-based repository of human miRNA (TissueAtlas, https://ccb-web.cs.uni-saarland.de/tissueatlas/) to determine the abundance of miRNAs identified in our meta-analysis in a total of 24 tissue biopsies of different organs from individuals collected post-mortem. To define the distribution of miRNAs, we utilized a tissue specificity index, described in Ludwig *et al.*[14]

miRNA-target prediction and network analysis

The miRNA target prediction was carried out by using the online tool MIENTURNET (MicroRNA ENrichment TURned NETwork; http://userver.bio.uniroma1.it/apps/mienturnet/), followed by pathway analysis for KEGG, Gene Ontology (GO), REACTOME, WikiPathways, and Disease Ontology analysis.[15] The miRTarBase database was used to assess experimentally validated miRNA/target interactions.[16] Additionally, a protein–protein interaction (PPI) analysis was done using STRING (https://string-db.org/)[17] and Metascape (http://metascape.org/gp/index.html#/main/step1)[18] for visualization of the miRNA predicted targets.

Statistical analysis

We calculated the TP, FN, FP, and TN from reported sensitivity and specificity defined at the considered thresholds from each study. We calculated the absolute and relative sensitivities and the specificities of the tests for the detection of CHF, followed by subgroup analysis in HFpEF and HFrEF at various thresholds and computed sensitivity [TP/(TP + TN)], specificity [TN/(TN + FP)], diagnostic odds ratio (DOR), and likelihood ratios (LRs), separately for each biomarker. To preserve the two-dimensional nature of original data, we used a bivariate random-effects model analysis (BRMA) followed by a hierarchical summary receiver operating characteristic (HSROC) model.

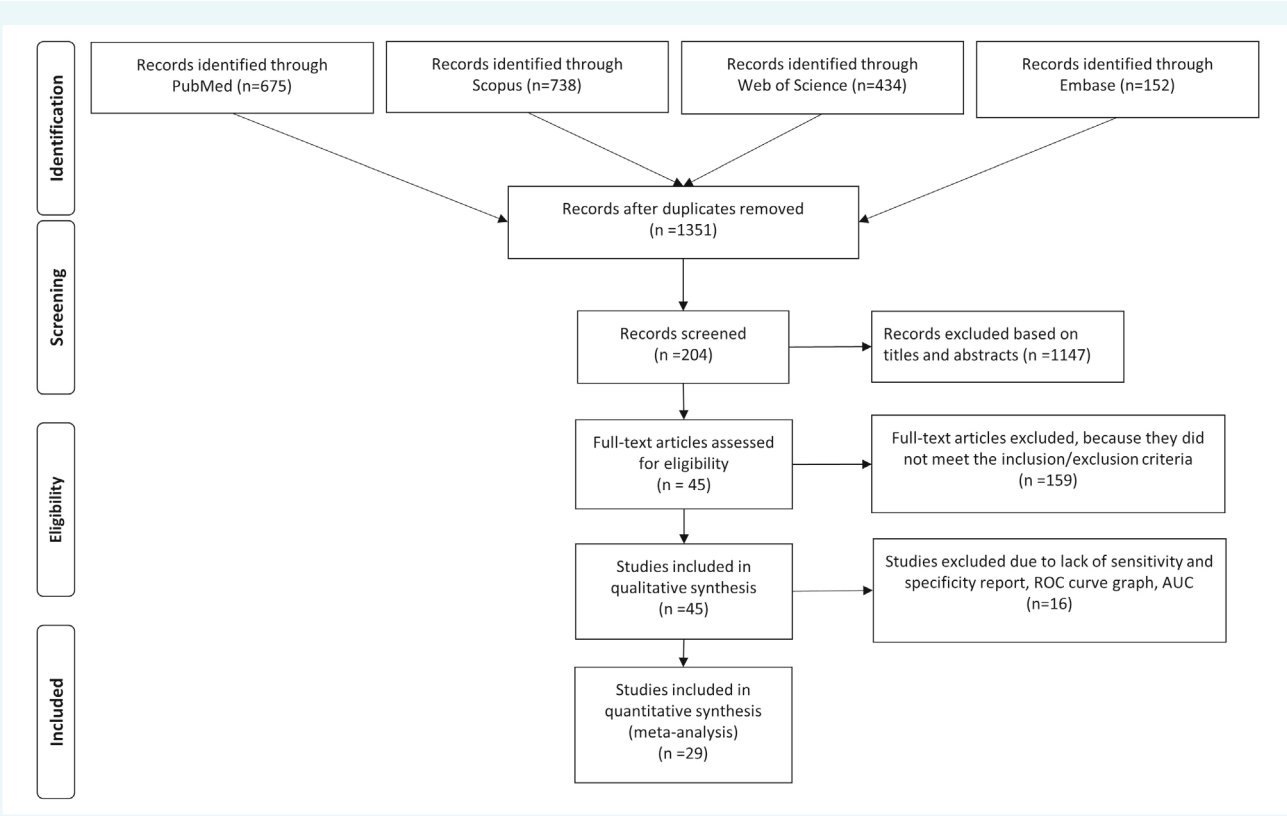


Figure 1 Flow diagram of study selection. AUC, area under the curve; ROC, receiver operating characteristic.

Inter-study heterogeneity was assessed using *Q* test and inconsistency index (I^2). A potential source of heterogeneity was assessed using meta-regression. The predictive accuracy of miRNA-based models and the addition of the miRNA-based prediction model to NT-proBNP was assessed by calculating the AUC. Publication bias was measured by Deek's funnel plot and a *p*-value <0.1 was considered in Deek's asymmetry test for indicative of publication bias.[19–21] All statistical models were programmed and fitted using MIDAS module in STATA (version 17). Z-test was performed to compare the AUCs and variance analysis using SPSS (version 28.0.1.1).

Results

Study selection process and characteristics

Figure 1 depicts the selection process based on PRISMA flow diagram. A total of 1999 studies were identified using our comprehensive search strategy using the following databases: PubMed ($n = 675$), Scopus ($n = 738$), Web of Science ($n = 434$), and EMBASE ($n = 152$). After removal of duplicate studies, 1351 articles entered the 'title' and 'abstract' screening step. We removed 1147 irrelevant studies, reviews, book chapters, or conferences/abstracts. We then retrieved the full text of the remaining 204 articles and assessed their eligibility to enter the process of qualitative and quantitative analysis. Based on inclusion/exclusion criteria, 45 studies became eligible for qualitative analysis (systematic review), and 29 out of the 45 eligible studies reported the ROC analysis data and were included in the quantitative (meta-analysis) assessments.

Overall, 3419 diagnosed CHF patients entered the current study, ranging in age from 47 to 81 years old, as well as 2590 control individuals, either healthy or non-CHF patients. Most of the studies ($n = 24$) measured miRNAs in serum samples. In addition, one of the studies collected blood from two different locations from the same patients (vein and coronary sinus); 24% of studies used TaqMan assay to measure expression levels of miRNAs; 42% and 29% of studies were conducted in China and Europe between 2010 and 2021, respectively (*Table 1* and online supplementary *Table Appendix S1*).[22–66]

Quality assessment of included studies using the QUADAS-2 tool

Concerning patient selection, only 18% of studies showed a low risk of bias; 29% of studies did not have a case-control design and 29% had inappropriate exclusion criteria. In the case of the index test domain, there was a high risk of bias mostly due to failure to use pre-specified cut-offs for miRNAs expression levels. A total of 47% of studies were classified as intermediate risk of bias in reference tests mostly because of different reference standard usage. Of note, 82% of studies suffered from intermediate risk of bias level in the flow and timing domains which is mainly due to the unclear status of the interval between reference test and index

Table 1 Characteristics of the 45 included studies

First author	Year	Country	Disease type	Number		Age (mean, years)	Sample type	Method	Reference test and guidelines	Ref
				Patient (F%)	Control (F%)					
Adachi	2010	Japan	CHF_II	9 (22%)	10 (50%)	61.5	Plasma	TaqMan	NYHA criteria BNP 175 ng/L	22
			CHF_III	6 (33%)		71.6			NYHA criteria BNP 674 ng/L	
Akat	2014	USA	HF	24 (8%)	13 (31%)	NP	Plasma	Microarray	NC	23
Al-Hayali	2019	Turkey	DM + CAD	45 (44%) 61.61	45 (51%)	66	Serum	RT-PCR	2 or 3 vascular occlusion	24
Baptista	2018	Portugal	PH PAH	88 (68%) 64 (72%)	34 (56%)	53.5 47	Plasma	RT-PCR	2015 PH	25
Beaumont	2017	Spain	Aortic stenosis	13 (62%)	15 (80%)	72	Serum	TaqMan	Framingham criteria	26
Ben-Zvi	2020	Israel	HFrEF	39 (36%)	21 (38%)	69.5	Serum	TaqMan	ESC-HFA/AHA	27
Cakmak	2015	Turkey	HFrEF	42 (29%)	15 (47%)	56.57	Serum	Microarray	2009 ACCF/AHA	28
Chang	2021	China	CHF	92 (42%)	80 (39%)	67.4	Serum	RT-PCR	2013 ACCF/AHA 2016 ESC	29
Chen	2020	China	CHF	30 (43%)	30 (47%)	60.5	Serum	RT-PCR	NYHA criteria	30
Chen	2018	China	HF	33 (36%)	20 (20%)	67	Plasma	TaqMan	Expert cardiologist LVEF <40% (HFrEF) LVEF ≥50% (HFpEF)	31
D'Alessandra	2020	Italy	HFrEF	61 (33%)	10 (10%)	62.7	Plasma	TaqMan	NYHA criteria	32
Ellis	2013	New Zealand	HF	44 (30%)	15 (40%)	76.6	Plasma	RT-PCR	Transaortic echocardiography	33
Endo	2013	Japan	CHF	13 (NP)	8 (ND)	NP	PBMCs	RT-PCR	NP	34
Galluzzo	2021	Italy	CHF	30 (13%)	36 (11%)	65	Plasma	RT-PCR	Echocardiography NYHA criteria	35
Gao	2019	China	HFrEF	32 (53%)	16 (56%)	62.4	Plasma	TaqMan	NT-proBNP >125 pg/ml	36
Goldraich	2014	Brazil	HF	17 (24%)	10 (50%)	58	Plasma	TaqMan	LVEF <40% NYHA criteria	37
Goren	2012	Israel	HFrEF	30 (13%)	30 (30%)	64.5	Serum	RT-PCR	LVEF <40% ACC/AHA	38
Han	2020	China	CHF	50 (46%)	30 (47%)	62.8	Serum	TaqMan	2012 ESC	39
He	2017	China	IHF, NIHF	108 (NP)	35 (14%)	62.35	Plasma	RT-PCR	Expert cardiologist NYHA criteria ACC/AHA	40
Ikitimur	2015	Turkey	HF	42 (29%)	15 (40%)	56.2	Serum	Microarray	LVEF ≤40% NYHA criteria 2009 ACC/AHA	41
Jin	2021	China	HFpEF	60 (55%)	30 (57%)	68.3	Serum	RT-PCR	LVEF ≥50% NYHA criteria	42
Jin	2017	China	PH-LHD	35 (51%)	36 (50%)	64	Serum	RT-PCR	2015 PH	43
Lai	2015	Hong Kong	HF	17 (18%)	17 (18%)	54	Tissue	RT-PCR	LVEF <50%	44
Li	2020	China	HFrEF	60 (30%)	60 (30%)	57	Serum	RT-PCR	LVEF <40% (HFrEF) LVEF >50% (HFpEF) ASE	45
			HFpEF	60 (22%)		63				
Li	2021	China	CHF	60 (NP)	60 (NP)	NP	Serum	RT-PCR	NP	46
Li	2016	China	CHF	45 (44%)	45 (47%)	60.8	Plasma	RT-PCR	ACC/AHA	47
Luo	2015	China	CHF	51 (NP)	23 (NP)	NP	Plasma	RT-PCR	NT-proBNP >1000 ng/L Framingham criteria	48

Table 1 (Continued)

First author	Year	Country	Disease type	Number		Age (mean, years)	Sample type	Method	Reference test and guidelines	Ref
				Patient (F%)	Control (F%)					
Marfella	2013	Italy	HF	81 (38%)	60 (35%)	70	Serum	RT-PCR	LVEF <35% QRS duration >120 ms NYHA criteria	49
Marketou	2018	Greece	HFpEF	56 (57%)	42 (69%)	67.2	PBMCs	RT-PCR	LVEF >50% E/e' ≥ 15 or 8 < E/e' < 15 NT-proBNP >220 pg/ml ESH/ESC	50
Marques	2016	Australia	HF	9 (33%)	8 (25%)	50.4	Plasma	RT-PCR	Balloon-tipped thermodilution catheter	51
Olivieri	2012	Italy	CHF	81 (58%)	99 (60%)	81.3	Plasma	RT-PCR	ESC guidelines	52
Scrutinio	2017	Italy	HF	29 (21%)	15 (27%)	60	Serum	RT-PCR	NYHA criteria ESC/ACC/AHA	53
Sun	2020	China	CHF	92 (37%)	60 (37%)	62.5	Serum	RT-PCR	AHA	54
Tijsen	2010	Netherlands	HF	30 (47%)	39 (62%)	68.2	Plasma	RT-PCR	NT-proBNP >1000 ng/L Framingham criteria	55
Tran	2019	USA	HF	31 (23%)	265 (34%)	68	Plasma	RT-PCR	Active surveillance methods by trained study staff	56
Vogel	2013	Germany	HFrEF	53 (8%)	39 (41%)	60	Serum	RT-PCR	LVEF <50% ESC	57
Watson	2015	Ireland	HF	150 (33%)	75 (41%)	72.5	Serum	TaqMan	LVEF ≥50% for HFpEF LVEF <50% for HFrEF NYHA criteria	58
Wong	2015	Singapore New Zealand	HF	60 (NP)	30 (NP)	64.73	Whole blood	TaqMan	LVEF ≥50% as HFpEF LVEF ≤40% as HFrEF ASE	59
Wong	2019	Singapore New Zealand	HF	338 (32%) 207 (32%) 358 (32%)	208 (40%) 241 (40%) 358 (33%)	64.6 60.5 74	Plasma	RT-PCR	LVEF ≥50% as HFpEF, LVEF ≤40% as HFrEF, ASE	60
Wu	2018	China	HFrEF	28 (36%)	30 (27%)	59.7	Serum	NC	LVEF ≤40% ESC	61
Wu	2021	China	HFrEF	144 (51%)	48 (52%)	59	Serum	RT-PCR	NYHA criteria 2016 ESC	62
Zhang	2017	China	HFrEF	80 (29%)	40 (50%)	59.58	Serum (vein and coronary sinus)	RT-PCR	LVEF <50% 2012 ESC	63
Zhang	2020	China	HF	200 (25%)	100 (27%)	60.5	Serum	TaqMan	LVEF >50% as HFpEF LVEF <40% as HFrEF ASE	64
Zhang	2021	China	CHF	70 (47%)	62 (45%)	67.24	Serum	RT-PCR	LVEF <40% NYHA criteria 2013 ACCF/AHA 2016 ESC	65
Zhao	2013	China	CHF	22 (23%)	18 (61%)	59.1	Serum	RT-PCR	proBNP ≥1000 ng/L NYHA Framingham	66

ACCF, American College of Cardiology Foundation; AHA, American Heart Association; ASE, American Society of Echocardiography; BNP, B-type natriuretic peptide; CAD, coronary artery disease; CHF, chronic heart failure; DM, diabetes mellitus; ESC, European Society of Cardiology; ESH, European Society of Hypertension; F%, female%; HF, heart failure; HFA, Heart Failure Association; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IHF, idiopathic heart failure; LHD, left heart disease; LVEF, left ventricular ejection fraction; NC, not clear; NIHF, non-idiopathic heart failure; NP, not provided; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; NYHA, New York Heart Association; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; RT-PCR, real-time polymerase chain reaction.

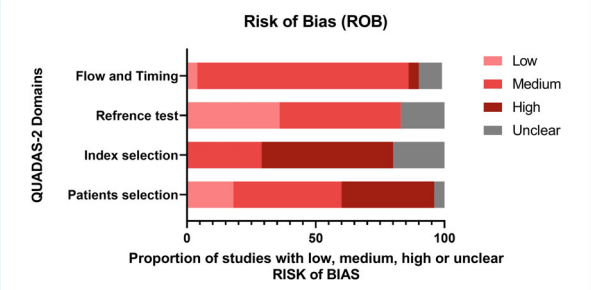


Figure 2 Risk of bias assessments of the included studies using QUADS-2 tool.

test (*Figure* 2). In the case of applicability, 44% of studies had an intermediate risk due to inappropriate exclusions criteria (online supplementary *Figure Appendix S1*).

Potential value of miRNAs to detect chronic heart failure

The diagnostic meta-analysis was conducted with the data retrieved from 29 studies, comprising 2468 CHF patients and 1838 non-CHF controls. The diagnostic performance of conventional biomarkers (NT-proBNP + BNP) for CHF presented a sensitivity value of 0.94 (95% confidence interval [CI] 0.90–0.96), specificity of 0.97 (95% CI 0.91–0.99) and AUC of 0.98 (95% CI 0.96–0.99) (*Table* 2). Remarkably, the pooled sensitivity and specificity of miRNAs to detect CHF were 0.80 (95% CI 0.77–0.84) and 0.79 (95% CI 0.73–0.85), respectively. The heterogeneity analysis using I^2 showed significantly higher heterogeneity in both pooled sensitivity ($I^2 = 83.72$) and pooled specificity ($I^2 = 91.66$). Overall, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and DOR were 3.9 (95% CI 2.9–5.2), 0.25 (95% CI 0.20–0.30), and 16 (95% CI 10–25), respectively. The SROC analysis for the pooled miRNAs showed an AUC of 0.86 (95% CI 0.83–0.89) for the diagnosis of CHF (*Table* 2). Of note, Deek's funnel plot showed symmetrical distribution which suggests no publication bias (online supplementary *Figure S2*). We then performed meta-regression analysis based on the age of patients, sample source, and method of analysis to find the potential source of heterogeneity. In this regard, our results showed lower heterogeneity when using the TaqMan method in both sensitivity and specificity (online supplementary *Table S2*). When conventional biomarkers (NT-proBNP + BNP) were combined with miRNAs, our model showed a pooled sensitivity of 0.84 (95% CI 0.81–0.88) and pooled specificity of 0.83 (95% CI 0.77–0.87). The DOR and SROC analysis showed values of 26 (95% CI 16–45) and 0.90 (95% CI 0.87–0.93), respectively.

miRNAs showed high potential for diagnosis of HFrEF and HFpEF

We then divided the studies into two subgroups, HFrEF and HFpEF, and compared these disease groups against its corresponding non-HF or non-HFpEF controls, respectively. Among the 29 studies, 17 investigated HFrEF and four HFpEF patients, including patients diagnosed with pulmonary hypertension and aortic stenosis. We excluded eight studies that did not differentiate the different HF forms. The pooled sensitivity analysis of miRNA biomarkers showed a value of 0.81 (95% CI 0.73–0.86) for HFrEF and 0.82 (95% CI 0.78–0.86) for HFpEF subgroups. The pooled specificity was 0.79 (95% CI 0.75–0.84) for HFrEF and 0.61 (95% CI 0.55–0.67) for HFpEF. The I^2 analysis showed significantly higher heterogeneity in HFrEF for both sensitivity ($I^2 = 91.50$) and specificity ($I^2 = 80.60$) (*Figure 3A*). AUCs were 0.86 (95% CI 0.83–0.89) for HFrEF and 0.79 (95% CI 0.76–0.83) for HFpEF (*Figure 3B,E*). Meta-regression analysis showed that the detection method of

Table 2 Diagnostic value of microRNAs for the detection of chronic heart failure

	Sensitivity		Specificity		AUC (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)
	Sensitivity (95% CI)	Heterogeneity	Specificity (95% CI)	Heterogeneity				
NT-proBNP + BNP	0.94 (0.90–0.96)	$Q = 49.69$ df = 11.00 $p < 0.01$ $I^2 = 77.86$ (65.72–90.01)	0.97 (0.91–0.99)	$Q = 154.20$ df = 11.00 $p < 0.01$ $I^2 = 92.87$ (90.02–95.71)	0.98 (0.96–0.99)	32.7 (9.4–113.4)	0.06 (0.04–0.11)	508 (111–2324
miRNAs	0.80 (0.77–0.84)	$Q = 325.48$ df = 53.00 $p < 0.001$ $I^2 = 83.72$ (79.92–87.51)	0.79 (0.73–0.85)	$Q = 635.84$ df = 53.00 $p < 0.001$ $I^2 = 91.66$ (90.07–93.26)	0.86 (0.83–0.89)	3.9 (2.9–5.2)	0.25 (0.20–0.30)	16 (10–25)
Combination of miRNAs + [NT-proBNP + BNP]	0.84 (0.81–0.88)	$Q = 500.70$ df = 62.00 $p < 0.001$ $I^2 = 87.62$ (85.15–90.08)	0.83 (0.77–0.87)	$Q = 897.65$ df = 62.00 $p < 0.001$ $I^2 = 93.09$ (91.94–94.25)	0.90 (0.87–0.93)	4.8 (3.8–6.1)	0.18 (0.13–0.26)	26 (16–45)

AUC, area under the curve; BNP, B-type natriuretic peptide; CI, confidence interval; df, degree of freedom; DOR, diagnostic odds ratio; I^2, *I*-square; miRNA, microRNA; NLR, negative likelihood ratio; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; PLR, positive likelihood ratio; *Q*, Chi-square.

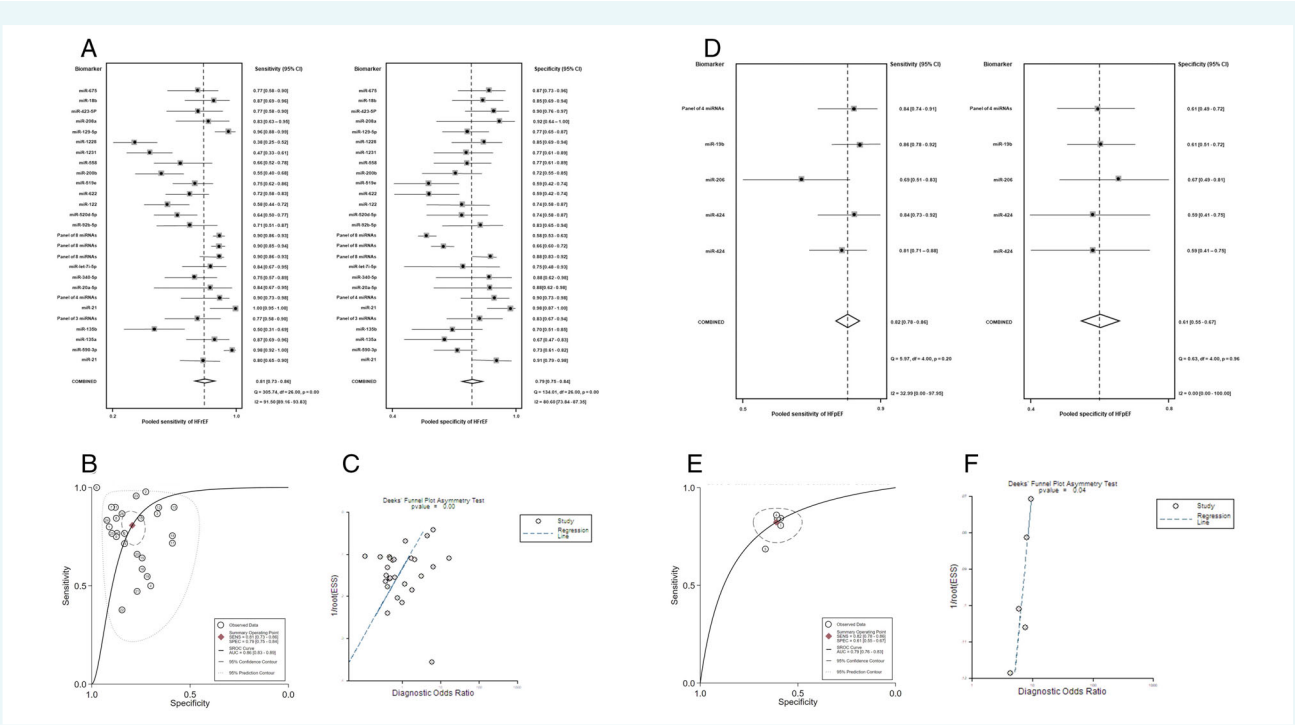


Figure 3 Sensitivity and specificity of circulating miRNAs for both heart failure with reduced (HFrEF) and preserved ejection fraction (HFpEF) patients. (*A*) Forest plot of pooled sensitivity and specificity of microRNAs (miRNAs) for HFrEF. (*B*) Receiver operating characteristic (ROC) curve of miRNAs extracted from HFrEF studies. (*C*) Deek's funnel plot for HFrEF studies. (*D*) Forest plot of pooled sensitivity and specificity of miRNAs for HFpEF. (*E*) ROC curve of miRNAs extracted from HFpEF studies. (*F*) Deek's funnel plot for HFpEF studies. SROC, summary receiver operating characteristic.

the miRNAs could be a potential source of heterogeneity, with TaqMan detection presenting the lowest heterogeneity (online supplementary *Table S2*). Moreover, our data showed relatively lower heterogeneity in studies with patients older than 65 years in the HFrEF group (online supplementary *Table S2*). Deek's funnel plot analysis showed publication bias for both HFrEF and HFpEF studies which can lead to an overestimation of the diagnostic performance of biomarkers (*Figure 3C,F*).

In addition, the combination of conventional biomarkers (NT-proBNP and BNP) with miRNAs led to a sensitivity of 0.84 (95% CI 0.78–0.89) and a specificity of 0.86 (95% CI 0.77–0.88) for HFrEF. SROC analysis, an indicator of diagnostic accuracy, showed an improvement of AUC up to 0.92 (95% CI 0.89–0.94) for HFrEF (*Table 3*). Also, combination of miRNAs with conventional biomarkers (NT-proBNP and BNP) showed a sensitivity of 0.80 (95% CI 0.74–0.86), a specificity of 0.60 (95% CI 0.54–0.65) and an AUC of 0.69 (95% CI 0.65–0.73).

Panels of miRNAs with high diagnostic potential for HFrEF and HFpEF

Based on the data extracted from all the manuscripts included in this meta-analysis, we were able to list a total of 83 miRNAs for HErEF and 23 miRNAs for HFpEF, with an overlap of six miRNAs (online supplementary *Figure S4*). We then used the ROC cut-off strategy to select only the high-performance miRNAs that can assist the identification and stratification of HFrEF and HFpEF patients. In regard with the diagnosis of HFrEF, a panel of eight miRNAs (hsa-miR-18b-3p, hsa-miR-129-5p, hsa-miR-423-5p, hsa-miR-320a-5p, hsa-miR-22-3p, hsa-miR-92b-3p, hsa-miR-675-5p, and hsa-miR-21-5p) was identified (*Table 4*). To obtain this panel, we used sensitivity and specificity values of pooled miRNAs as a cut-off in the SROC curve and selected the miRNAs presenting higher sensitivity and specificity. These miRNAs were found upregulated in the circulation (both in plasma and serum). The AUC of each miRNA ranges from 0.86 and 0.944 (*Table 4*). Sensitivity of this 8-miR panel was 0.85 (95% CI 0.79–0.89), specificity was 0.88 (95% CI 0.82–0.92), and AUC was 0.91 (online supplementary *Figure S3*). For HFpEF, a panel of seven miRNAs (hsa-miR-424-5p, hsa-miR-206, hsa-miR-328-5p, hsa-miR-30c-5p, hsa-miR-221-3p, hsa-miR-375-3p and hsa-miR-19b-3p) was retrieved from included studies with sensitivity of 0.82 (95% CI 0.78–0.86), specificity of 0.61 (95% CI 0.55–0.67), and AUC of 0.79 (95% CI 0.76–0.83) for diagnosis of HFpEF either from HFrEF or non-HF controls (*Figure 3D,E*, and *Table 3*).

miRNA target prediction, network analysis, and tissue distribution

In order to better understand the processes associated with these miRNA panels, we performed a target prediction analysis using the MIENTURNET algorithm that combines the TargetScan tool for

Table 3 Diagnostic values of biomarkers (microRNAs + [NT-proBNP + BNP]) for both heart failure with reduced and preserved ejection fraction

	Sensitivity		Specificity		AUC (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)
	Sensitivity (95% CI)	Heterogeneity	Specificity (95% CI)	Heterogeneity				
HFrEF	0.84 (0.78–0.89)	$Q = 397.48$ df = 33.00 $p < 0.001$ $I^2 = 91.70$ (89.69–93.71)	0.86 (0.77–0.88)	$Q = 376.38$ df = 33.00 $p < 0.001$ $I^2 = 91.23$ (89.07–93.39)	0.92 (0.89–0.94)	6.1 (4.1–9.0)	0.18 (0.13–0.26)	33 (17–65)
HFpEF	0.80 (0.74–0.86)	$Q = 24.18$ df = 7.00 $p < 0.001$ $I^2 = 71.06$ (50.05–92.66)	0.60 (0.54–0.65)	$Q = 7.46$ df = 7.00 $p = 0.38$ $I^2 = 6.18$ (0.00–100.00)	0.69 (0.65–0.73)	2.0 (1.7–2.4)	0.33 (0.23–0.47)	6 (4–10)

AUC, area under the curve; BNP, B-type natriuretic peptide; CI, confidence interval; df, degree of freedom; DOR, diagnostic odds ratio; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; I^2, *I*-square; NLR, negative likelihood ratio; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; PLR, positive likelihood ratio; *Q*, Chi-square.

Table 4 Characteristics of panels of microRNAs for the diagnosis for both heart failure with reduced and preserved ejection fraction

miRNAs	miRBase ID	HF type	Expression level	Sensitivity	Specificity	AUC	Ref
hsa-miR-18b-3p	MIMAT0004751	HFrEF	Up	0.87[a]	0.85[a]	0.86	55
hsa-miR-129-5p	MIMAT0000242	HFrEF	Up	0.957	0.774	0.921	65
hsa-miR-22-3p	MIMAT0004748	HFrEF	Up				
hsa-miR-92b-3p	MIMAT0037311	HFrEF	Up	0.9[a]	0.9[a]	0.9	38
hsa-miR-320a-5p	MIMAT0000077	HFrEF	Up				
hsa-miR-423-5p	MIMAT0003218	HFrEF	Up				
hsa-miR-675-5p	MIMAT0004284	HFrEF	Up	0.77[a]	0.87[a]	0.89	55
hsa-miR-21-5p	MIMAT0000076	HFrEF	Up	0.800	0.911	0.944	24
hsa-miR-424-5p	MIMAT0001341	HFpEF	Up	0.847	0.587	0.709	25
hsa-miR-206	MIMAT0000462	HFpEF	Down	0.686	0.658	0.720	43
hsa-miR-30c-5p	MIMAT0026486	HFpEF	Down				
hsa-miR-221-3p	MIMAT0000244	HFpEF	Down	0.834[a]	0.61[a]	0.796	58
hsa-miR-328-5p	MIMAT0000278	HFpEF	Down				
hsa-miR-375-3p	MIMAT0000728	HFpEF	Down				
hsa-miR-19b-3p	MIMAT0000074	HFpEF	Down	0.859[a]	0.609[a]	–	64

Up, up-regulation; Down, down-regulation, the comparator is non-HF or healthy controls.
AUC, area under the curve; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; miRNA, microRNA.
[a]Estimated values are derived from the receiver operating characteristic curve presented in the corresponding study.

sequence-based miRNA target predictions with the miRTarBase tool for validated interactions. A total of 273 target genes were identified for the 8-miRNA HFrEF panel, and 225 targets for the 7-miRNA HFpEF panel, with an overlap of 36 genes (online supplementary *Figure S4*). This analysis was followed by PPI and pathway/network analysis to garner insights into specific and/or common biological processes associated with the identified miRNAs minimal panels. The PPI analysis identified SP1, PTEN, and HMGB1 presenting the highest degree of centrality among predicted targets of the eight HFrEF miRNAs. SP1 and HMGB1 were predicted targets of hsa-miR-21-5p, hsa-miR-22-3p and hsa-miR-129-5p, and PTEN was a predicted target of hsa-miR-21-5p, hsa-miR-22-3p and hsa-miR-92b-3p. Interestingly, these miRNAs affect the transforming growth factor (TGF)-β pathway, syndecan signalling, and cardiomyocyte hypertrophy which are well-known pathways associated with the pathogenesis of HF (*Figure 4A–C*, and online supplementary *Figures S5* and *S6*). Regarding miRNAs associated with HFpEF (hsa-miR-424-5p, hsa-miR-206, hsa-miR-328-5p, hsa-miR-30c-5p, hsa-miR-221-3p, hsa-miR-375-3p, and hsa-miR-19b-3p), we identified ESR1 and TP53 target genes as the most central genes in our miRNA-target network analysis. The miRNAs found enriched in the GO term 'cardiac myocyte' are also associated with the TGF-β pathway, RUNX2 pathways, and apoptosis (*Figure 4D–F*, and online supplementary *Figures S7* and *S8*).

Last, we used the TissueAtlas repository database to determine the expression levels of the eight miRNAs (hsa-miR-18b-3p, hsa-miR-129-5p, hsa-miR-423-5p, hsa-miR-320a-5p, hsa-miR-22-3p, hsa-miR-92b-3p, hsa-miR-675-5p, and hsa-miR-21-5p) identified for diagnosis of HFrEF and seven miRNAs (hsa-miR-424-5p, and hsa-miR-206, hsa-miR-328-5p, hsa-miR-30 c-5p, hsa-miR-221-3p, hsa-miR-375-3p, hsa-miR-19b-3p) for diagnosis of HFpEF in the current study (online supplementary *Figure S9*). Of note, no expression data were available for two of those miRNAs (hsa-miR-328-5p and hsa-miR-675-5p). Overall,

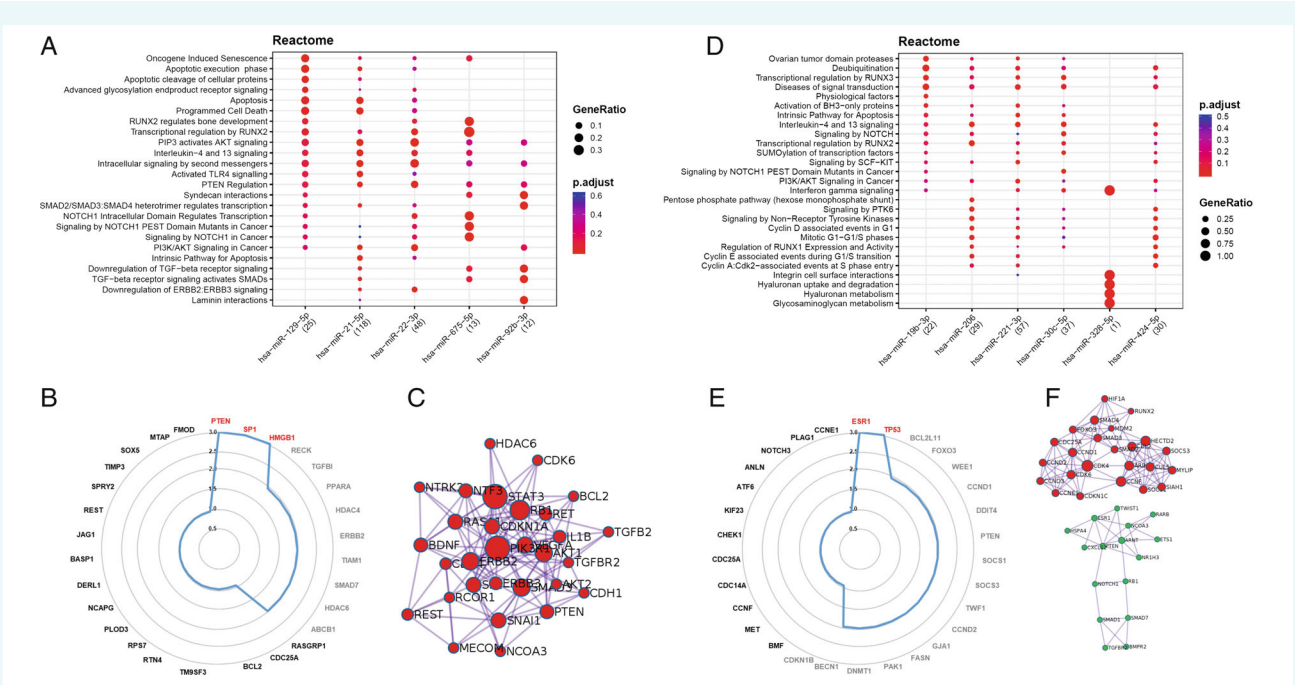


Figure 4 MicroRNA (miRNA) target prediction and pathway analysis for both heart failure with reduced (HFrEF) and preserved ejection fraction (HFpEF). (*A*) Dot blot of REACTOME for HFrEF. (*B*) Identified genes with higher degrees of centrality in related pathways for HFrEF miRNAs. (*C*) Protein–protein interaction networks for identified miRNAs of HFrEF. (*D*) Dot blot of pathways retrieved from REACTOME for HFpEF. (*E*) Identified genes with higher degrees of centrality in related pathways for HFpEF miRNAs. (*F*) Protein–protein interaction networks for identified miRNAs of HFpEF.

all miRNAs presented ubiquitous expression patterns across the interrogated tissues regardless of the magnitude of expression. While hsa-miR-92b-3p and hsa-miR-375-3p presented low levels of expression, hsa-miR-21-5p and hsa-miR-22-3p were highly abundant in all tissues, and hsa-miR-206 was found enriched in muscle tissue (online supplementary *Figure S9*).

Discussion

To our knowledge, this is the first meta-analysis to provide a comprehensive overview of the diagnostic performance of miRNAs in detecting CHF, as well as present specific panels of miRNAs associated with HFrEF or HFpEF. A total of 45 out of 1999 studies were initially included in the current meta-analysis, comprising 3464 CHF patients and 2666 non-HF or healthy controls analysed. In this population, our results showed a diagnostic performance of miRNAs with an AUC of ~0.86, with approximately 3.9-fold odds of a positive outcome of the index test (PLR), and only 25% of negative outcomes (NLR). Additionally, the combination of miRNAs with conventional biomarkers (e.g. NT-proBNP and BNP) improved both PLR and NLR in CHF. In regard to the CHF forms, we performed the HSROC curve and selected a minimal panel of eight miRNAs for HFrEF (hsa-miR-18b-3p, hsa-miR-21-5p, hsa-miR-22-3p, hsa-miR-92b-3p, hsa-miR-129-5p, hsa-miR-320a-5p, hsa-miR-423-5p, and hsa-miR-675-5p) which showed a sensitivity of 0.85, a specificity of 0.88 and an AUC of 0.91. Similarly, seven miRNAs (hsa-miR-19b-3p, hsa-miR-30c-5p, hsa-miR-206, hsa-miR-221-3p, hsa-miR-328-5p, hsa-miR-375-3p, and hsa-miR-424-5p) were identified with a sensitivity of 0.82 and a specificity of 0.61 for detection of HFpEF (*Graphical Abstract*). Interestingly, miRNAs alone showed a good capacity for 'ruling out' patients with HFrEF or HFpEF.

However, higher heterogeneity was observed between studies. Heterogeneity indicates between-study variation which is usually higher in the diagnostic meta-analysis due to variation in the protocol of conduction, sampling, reporting, etc.[20] In this study, we observed significant heterogeneity, as expected. Variations in the protocol of RNA isolation, inclusion/exclusion criteria of sampling, case-control design, age of patients, source of samples, different miRNA expression cut-offs in various studies, and method of analysis are factors that can be considered a potential source of heterogeneity. Based on the QUADS-2 assessment, the studies included in the current meta-analysis were suffering from patient selection domain mainly because of inappropriate inclusion/exclusion criteria and index test due to not pre-defined cut-offs. Patient selection is an essential step in case-control study design. Therefore, inappropriate inclusion/exclusion criteria lead to a different population of patients enrolled that will not reflect the reality in clinics. Additionally, controls may not necessarily reflect the suspicious population with HF.[5] Corroborating our findings, Henkens *et al.*[12] performed a comprehensive risk of bias assessment of different diagnostic biomarkers for HFpEF patients using the QUADS-2 tool. The authors concluded that most of the studies on the high-risk zone presented an inappropriate inclusion/exclusion criteria, lack

of pre-defined thresholds, or lack of information on the interval between index test and reference test.[12] Overall, we have to take this into account when performing the diagnostic study; otherwise, the results would be overestimated for the diagnostic performance of biomarkers and cannot be used in clinical practice. Based on approaches to observed heterogeneity described by Petitti,[67] we performed a subgroup analysis to find possible source of heterogeneity and identified the source of the blood sample collection (serum or plasma), the miRNA method of analysis used (TaqMan or real time-polymerase chain reaction assays), and the age of patients (<65 or ≥65) as potential sources of heterogeneity. Our data have shown that the TaqMan assay, as a method of miRNA expression detection, reduced the heterogeneity significantly in CHF and HFrEF. Quantitative polymerase chain reaction is known as the gold standard method for quantification of nucleic acids including miRNAs.[68] However, there is still no clinically standard setup for the use of it in diagnostic labs as a method for biomarker quantification.[69] Similarly, we found that patients younger than 65 years old presented lower heterogeneity in the HFrEF subgroup. Of note, due to the small number of studies on HFpEF patients, we were unable to run a meta-regression analysis to find the potential source of heterogeneity for the clinical phenotype of HF. Another important source of heterogeneity observed was the pooling of all miRNAs to estimate the diagnostic accuracy which is due to the lack of enough studies for each miRNA individually. Our study pointed toward a good diagnostic performance of circulating miRNAs in the detection of HFrEF and HFpEF. For both HFrEF and HFpEF, pooled sensitivity of miRNAs (0.81 for HFrEF and 0.82 for HFpEF) was higher than specificity (0.79 for HFrEF and 0.61 for HFpEF). Of note, the reduced number of studies, accompanied by lack of complete data, comparing directly HFrEF versus HFpEF limited us to perform further sub-analysis discriminating the miRNAs with diagnostic performance between both HF forms.

Natriuretic peptides are established biomarkers of HF.[70] Their use is recommended as diagnostic biomarkers in guidelines with defined cut-offs besides echocardiography.[3] In this regard, we also extracted the data of NPs from the same group of studies and showed an improvement in the diagnostic performance of miRNAs in presence of NPs for both HFrEF and HFpEF. However, the performance of NPs for the diagnosis of HFrEF remains superior to that of miRNAs used in this analysis. Recently, Remmelzwaal *et al.*[5] performed a comprehensive meta-analysis on NP detection of diastolic dysfunction and HFpEF. The authors reported a pooled sensitivity of ∼65% and a pooled specificity of ∼80%. Based on these observations, the authors suggested that NPs could be used in 'ruling out' HFpEF patients. Taken together, miRNAs detection in the circulation has good potential to be used as an additional biomarker for HFpEF, however, more studies with larger populations need to be performed. Likewise, our results showed a combination of NPs and miRNAs can lead to better ruling out in primary care of HFpEF rather than diagnosis.

Our data indicate the cumulative sensitivity and specificity of miRNAs (hsa-miR-18b-3p, hsa-miR-129-5p, hsa-miR-423-5p, hsa-miR-320a-5p, hsa-miR-22-3p, hsa-miR-92b-3p, hsa-miR-675-5p, and hsa-miR-21-5p) collected from available studies for HFrEF patients. In this regard, Tijsen *et al.*[55] identified miR-423-5p as a potential biomarker for the diagnosis of HF in general. Moreover, the authors reported a significant correlation between the upregulation of miR-423-5p, NT-proBNP, and New York Heart Association (NYHA) functional classification.[55] However, it was performed no additional analysis in subpopulations which limits their work for better clinical understanding.[55] On the other hand, Fan *et al.*[71] did not find any correlation between miR-423-5p upregulation, NYHA functional classification, and left ventricular EF in patients with dilated cardiomyopathy which is in contradiction with Tijsen et al.[55] Another meta-analysis conducted by Yan *et al.*,[8] reported miR-423-5p as a potential biomarker for HF in general with higher specificity and sensitivity compared to other pools of different miRNAs. Interestingly, we also found miR-423-5p in our meta-analysis as one important member of a circulating miRNA panel for the detection of HFrEF. Also, evidence from Wu *et al.*[61] showed a negative correlation between left ventricular EF, fractional shortening, and miR-92b-3p circulating levels in HFrEF patients. The authors identified a panel of 11 miRNAs, named 'ThrombomiR', including miR-320a-5p, which is also included in our miRNA panel for HFrEF patients. In the other side, there is a panel of miRNA (hsa-miR-424-5p, hsa-miR-206, hsa-miR-328-5p, hsa-miR-30c-5p, hsa-miR-221-3p, hsa-miR-375-3p, hsa-miR-19b-3p) identified for HFpEF detection. So far, Baptista *et al.*[25] showed a negative correlation between cardiac output and level of miR-424-5p in pulmonary hypertension patients. In a study conducted by Watson *et al.*,[58] it was shown a miRNAs panel (miR-328-5p, miR-30c-5p, miR-221-3p, and miR-375-3p) differentiated HFpEF from HFrEF even better than BNP. Following this study, Zhang *et al.*[64] published a study on the diagnostic performance of miR-19b to differentiate HFpEF from HFrEF. Also, the authors showed higher sensitivity and specificity of miRNA-based biomarkers including miR-19b for discrimination of HFrEF and HFpEF.[64] In this regard, our results confirmed higher pooled diagnostic performance of miRNA-based biomarkers for the detection of HFpEF.

The miRNA detection in different biofluids has been explored as novel diagnostic and prognostic biomarkers for cardiovascular diseases,[10,72] but might also serve as a potential mediator of cell-to-cell communication, due to its paracrine properties.[73] A better understanding of the pathophysiological processes associated with the miRNA panels identified for both HFrEF and HFpEF conditions is fundamental to improve the current diagnostic approaches, and might help to develop novel therapeutic strategies or guide current treatments. Variations in the expression levels of miRNAs associated with the pathophysiological processes of cardiac disease have been revealed for both HFrEF and HFpEF.[58] Another key aspect here is related to the tissue source of those extracellular miRNAs found associated with the HF forms. Therefore, we performed an *in silico* evaluation of miRNA tissue distribution in different human tissues, followed by target prediction and pathway/network analysis. We used the miRTarBase database because it is representative of all validated targets of miRNAs. We found several pathways including the TGF-β, interleukin (IL)-4 and IL-13, apoptosis, mitogen-activated protein kinase (MAPK), phosphatase and tensin homologue deleted on chromosome 10 (PTEN), Wnt and syndecan signalling pathways.

Among others, the TGF-β signalling pathway was found affected by the miRNAs identified in both HFrEF and HFpEF. By binding TGF-β cytokines to their receptors, SMAD molecules are to phosphorylate and translocate to the nucleus as a downstream canonical signaling cascade.[74] Overall, TGF-β signalling pathways lead to cardiac remodelling by cardiomyocyte hypertrophy, fibrosis, and apoptosis.[7] Interestingly, among the pathways predicted to be affected by miR-22-3p, miR-129-5p, miR-206, and miR-675-5p, we found the transcriptional regulation by transcription factor RUNX2. RUNX2 is one of the cofactors of SMAD molecules which lead to cellular proliferation and extracellular matrix protein production.[74] MAPK signalling is another pathway found to be affected by HFpEF/HFrEF circulating miRNAs (miR-21-5p, and miR-22-3p). Consistent with our data, Peterlin et al.[7] found MAPK pathways to be affected by several miRNAs in a systematic review study. MAPK pathway is one of the non-canonical pathways that can be activated by TGF-β and leads to the cardiac response to hypertrophic stimuli including angiotensin II.[75] The activation of the MAPK pathway via TGF-β and miR-21-5p is known to lead to myocardial growth, proliferation, and differentiation of cardiac fibroblasts which can contribute to the formation of fibrotic tissue and stiffness of the heart.[76] Based on our results, syndecan signalling is among the pathways that can be affected by miR-92b-3p, miR-129-5p, and miR-675-5p. Syndecans are one of the families of glycoproteins that are expressed in the myocardium and present a key role in the extracellular matrix remodelling of the heart. Syndecan activity regulated the physiology of cardiac fibroblasts via TGF-β signalling. For instance, syndecan 4 is among the key players in cardiac myofibroblast differentiation and collagen cross-linking. Therefore, the syndecan signalling is important for heart remodelling that can be regulated by miRNAs.[75] Equally importantly, IL-4 and IL-13 are cytokines mainly produced by T-helper 2 cells. Their binding to corresponding receptors leads to the activation Janus kinases (JAK) family of enzymes. It has been reported a high level of IL-4 is associated with the development of hypertension, atherosclerosis, as well as valvular, and non-valvular cardiovascular diseases.[76] It has been revealed that high levels of IL-4 and IL-13 in the circulation lead to the recruitment of bone-marrow-derived fibroblast cells.[77] Of note, both IL-4 and IL-13 have important roles in cardiac remodelling and fibrosis deposition which is regulated by different miRNAs, including miR-21-5p, miR-22-3p, miR-30c-5p, miR-129-5p, miR-206, and miR-221-3p. The Toll-like receptor (TLR)-4 signalling pathway was also found enriched in our study that can be affected by miR-21-5p and miR-129-5p. TLR-4 is a plasma membrane-embedded receptor that has a high expression level in the heart compared with other TLRs. Its binding to endogenous ligands such as HMGB1 activating a cascade that ends up with translocation of nuclear factor-κB to the nucleus and triggers the inflammatory genes. It has been reported the importance of these pathways in the progression of hypertension, myocardial inflammation, and CHF.[78]

Study limitations

Despite the large number of CHF patients included in the current meta-analysis, the following limitations should be considered. Only four out of the 45 studies included more than 100 patients, which considerably limits the interpretation of the results. Moreover, the sensitivity and specificity values were not available in some studies[28,30,31,35,36,46,47,49,51,58,59,64,79–82] and prevented the calculation of TP, FP, TN, and FN. Individual electronic mails were sent to the corresponding authors of those studies and received responses for studies referenced in.[35,49,59,81,82] For those studies that did not respond to our requests, we decided to estimate the sensitivity and specificity reported in the ROC curves. Another limitation was the lack of data on NT-proBNP and BNP measurements, especially for the HFpEF studies. In addition, the case-control design of studies with inappropriate control groups did not fully reflect the clinical scenario of patients suspicious of CHF. Equally important, there is also a reduced number of clinical trials that directly compared circulating miRNAs in HFrEF against HFpEF patients,[58,60,64] and some of these studies[58] lack reporting complete data (i.e. sensitivity and specificity data from the ROC) needed to perform panel discrimination analysis. Therefore, we were not able to perform further sub-analysis to directly discriminate the diagnostic performance of miRNAs between HFrEF and HFpEF. Alternatively, we made use of data available on case-control studies to identify dedicated minimal miRNA panels with high diagnostic performance (eight miRNA panel for HFrEF and seven miRNA panel for HFpEF). Finally, we could not include patients with HFmrEF in the current meta-analysis. This third sub-group of HF patients was initially introduced to the ESC HF guidelines in 2016,[83] and our search strategy did not retrieve any clinical study that has investigated circulating miRNAs levels in HFmrEF patients up to September 2021. Clinical studies and meta-analyses including this distinct HF phenotype should be stimulated in the near future.

Conclusion

This meta-analysis included 45 studies that addressed the diagnostic performance of miRNAs in CHF (as summarized in the *Graphical Abstract*). Although our meta-analysis did not show superior diagnostic performance of miRNAs compared to conventional biomarkers, these studies showed a good performance of miRNAs as biomarkers for the detection of CHF. Regarding the CHF forms, it was identified signature panels comprising eight miRNAs for HFrEF and seven miRNAs for HFpEF can be potentially useful for ruling out the patients. Nevertheless, previous studies showed lower diagnostic performance of NPs for the diagnosis of HFpEF. In contrast, our results indicate a higher potential for the use of miRNAs as novel biomarkers. Indeed, our analysis suggests that miRNAs can improve the diagnostic power of NT-proBNP in HFpEF, a frequent morbid condition where this cardiac hormone has shown reduced sensitivity. Thus, our study suggests a possible role of miRNAs, in combination with NT-proBNP, in the diagnosis of HFpEF. However, to thoroughly evaluate the potential role in the diagnosis of the different forms of HF of the miRNA panel, either alone or in combination with other biomarkers, further studies with larger populations are warranted. Novel miRNAs involved in the evolution of HF, not included in this meta-analysis, are constantly identified and their role as potential novel biomarkers should also be studied in future, well-designed, cross-sectional studies with adequate statistical power and proper control groups.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Acknowledgements

We gratefully acknowledge all the study's participants and patients.

Funding

This study was supported by the Nasjonalforeningen for folkehelsen under grant agreements of No. 19560 (RP) and No. 13173 (AC). Additional support was provided by The South-Eastern Norway Regional Health Authority (GJJS, No. 25674; and AC, No. 2016089 and 2019009).

Conflict of interest: Y.D. holds patents on RNA biomarkers of cardiovascular disease. A.C. serves as a consultant for the advisory board of Madeleine Pharmaceuticals, Inc. All other authors have nothing to disclose.

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