Heliyon 10 (2024) e37929

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Potential diagnostic value of circulating miRNAs in HFrEF and bioinformatics analysis

Zheng Kuai [a,1], Yuanji Ma [b,1], Wei Gao [b,1], Xiaoxue Zhang [b], Xiaoyan Wang [b], Yangli Ye [a], Xiaoyi Zhang [a,**], Jie Yuan [b,*]

[a] Department of Geriatrics, Zhongshan Hospital, Fudan University, Shanghai, China
[b] Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai, China

ARTICLE INFO

Keywords:
microRNA
Heart failure
Heart failure with reduced ejection fraction
Biomarker

ABSTRACT

Background: Few studies have compared the performances of those reported miRNAs as biomarkers for heart failure with reduced EF (HFrEF) in a population at high risk. The purpose of this study is to investigate comprehensively the performance of those miRNAs as biomarkers for HFrEF.

Methods: By using bioinformatics methods, we also examined these miRNAs' target genes and possible signal transduction pathways. We collected serum samples from patients with HFrEF at Zhongshan Hospital. Receiver operating characteristic (ROC) curves were used to evaluate the accuracy of those miRNAs as biomarkers for HFrEF. miRWALK2.0, Gene Ontology (GO) analysis, and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were performed to predict the target genes and pathways of selected miRNAs.

Results: The study included 48 participants, of whom 30 had HFrEF and 18 had hypertension with normal left ventricular ejection fraction (LVEF). MiR-378, miR-195-5p were significantly decreased meanwhile ten miRNAs were remarkably elevated (miR-21-3p, miR-21-5p, miR-106-5p, miR-23a-3p, miR-208a-3p, miR-1-3p, miR-126-5p, miR-133a-3p, miR-133b, miR-223-3p) in the serum of the HFrEF group.

Conclusion: The combination of miR 133a-3p, miR 378, miR 1-3p, miR 106b-5p, and miR 133b has excellent diagnostic performance for HFrEF, and there is a throng of mechanisms and pathways by which regulation of these miRNAs may affect the risk of HFrEF.

1. Introduction

Heart failure (HF) is a complex clinical syndrome characterized by dyspnea or fatigue resulting from impaired ventricular filling, blood ejection, or both. It is a significant public health concern around the world. The World Health Organization (WHO) estimates that 26 million people have heart failure worldwide [1]. In developed countries, approximately 1–2% of adults [2] and up to 4–5% in developing countries [3]. HF is estimated to affect approximately 1.3 % of the Chinese population, with prevalence increasing among people over 65 [4]. There are three categories of HF based on the left ventricular (LV) ejection fraction (EF): heart failure with

* Corresponding author.
** Corresponding author.
E-mail addresses: zhang.xiaoyi@zs-hosptial.sh.cn (X. Zhang), yuan.jie@zs-hosptial.sh.cn (J. Yuan).
[1] These authors contributed equally.

https://doi.org/10.1016/j.heliyon.2024.e37929
Received 15 June 2024; Received in revised form 28 August 2024; Accepted 13 September 2024
Available online 20 September 2024

preserved ejection fraction (HFpEF) (LVEF ≥50 %), heart failure with mid-range ejection fraction (HFmEF) (LVEF 41%–49 %), and heart failure with reduced ejection fraction (HFrEF) (LVEF ≤40 %) [5].

N-terminal fragment of B-type natriuretic peptide (NT-pro-BNP)measurement has proved to be an effective screening tool for patients with various heart diseases, regardless of underlying etiology and the degree of systolic dysfunction of the left ventricle, which is associated with an increased risk of producing cardiovascular events [6].

A subgroup of non-coding RNA species known as microRNAs(miRNAs) is recognized to play critical roles in post-transcriptional regulation of the expression of most protein-coding genes [7]. Functional miRNA studies have revealed that some miRNAs play a role in pathogenic mechanisms leading to heart failure, such as remodeling, hypertrophy, and apoptosis [8,9]. Nevertheless, extracellular circulating miRNAs were first discovered in 2008 [10–12]. In response to these findings, many studies have investigated miRNAs as potential disease biomarkers, including heart failure [13–15].

Compared to traditional biomarkers such as NT-pro-BNP, miRNAs offer several advantages as biomarkers for heart failure. The levels of NT-pro-BNP are more affected by confounding factors such as renal function than miRNAs, which are more stable in circulation [16]. Additionally, miRNAs have high sensitivity and specificity for diagnosing heart failure across a variety of patient demographics, as opposed to NT-pro-BNP which may vary with age and obesity [17]. Furthermore, miRNAs can be directly linked to cellular mechanisms of heart failure, offering insight into disease pathophysiology and progression not available via NT-pro-BNP [18]. As a result, circulating miRNAs provide a option for diagnosing heart failure. However, there is another problem. Even if there are systematic studies on miRNAs and HFrEF, they select healthy individuals who do not have hypertension or diabetes as control group [19]. In any case, screening for HF patients in a population at high risk for a potential diagnostic marker may be more meaningful.

Thus, we aim to this screen out the potential biological markers of potential miRNAs for HFrEF based on the hypertensive population as control while focusing on these miRNAs and studying their target genes and possible signal transduction pathways by bioinformatics methods.

2. Materials and methods

2.1. Patients and control subjects

Patients registered in the China National Heart Failure Registry (CN-HF) had their serum samples taken (Approval No. B2012-140 (2)). The Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital Fudan University (the head unit) is leading the national, multicentered, prospective, and observational registry project known as the CN-HF, which also includes 50 to 100 secondary and tertiary hospitals.

HFrEF is defined as LVEF≤40 %. We recruited 30 HFrEF patients from April 2012 to October 2013. 23 cases were confirmed HF after MI, along with 6 cases of dilated cardiomyopathy, and 1 case of valvular cardiomyopathy. 30 hypertension patients were enrolled in the control group (NoHF). These hypertension patients should meet the following criteria: 1. No signs or symptoms of HF; 2. LVEF ≥50 %; 3. NT-proBNP <100 pg/mL. ALL patients with severe renal failure or comorbid diseases that indicated a life expectancy of less than a year were excluded.

2.2. Clinical assessment

All study patients got a thorough history and physical examination, NYHA functional class assessment, and phlebotomy. Peripheral venous blood samples were tested for full blood count, liver function, serum creatinine(Scr), glycosylated hemoglobin (HbA1C), A2-macroglobulin, β2-microglobulin, NT-proBNP, and lipid profile.

2.3. Doppler echocardiography

In all cases, Doppler echocardiography was performed. In accordance with the recommendations of the European Society of Echocardiography, one of two blinded operators performed the Doppler echocardiographic assessment. All data represent the mean of three measurements on consecutive cardiac cycles. Left atrium diameter (LAD), left ventricular end-diastolic diameter (LVDd), left ventricular end-systolic diameter (LVDs), interventricular septum diameter (IVSd), left ventricular posterior wall diameter (LVPWd), Pulmonary artery pressure (PAP) and left ventricular ejection fraction (LVEF) were measured.

2.4. Serum preparation and RNA isolation

Donor blood samples were collected and placed at room temperature for 2 h. Separation of serum was accomplished by centrifugation at 1000*g* for 10 min. The supernatant was centrifuged to completely remove the cell debris. Total RNA was extracted from 200 μl serum using miRcutes serum/plasma miRNA isolation kit (Tiangen, Beijing, China) according to the manufacturer's instructions. After RNA preparation, each portion was separated and frozen at −80 °C until qPCR could be performed. The purity and concentration of the isolated RNA, including miRNAs, were assessed using a NanoDrop spectrophotometer (Thermo Scientific).The A260/A280 ratio was measured to determine RNA purity, with acceptable values typically ranging between 1.8 and 2.1.RNA concentration was calculated based on the absorbance at 260 nm. The concentration of isolated miRNAs ranged from 20 to 50 ng/μL, depending on the sample type and quality.

Table 1
Characteristics of the enrolled individuals.

Baseline characteristics	NoHF patient (n = 30)	HFrEF patient (n = 30)	P-value
Age	59.03 ± 9.10	66.80 ± 10.32	<0.01
Male (%)	18 (60.0 %)	16(53.3 %)	0.610
Hypertension (%)	30 (100 %)	27(90 %)	0.282
Ischemic heart disease (%)	0 (0 %)	23(76.7 %)	<0.01
Clinical assessment			
CTnT (ng/ml)	0.01 ± 0.01	0.11 ± 0.27	0.055
A2-macroglobulin (mg/L)	1.56 ± 0.66	1.79 ± 0.43	0.116
β2-microglobulin (mg/L)	1.87 ± 0.54	3.79 ± 2.55	<0.01
NT-proBNP (pg./ml)	42.93 ± 27.98	4933.59 ± 6179.34	<0.01
Hb (g/L)	134.60 ± 14.07	130.43 ± 17.45	0.313
WBC (X10^9/L)	6.30 ± 1.52	6.81 ± 2.00	0.269
ALT (U/L)	23.90 ± 11.69	20.33 ± 12.94	0.067
AST (U/L)	20.67 ± 5.29	25.60 ± 1.65	0.058
Alb (g/L)	41.47 ± 2.78	38.00 ± 4.07	<0.001
BUN (mmol/L)	5.78 ± 1.69	9.11 ± 5.67	<0.001
Scr (μmol/L)	76.43 ± 18.12	106.93 ± 53.73	<0.001
eGFR (mL/min/1.73m^2)	88.30 ± 25.78	67.52 ± 25.52	0.031
HbA1C (%)	6.35 ± 1.61	6.15 ± 1.06	0.430
TC (mmol/L)	4.25 ± 0.96	3.80 ± 0.81	0.393
TG (mmol/L)	2.04 ± 1.49	1.34 ± 0.45	0.251
HDL (mmol/L)	1.20 ± 0.28	1.24 ± 0.80	0.451
LDL (mmol/L)	2.58 ± 2.07	2.09 ± 0.73	0.961
Echocardiography			
LAD (mm)	38.65 ± 4.56	46.47 ± 8.48	<0.01
LVDd (mm)	47.00 ± 4.00	65.97 ± 8.35	<0.01
LVDs (mm)	28.77 ± 3.27	54.23 ± 10.53	<0.01
IVSd (mm)	9.35 ± 2.08	8.90 ± 1.71	0.382
LVPWd (mm)	8.85 ± 1.19	8.97 ± 1.43	0.735
PAP (mmHg)	26.54 ± 7.11	42.57 ± 15.53	<0.01
LVEF (%)	68.19 ± 5.07	32.60 ± 5.99	<0.01

2.5. cDNA synthesis and RT-qPCR

The miRNA isolated from blood sample was polyadenylated and reverse transcribed to cDNA in a final volume of 20 μl using miRcute miRNA First-Strand cDNA Synthesis Kit (Tiangen, Beijing, China). Real-time PCR was performed in duplicate using the miRcute Plus miRNA qPCR Detection Kit (SYBR Green) (Tiangen, Beijing, China). The miRNA-specific primer sequences were designed by a biologics company (Tiangen, Beijing, China). Each amplifying reaction was conducted in a final volume of 20 μl containing 1 μl of the cDNA, 0.2 mM of each primer and 1 × miRcute Plus miRNA Premix (with SYBR ROX). Cat number of the primers for microRNA (Tiangen, Beijing, China) are listed in Extended data table 1. MiR-16 was used as endogenous control [20]. Then qRT-PCR was performed in triplicate using an ABI Prism 7500 sequence detection system (Applied Biosystems). The amplification reactions were incubated at 95 °C for 30 min followed by 40 cycles at 94 °C for 15 s, 55 °C for 30 s, and 70 °C for 30 s. At the end of the PCR cycles, melting curve analyses as well as electrophoresis of the products on 3.0 % agarose gels were performed. This was done to validate the specific generation of the expected PCR product. Analyses were conducted in duplicate on each sample. The expression level of the miR-19b was quantified in accordance with the cycle threshold (Ct) method. The relative expression level was calculated as follows: relative microRNA expression $= 2^{-\ (\Delta Ct\ sample - \Delta Ct\ miR-16)}$.

2.6. MicroRNA target gene prediction and pathway analysis

MiRWALK2.0 (http://mirwalk.umm.uni-heidelberg.de) was used for the prediction of miRNA target [21,22]. Venn' s diagram was plotted using Jvenn [23]. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis (mirPath V.3) was applied to identify molecular pathways that were potentially altered. Gene Ontology (GO) analysis (mirPath V.3), which included cellular component (CC), molecular function (MF), and biological process (BP), was performed to analyze the primary function of putative target genes. Bar charts and bubble diagrams, respectively, were used to visualize the results of the KEGG pathway and GO terms enrichment analyses [24].

3. Statistics

Data were expressed in terms of mean ± standard error or standard deviation. For relative gene expression, the mean value of the control group is defined as 1 or 100 %. Comparisons of continuous variables amongst groups were performed by the student's *t*-test for Gaussian data and Mann-Whitney test for non-Gaussian data. For comparison of categorical variable, chi-square test was used. The correlation ship between miRNAs and biochemical indicators in HFrEF patients and NoHF group was evaluated by Pearson correlation coefficient. Diagnostic potential of microRNA in differentiating HFrEF from the controls was conducted by sensitivity, specificity, and

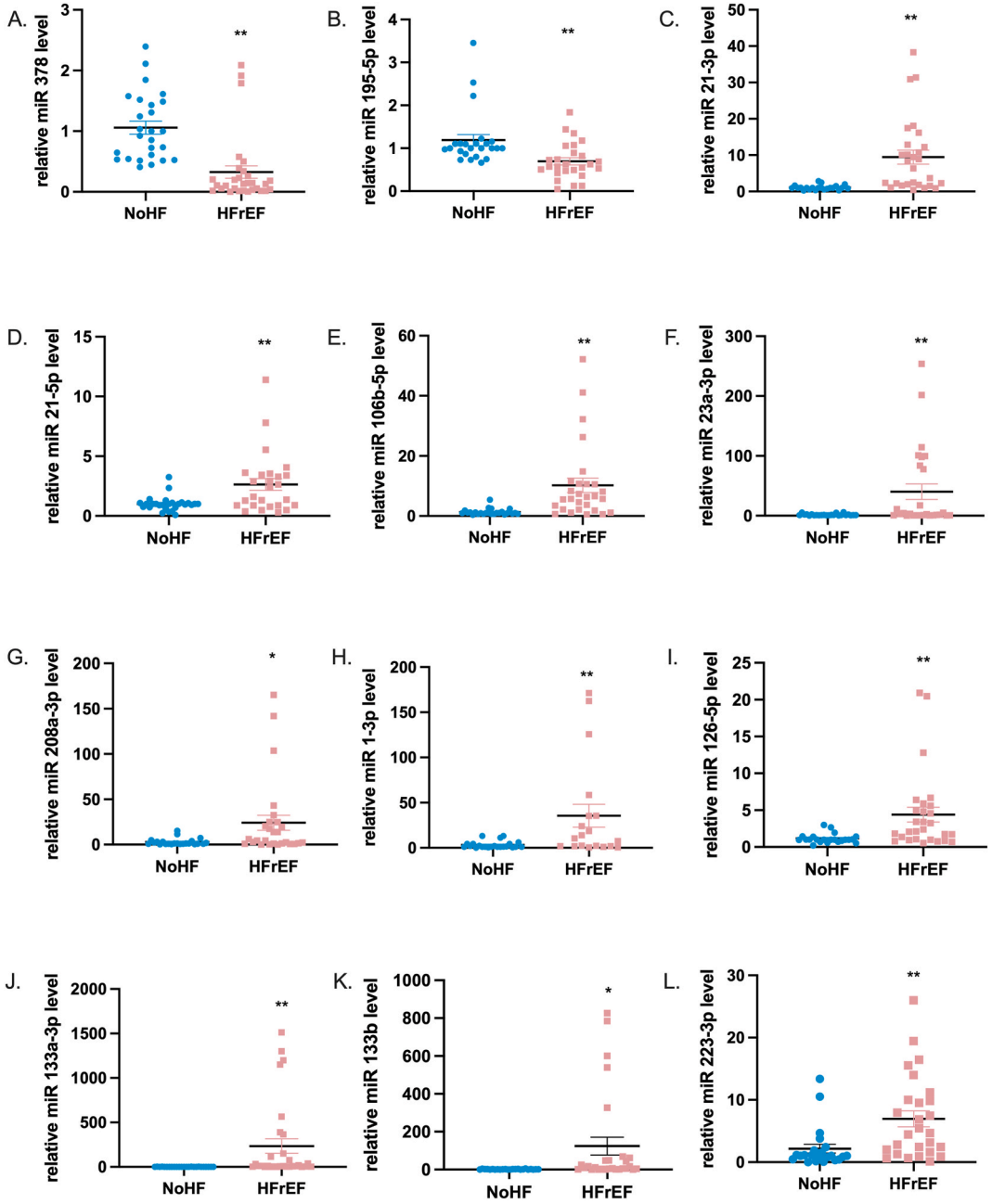

Fig. 1. Comparative expression levels of the miRNA biomarker candidates within the independent validation cohort. The circulating levels of 28 miRNAs were quantified in hypertension and HFrEF study cohorts. **A-B:** miR-378 and miR-195-5p were decreased in the serum of HFrEF patients. **C-L:** miR-21-3p, miR-21-5p, miR-106-5p, miR-23a-3p, miR-208a-3p, miR-1-3p, miR-126-5p, miR-133a-3p, miR-133b, miR-223-3p were increased in the serum of HFrEF patients. Data represent mean ± S.E.M. *P < 0.05, **P < 0.01.

area under the curve analysis (AUC). The cut-off value was calculated using the Jordan index. Statistical analysis was performed with SPSS 26.0 (SPSS Inc., Chicago, Illinois, U.S.A.) and GraphPad Prism 9.20 software (GraphPad Software Inc., La Jolla, CA, U.S.A.). A p value < 0.05 was considered significant.

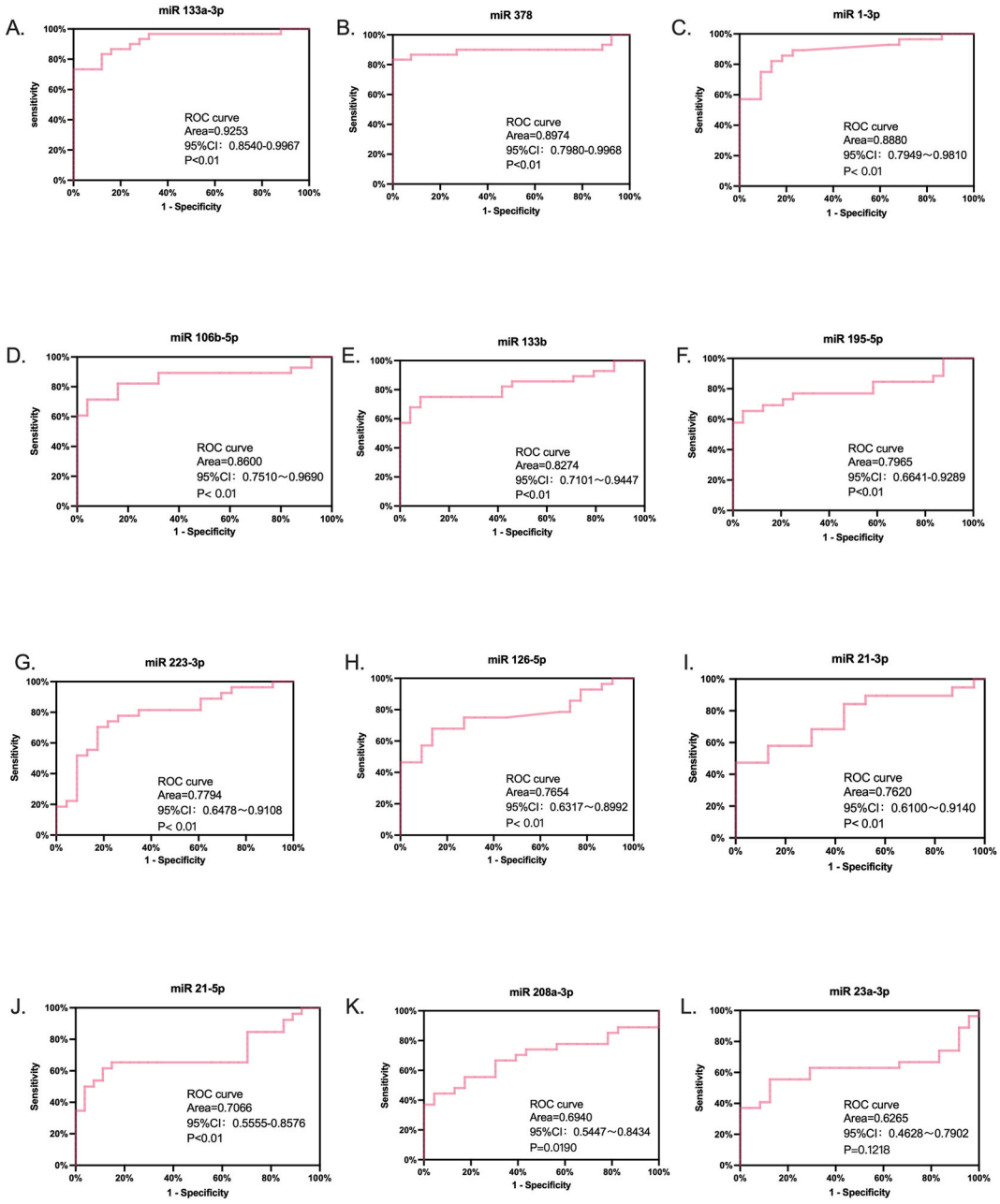

Fig. 2. Receiver operating characteristic (ROC) analysis for the use of miRNAs to diagnosis HFrEF. A-L: ROC analysis for miRNA biomarker candidate.

4. Result

4.1. Characteristics of the enrolled individuals

A total of 30 HFrEF patients and 30 NoHF patients were included in this study according to the eligibility criteria. As was shown in Table 1, all populations had a similar gender split and a comparable prevalence of hypertension. The HFrEF cohort exhibits numerous traits specific to this HF subtype, including being significantly older than the NoHF cohort and demonstrating a dominant ischemic etiology. In contrast to the NoHF group, HFrEF patients had significantly higher NT-proBNP, β2-microglobulin, poorer renal function (indicated by elevated blood urea nitrogen (BUN) and serum creatinine (Scr), as well as considerably lower glomerular filtration rate levels (eGFR), and poorer nutritional status (indicated by decreased albumin (Alb) levels). Once more, these groups' echocardiographic findings are characterized by noticeably larger LAD, LVDd, LVDs, PAP and remarkable reduced EF in the HFrEF cohort.

Table 2
Cut-off levels for miRNA biomarker candidates in HFrEF.

miRNA	Cut-off value (change fold)	Sensitivity%(95%CI)	Specificity%(95%CI)
miR 133a-3p	3.865	73.33(55.55–85.82)	100(86.68–100.0)
miR 378	0.3976	83.33(66.44–92.66)	100(87.13–100.0)
miR 1-3p	1.629	82.14(64.41–92.12)	86.36(66.67–95.25)
miR 106b-5p	3.111	71.43(52.94–84.75)	96(80.46–99.79)
miR 133b	3.519	75(56.64–87.32)	91.67(74.15–98.52)
miR 195-5p	0.7269	65.38(46.22–80.59)	95.83(79.76–99.79)
miR 223-3p	2.419	70.37(51.52–84.15)	82.61(62.86–93.02)
miR 126-5p	1.579	67.86(49.34–82.07)	86.36(66.67–95.25)
miR 21-3p	13.56	47.37(27.33–68.29)	100(85.69–100.0)
miR 21-5p	1.210	65.38(46.22–80.59)	85.19(67.52–94.08)
miR 208a-3p	12.64	44.44(27.59–62.69)	95.65(79.01–99.78)

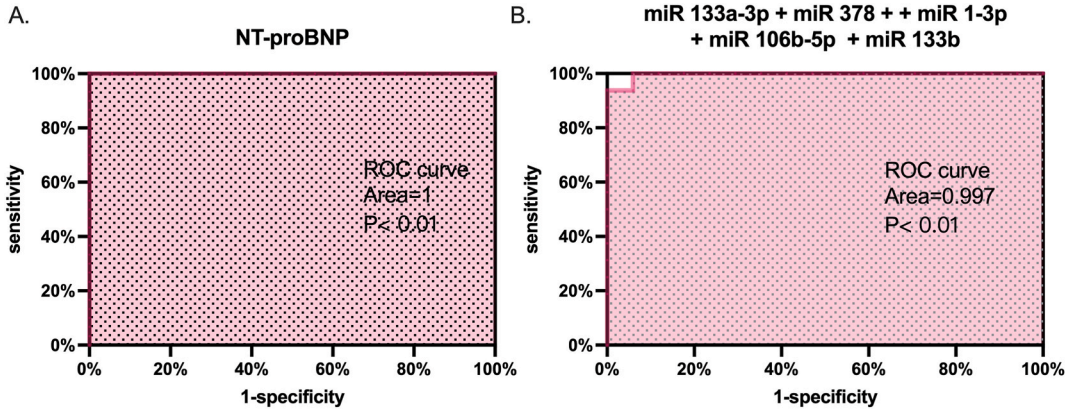

Fig. 3. Receiver operating characteristic (ROC) analysis highlights that the diagnostic value of using miRNA combinations to differentiate HFrEF from NoHF is approaching to using NT-proBNP on its own. A. ROC analysis of NT-proBNP. **B.** ROC anlysis of combined miRNAs (miR 133a-3p+ + miR 378 + miR 1-3p + miR 106b-5p + +miR 133b).

4.2. Independent validation of selected microRNA candidates as HFrEF diagnostics

We performed miRNA profiling from the plasma RNA pool between NoHF patients and HFrEF patients. According to literature reports, candidate miRNAs related to heart disease and cardiac development were selected. Out of the 28 candidate miRNAs, the expression levels of two miRNA were significantly decreased (miR 378, miR 195-5p) (Fig. 1A and B) meanwhile ten miRNAs were remarkably elevated (miR 21-3p, miR 21-5p, miR 106-5p, miR 23a-3p, miR 208a-3p, miR 1-3p, miR 126-5p, miR 133a-3p, miR 133b, miR 223-3p) in the serum of HFrEF group compared to the NoHF cohort(Fig. 1C–L). The expression of other 16 miRNAs was shown in Extended data figure S1.

These differentially expressed miRNA biomarker candidates were further evaluated for HFrEF diagnostic utility. We analyzed these selected miRNAs' receiver operating characteristics (ROC) to assess their diagnostic performance. For distinguishing HFrEF from NoHF, all miRNAs displayed their potential diagnostic value based on area under the ROC curve (AUC), sensitivity, or specificity (Fig. 2A–K) except for miR 23a-3p (Fig. 2L).

The optimum cut-off values for these miRNA biomarker candidates were identified by drawing ROC curves. The cut-off values are shown in Table 2.

Notably, five miRNAs (miR 133a-3p, miR 378, miR 1-3p, miR 106b-5p, and miR 133b) exhibited the greatest estimated AUCs (from 0.8274 to 0.9253). The ROC curves of these five miRNAs were integrated to test whether this improves diagnostic accuracy. As shown in Fig. 3B, the combined analysis showed an AUC of 0.997 ($P < 0.01$). By contrast, NT-proBNP used alone to predict HFrEF in this study yielded an AUC of 1 (Fig. 3A).

4.3. Correlation of the selected miRNA biomarker candidates with the echocardiographic parameters

A linear correlation analysis was performed between several Echocardiographic Parameters and circulating miRNA candidates in HFrEF and NoHF patients.

Briefly, there was a negative correlation between NT-proBNP and LVEF (Fig. 4A). Interestingly, a similar trend was also found in the relationship between LVEF and the circulating miRNA candidates (miR 133a-3p, miR 1-3p, miR 106b-5p, and miR 126-5p) (Fig. 4B–E). Also, LVEF positively correlated with miR 195-5p expression (Fig. 4F). LAD showed statistically significant correlations with NT-

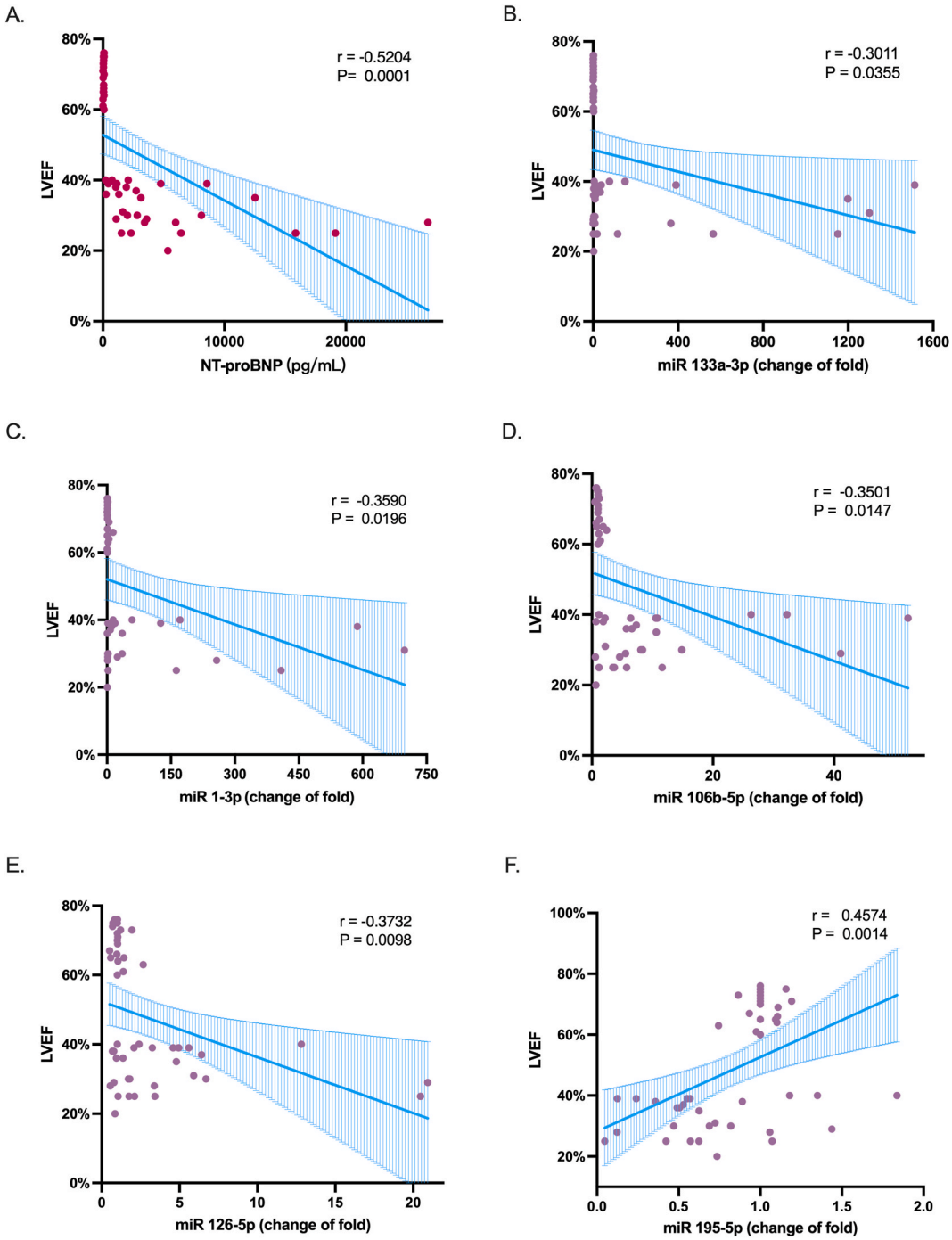

Fig. 4. Correlation of NT-proBNP and the selected miRNA biomarker candidates with LVEF. A-E. NT-proBNP, miR-133a-3p, miR 1-3p, miR 106b-5p, miR 126-5p were negatively related with LVEF were observed. **F.** There was a positive correlation between miR 195-5p with LVEF.

proBNP, miR 133a-3p and miR 378 (Fig. 5A–C); NT-proBNP, miR 126-5p, miR 21-3p and miR 195-5p were all related with LVDd (Fig. 6A–D).

The analysis revealed a positive correlation between LVDs and NT-proBNP, miR 133a-3p, and miR 126-5p expression, but a negative relationship with miR 195-5p (Fig. 7A–D).

Although IVds showed no correlation with NT-pro-BNP, there was a positive correlation with miR 195-5p (Fig. 8). Similarly, a marked and relevant negative correlation was also noted between PAP and miR 378 (Fig. 9A and B).

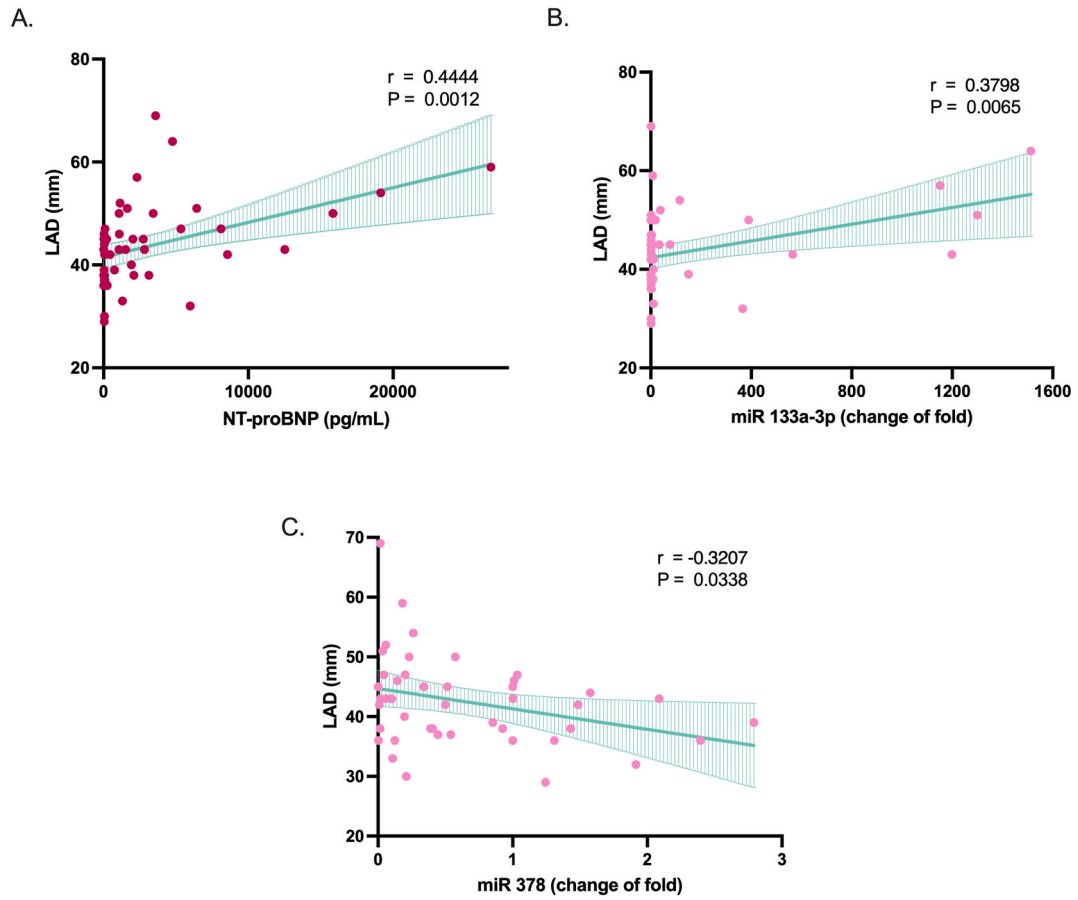

Fig. 5. Correlation of NT-proBNP and the selected miRNA biomarker candidates with LAD. A-B. NT-proBNP, miR-133a-3p were positively related with LAD were observed. **C.** There was a negative correlation between miR 378 with LAD.

4.4. Biomarker candidates MicroRNA target gene prediction and pathway analysis

The target genes of the five potential biomarker candidates (miR 133a-3p, miR 378, miR 1-3p, miR 106b-5p, and miR 133b) were predicted using the miRWALK2.0 software. Among them, 130 overlapping genes (Extended data table 2.) were extracted and plotted in the Venn diagram (Fig. 10A).

A KEGG analysis was conducted to identify the main pathways in which the candidate target genes may be engaged, which will help us better understand the biological functions of the predicted target genes. The top five pathways were endocytosis, axon guidance, TGF-beta signaling, Ubiquitin mediated proteolysis pathway, and gap junction after cancer-related pathways were eliminated (Fig. 10B). The main biological processes, molecular functions, and pathway analyses determined by GO analysis of the target genes of the five analyzed miRNAs are summarized in Fig. 10B. It demonstrated that the overlapping differentially expressed genes were dramatically concentrated in the organelle, ion binding, cellular nitrogen compound metabolic process, biosynthetic process, and cellular component (Fig. 10C).

5. Discussion

There is a growing burden of cardiovascular disease associated with HF worldwide. Patients with chronic HF have a 1-year mortality rate of 7.2 % and a 1-year hospitalization rate of 31.9 %, while patients with acute HF have 17.4 % and 43.9 %, respectively [25]. Although the N-terminal fragment of NT-pro-BNP is a potential marker of heart failure as outlined in the European guidelines from 2008 [26], circulating diagnostic biomarkers-microRNAs have their advantages. Unlike mRNAs or peptides, microRNAs are stable at room temperature and remain so after repeated freeze-thawing [10]. The advantages of circulating miRNAs can be seen in small medical institutions or areas with less developed medical resources, where blood samples from heart failure patients cannot be tested immediately. The present study analyzed the circulating miRNA signature of patients with HFrEF. The results of a genome-wide microarray followed by an independent qRT-PCR analysis demonstrated that two plasma microRNAs (miR 378 and miR 195-5p) were significantly downregulated while ten circulating miRNAs (miR 21-3p, miR 21-5p, miR 106b-5p, miR 23a-3p, miR 208a-3p, miR 1-3p, miR 126-5p, miR 133a-3p, miR 133b and miR 223-3p) were remarkably upregulated in HFrEF patients compared

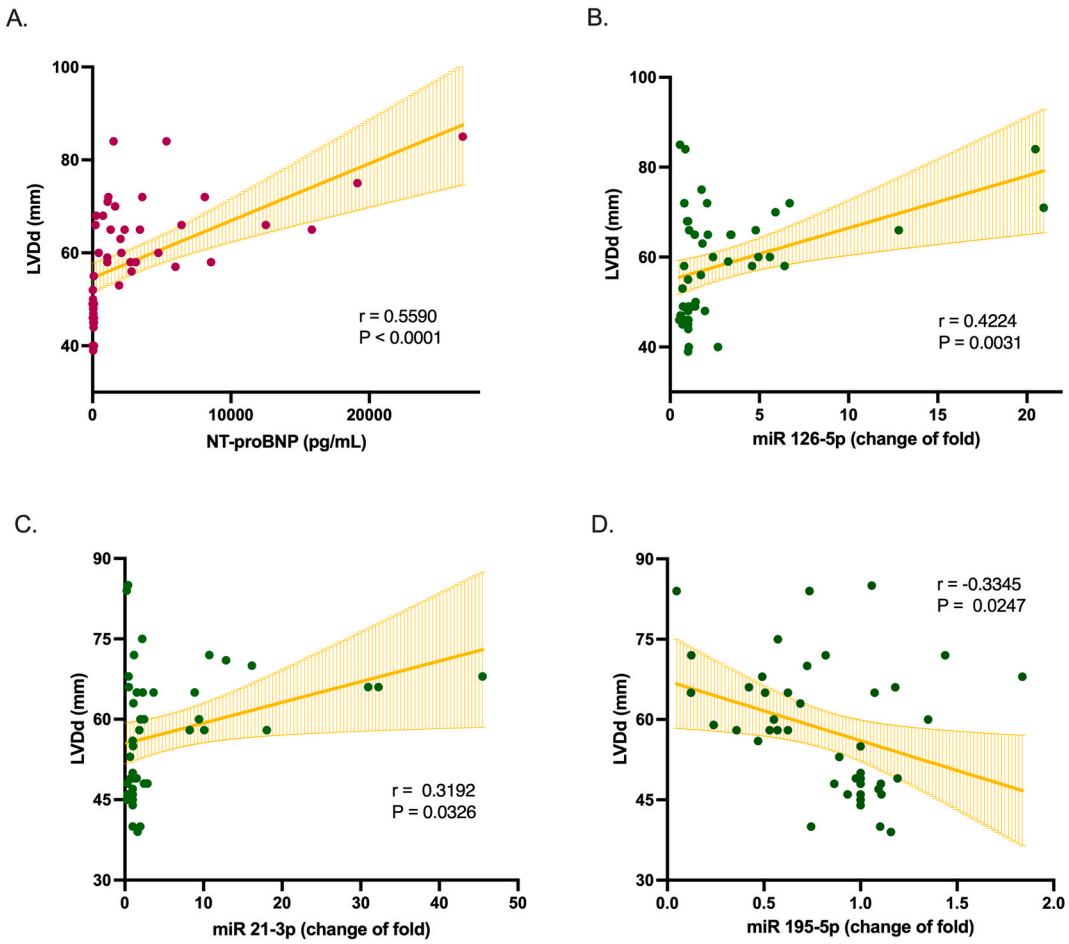

Fig. 6. Correlation of NT-proBNP and the selected miRNA biomarker candidates with LVDd. A-C. NT-proBNP, miR 126-5p and miR 21-3p were positively related with LVDd. **D.** There was a negative correlation between miR 195-5p with LVDd.

with their hypertensive controls(Fig. 1). All miRNAs displayed their potential diagnostic value based on area under the ROC curve (AUC), sensitivity, or specificity except for miR 23a-3p. ROC analysis revealed that the combination of miR 133a-3p, miR 106b-5p, miR 1-3p, miR 133b, and miR 378 had similar discriminatory abilities in identifying HFrEF as NT-proBNP, which is an acknowledged biomarker. Drawing ROC curves for these miRNA biomarker candidates were used to determine the cut-off points for these biomarker candidates. It should be noted that the cut-off values here were change-fold, which was a relative ratio. The absolute quantification of miRNA is challenging to define and is greatly influenced by each batch's reagents, operation, and other factors. Therefore, the diagnostic value of miRNA may be somewhat affected in practical operation. The absolute RT-qPCR method, on the other hand, is capable of determining the exact number of copies of a miRNA. The signal in an unknown sample is compared to a standard curve to achieve this [27]. Recently, digital RT-PCR has been used to quantify miRNA absolute levels. Digital PCR has the inherent advantage over conventional PCR in that it does not require external calibration (standard curves) or normalization to estimate the concentration of an unknown target [28].

It is reported that the overexpression of miR-133a significantly decreased fibrosis in rats with chronic heart failure by inhibiting the serine/threonine kinase Akt [29]. MiR-133 overexpression also suppresses the expression of multiple genes in fibroblasts, concurrently activating cardiac reprogram [30]. MiR-378 plays a dual role in suppressing cardiac hypertrophy and fibrosis through a paracrine mechanism [31]. In our study, some of these diagnostic candidate miRNAs are also associated with the LVEF (miR 133a-5p, miR 1-3p, miR 106b-5p, miR 126-5p, and miR 195-5p) (Fig. 4)and other echocardiographic parameters(LAD, LVDd, LVDs, IVSd, and PAP),which predicts that these miRNAs may be involved in myocardial hypertrophy or myocardial remodeling and deserve further investigation.

In addition, we conducted a bioinformatic analysis of these candidates for diagnosing HFrEF. It was determined that these five candidate miRNAs target 130 genes that are co-expressed using the miRWALK2.0 software. GO analysis demonstrated that the overlapping differentially expressed genes were dramatically concentrated in the organelle. Regarding KEGG analysis, we investigated if there are any common enriched pathways related to pathophysiological processes of heart failure. We found support for some familiar pathway enrichment results with heart failure for the Mitogen-activated protein kinas (MAPK) signaling pathway, ErbB signaling pathway, and TGF-beta signaling pathway.

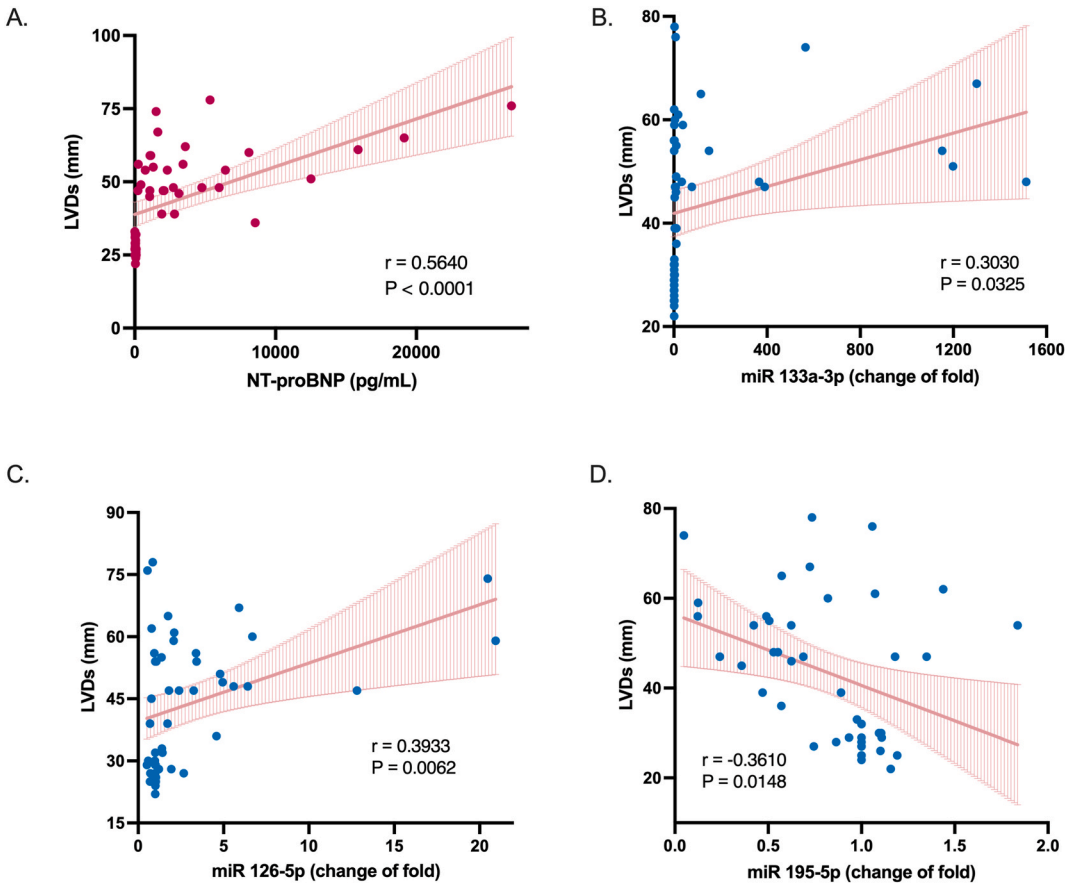

Fig. 7. Correlation of NT-proBNP and the selected miRNA biomarker candidates with LVDs. A-C.NT-proBNP, miR 133a-3p and miR 126-5p were positively related with LVDs. **D.** There was a negative correlation between miR 195-5p with LVDs.

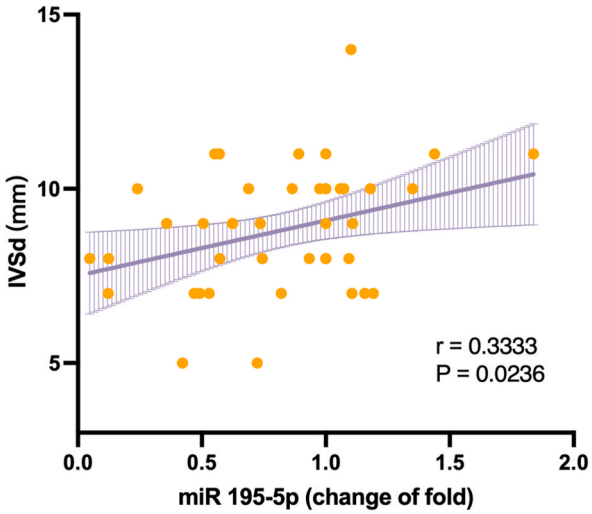

Fig. 8. Correlation of miR 195-5p with IVSd. MiR 195-5p was positively related with IVSd.

MAPK signaling cascades are critical regulators of cardiac hypertrophic response [32]. Liang reported that inhibiting p38 MAPK can reduce cardiomyocyte growth in response to hypertrophic stimuli [33]. In addition, chronic activation of the p38 MAPK pathway has been demonstrated to induce hypertrophic responses in cultured cardiomyocytes [34,35].

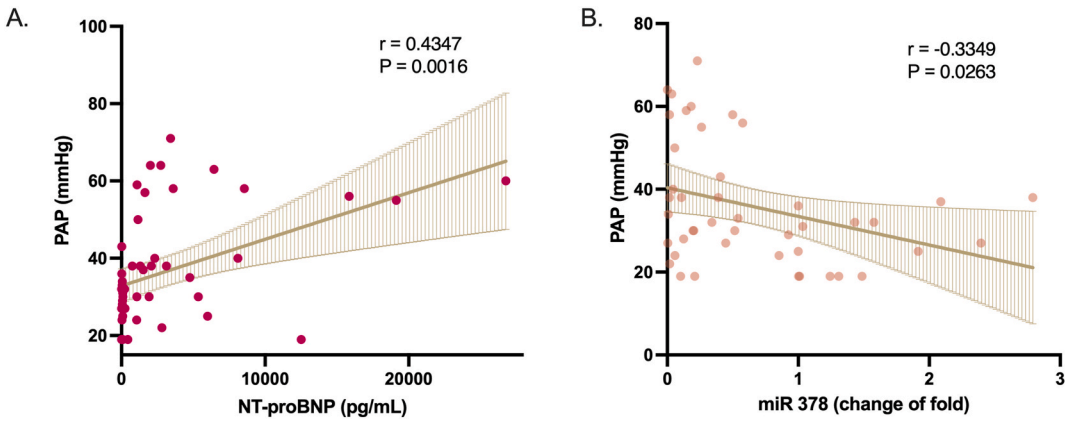

Fig. 9. Correlation of NT-proBNP and the selected miRNA biomarker candidates with PAP. A. NT-proBNP was positively related with PAP. **B.** There was a negative correlation between miR 378 with PAP.

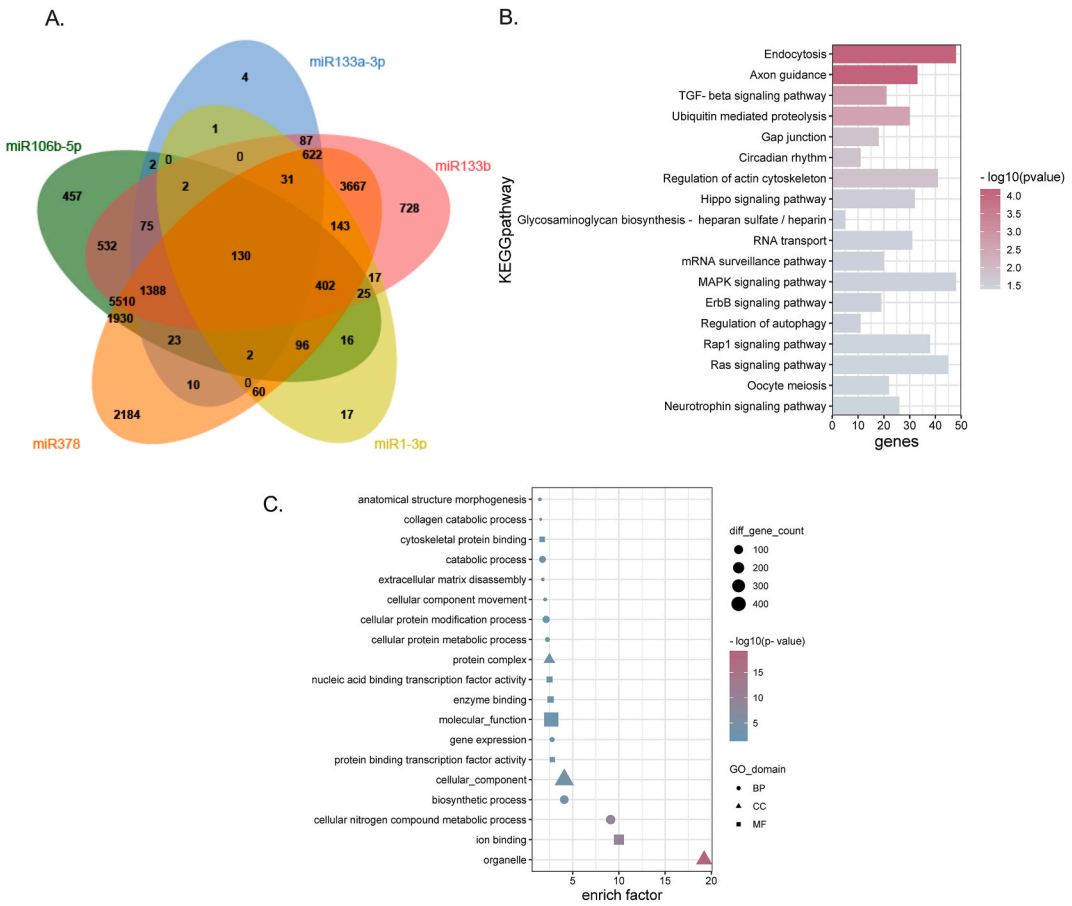

Fig. 10. Bioinformatic analysis for the five miRNA biomarker candidates. A. Venn's diagram of genes targeted by each of the five miRNA biomarker candidates. **B.** Statistically significantly enriched pathways in Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. **C.** Gene Ontology (GO) analysis for the target genes of the five miRNA biomarker candidates.

Animal models have demonstrated significant changes within the cardiac NRG-1/ErbB pathway during the progression of chronic HF. In the early stages of the disease, NRG-1/ErbB expression is elevated and declines just after the pump fails [36].

It has been demonstrated that the TGFβ signaling pathway plays a role in cardiac remodeling [37]. Increased TGFβ1 expression is instrumental in heart hypertrophy and cardiomyocyte apoptosis [38]. It is also reported that there is a climacteric link between

miR-34a, cardiovascular fibrosis, and Smad4/TGFβ1 signaling pathway [39].

New visions were also provided into Endocytosis, axon guidance, gap junction, and regulation of actin cytoskeleton.

Our study has some limitations: 1) the number of patients was relatively small, which reduced our statistical power. Therefore, more extensive studies should be conducted to confirm the diagnostic value of miRNAs. 2) it is skeptical whether these circulating miRNAs are released from cardiomyocytes, fibroblasts, macrophages, or even by non-cardiovascular tissues due to the secondary consequence of HF. Artificial intelligence (AI) algorithms, including machine learning and deep learning, are increasingly being used to predict the onset of diseases. Some researchers have reported using AI technology and profound learning algorithms to automatically detect and predict coronary artery disease, thereby improving the accuracy of medical outcomes [40]. Predictive models can analyze a wide range of data, including genetic information, patient history, and lifestyle factors, to estimate an individual's risk of developing a particular condition. In the future, we can also attempt to train AI algorithms on the CN-HF database to optimize the diagnosis and treatment of HFrEF.

6. Conclusion

The combination of miR 133a-3p, miR 378, miR 1-3p, miR 106b-5p, and miR 133b has excellent diagnostic performance for HFrEF, which can be an alternative to NT-proBNP in certain circumstances.

Ethical approval and consent to participate

All participants provided written informed consent to participate and consent to publish in the study, which was authorized by the Zhongshan Hospital Ethical Committee of Fudan University, China, in the declaration of Helsinki (approval number: B2012-140(2)).

Consent for publication

All authors approved the final manuscript and the submission to this journal.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Funding

This study was supported by the National Natural Science Foundation of China (Grant No. 82170279) and the Natural Science Foundation of Shanghai, Grant (No. 21ZR1412700).

CRediT authorship contribution statement

Zheng Kuai: Writing – original draft, Visualization, Data curation. **Yuanji Ma:** Writing – original draft, Methodology, Funding acquisition, Formal analysis. **Wei Gao:** Writing – original draft, Data curation. **Xiaoxue Zhang:** Data curation. **Xiaoyan Wang:** Data curation. **Yangli Ye:** Methodology. **Xiaoyi Zhang:** Writing – review & editing, Project administration, Conceptualization. **Jie Yuan:** Writing – review & editing, Supervision, Investigation, Funding acquisition.

Declaration of generative AI and AI-assisted technologies in the writing process

None.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We sincerely express our gratitude to all the patients and their families for their invaluable participation and support in our clinical study.

Abbreviations

HF	Heart failure
LV	left ventricular
EF	ejection fraction

HFpEF	heart failure with preserved ejection fraction
HFmEF	heart failure with mid-range ejection fraction
HFrEF	heart failure with reduced ejection fraction
NT-pro-BNP	N-terminal fragment of B-type natriuretic peptide
miRNA	microRNA
CN-HF	the China National Heart Failure Registry
BUN	blood urea nitrogen
Scr	serum creatinine
eGFR	estimated glomerular filtration rate
Alb	albumin
HbA1C	glycosylated hemoglobin
LAD	left atrium diameter
LVDd	left ventricular end-diastolic diameter
LVDs	left ventricular end-systolic diameter
IVSd	interventricular septum diameter
LVPWd	left ventricular posterior wall diameter
PAP	Pulmonary artery pressure
LVEF	left ventricular ejection fraction
AUC	area under the curve analysis
CTnT	troponin T
Hb	Hemoglobin
WBC	white blood cell
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
eGFR	estimated glomerular filtration rate
TC	total cholesterol
TG	total triglyceride
HDL:	high density lipoprotein
LDL:	low density lipoprotein
AI	Artificial intelligence

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37929.

References

[1] P. Ponikowski, S.D. Anker, K.F. AlHabib, et al., Heart failure: preventing disease and death worldwide, ESC Heart Failure 1 (1) (2014) 4–25.
[2] B. Ziaeian, G.C. Fonarow, Epidemiology and aetiology of heart failure, Nat. Rev. Cardiol. 13 (6) (2016) 368–378.
[3] G. Savarese, P.M. Becher, L.H. Lund, et al., Global burden of heart failure: a comprehensive and updated review of epidemiology, Cardiovasc. Res. 118 (17) (2023) 3272–3287.
[4] X. Wang, S. Yao, M. Wang, et al., Multimorbidity among two million adults in China, Int. J. Environ. Res. Publ. Health 17 (10) (2020) 3395.
[5] P. Ponikowski, A.A. Voors, S.D. Anker, et al., 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure, Eur. Heart J. 37 (27) (2016) 2129–2200.
[6] A. Raizada, S. Bhandari, M.A. Khan, et al., Brain type natriuretic peptide (BNP)—a marker of millennium in diagnosis of congestive heart failure, Indian J. Clin. Biochem. 22 (1) (2007) 4–9.
[7] R.S. Pillai, MicroRNA function: multiple mechanisms for a tiny RNA? RNA 11 (12) (2005) 1753–1761.
[8] A. Peterlin, K. Počivavšek, D. Petrovič, et al., The role of microRNAs in heart failure: a systematic review, Frontiers in Cardiovascular Medicine 7 (2020).
[9] H. Wang, J. Cai, The role of microRNAs in heart failure, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1863 (8) (2017) 2019–2030.
[10] P.S. Mitchell, R.K. Parkin, E.M. Kroh, et al., Circulating microRNAs as stable blood-based markers for cancer detection, Proc. Natl. Acad. Sci. USA 105 (30) (2008) 10513–10518.
[11] X. Chen, Y. Ba, L. Ma, et al., Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases, Cell Res. 18 (10) (2008) 997–1006.
[12] C.H. Lawrie, S. Gal, H.M. Dunlop, et al., Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma, Br. J. Haematol. 141 (5) (2008) 672–675.
[13] E.L. Vegter, P. Van der Meer, L.J. De Windt, et al., MicroRNAs in heart failure: from biomarker to target for therapy, Eur. J. Heart Fail. 18 (5) (2016) 457–468.
[14] G. Siasos, E. Bletsa, P.K. Stampouloglou, et al., MicroRNAs in cardiovascular disease, Hellenic J. Cardiol. 61 (3) (2020) 165–173.
[15] S. Zhou, J. Jin, J. Wang, et al., miRNAS in cardiovascular diseases: potential biomarkers, therapeutic targets and challenges, Acta Pharmacol. Sin. 39 (7) (2018) 1073–1084.
[16] R. Figueiredo, R. Adão, A.F. Leite-Moreira, et al., Candidate microRNAs as prognostic biomarkers in heart failure: a systematic review, Rev. Port. Cardiol. 41 (10) (2022) 865–885.
[17] L. Qian, Q. Zhao, P. Yu, et al., Diagnostic potential of a circulating miRNA model associated with therapeutic effect in heart failure, J. Transl. Med. 20 (1) (2022) 267.
[18] D. Scrutinio, F. Conserva, P. Guida, et al., Long-term prognostic potential of microRNA-150-5p in optimally treated heart failure patients with reduced ejection fraction: a pilot study, Minerva Cardiology and Angiology 70 (4) (2022).

[19] F. Chen, J. Yang, Y. Li, et al., Circulating microRNAs as novel biomarkers for heart failure, Hellenic J. Cardiol. 59 (4) (2018) 209–214.
[20] X. Wang, X. Zhang, J. Yuan, et al., Evaluation of the performance of serum miRNAs as normalizers in microRNA studies focused on cardiovascular disease, J. Thorac. Dis. 10 (5) (2018) 2599–2607.
[21] H. Dweep, N. Gretz, miRWalk2.0: a comprehensive atlas of microRNA-target interactions, Nat. Methods 12 (8) (2015) 697, 697.
[22] H. Dweep, C. Sticht, P. Pandey, et al., miRWalk – database: Prediction of possible miRNA binding sites by "walking" the genes of three genomes, J. Biomed. Inf. 44 (5) (2011) 839–847.
[23] P. Bardou, J. Mariette, F. Escudié, et al., jvenn: an interactive Venn diagram viewer, BMC Bioinf. 15 (1) (2014) 293.
[24] I.S. Vlachos, K. Zagganas, M.D. Paraskevopoulou, et al., DIANA-miRPath v3.0: deciphering microRNA function with experimental support, Nucleic Acids Res. 43 (W1) (2015) W460–W466.
[25] A.P. Maggioni, U. Dahlström, G. Filippatos, et al., EUR observational research programme: regional differences and 1-year follow-up results of the heart failure pilot survey (ESC-HF pilot), Eur. J. Heart Fail. 15 (7) (2013) 808–817.
[26] A. Palazzuoli, Natriuretic peptides (BNP and NT-proBNP): measurement and relevance in heart failure, Vasc. Health Risk Manag. (2010) 411.
[27] S. Bustin, Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays, J. Mol. Endocrinol. 25 (2) (2000) 169–193.
[28] S. Nazir, Medical diagnostic value of digital PCR (dPCR): a systematic review, Biomedical Engineering Advances 6 (2023) 100092.
[29] H.-Q. Sang, Z.-M. Jiang, Q.-P. Zhao, et al., MicroRNA-133a improves the cardiac function and fibrosis through inhibiting Akt in heart failure rats, Biomed. Pharmacother. 71 (2015) 185–189.
[30] N. Muraoka, H. Yamakawa, K. Miyamoto, et al., MiR-133 promotes cardiac reprogramming by directly repressing Snai1 and silencing fibroblast signatures, EMBO J. 33 (14) (2014) 1565–1581.
[31] J. Yuan, H. Liu, W. Gao, et al., MicroRNA-378 suppresses myocardial fibrosis through a paracrine mechanism at the early stage of cardiac hypertrophy following mechanical stress, Theranostics 8 (9) (2018) 2565–2582.
[32] X. He, T. Du, T. Long, et al., Signaling cascades in the failing heart and emerging therapeutic strategies, Signal Transduct. Targeted Ther. 7 (1) (2022) 134.
[33] Q. Liang, Redefining the roles of p38 and JNK signaling in cardiac hypertrophy: dichotomy between cultured myocytes and animal models, J. Mol. Cell. Cardiol. 35 (12) (2003) 1385–1394.
[34] J.M. Streicher, S. Ren, H. Herschman, et al., MAPK-activated protein kinase-2 in cardiac hypertrophy and cyclooxygenase-2 regulation in heart, Circ. Res. 106 (8) (2010) 1434–1443.
[35] R. Romero-Becerra, A. Santamans, C. Folgueira, et al., p38 MAPK pathway in the heart: insights in health and disease, Int. J. Mol. Sci. 21 (19) (2020) 7412.
[36] K. Lemmens, V.F.M. Segers, M. Demolder, et al., Role of neuregulin-1/ErbB2 signaling in endothelium-cardiomyocyte cross-talk, J. Biol. Chem. 281 (28) (2006) 19469–19477.
[37] M. Dobaczewski, W. Chen, N.G. Frangogiannis, Transforming growth factor (TGF)-β signaling in cardiac remodeling, J. Mol. Cell. Cardiol. 51 (4) (2011) 600–606.
[38] M. Vigil-Garcia, C.J. Demkes, J.E.C. Eding, et al., Gene expression profiling of hypertrophic cardiomyocytes identifies players in pathological remodelling, Cardiovasc. Res. 117 (6) (2021) 1532–1545.
[39] C.-C. Hua, X.-M. Liu, L.-R. Liang, et al., Targeting the microRNA-34a as a novel therapeutic strategy for cardiovascular diseases, Frontiers in Cardiovascular Medicine 8 (2022).
[40] H.M. Sarmad Muwfak Khazaal, Predicting coronary artery disease utilizing support vector machines: optimizing predictive model, Mesopotamian Journal of Artificial Intelligence in Healthcare (2023) 21–26.