Combined Use of Circulating miR-133a and NT-proBNP Improves Heart Failure Diagnostic Accuracy in Elderly Patients

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABEFG Meizi Guo
BC Jun Luo
BC Junli Zhao
D Dongya Shang
BE Qing Lv
BE Panpan Zang

Department of Gereology and Cardiology, Shanghai University of Medicine and Health Sciences Affiliated Zhoupu Hospital, Shanghai, P.R. China

Background: Circulating microRNAs (miRNAs) are emerging as novel biomarkers for detecting cardiovascular diseases. Here, circulating miR-133a and miR-221 were investigated as potential diagnostic biomarkers for heart failure (HF) patients, particularly in elderly patients.

Material/Methods: A total of 94 elderly HF patients (mean age=77.4 years old) and 31 healthy controls (age- and sex-matched) participated in this study. Plasma NT-proBNP levels were measured using an electrochemiluminescence immunoassay, and circulating miR-133a and miR-221 levels were examined using real-time quantitative PCR, with diagnostic efficacies determined for each independently and in combination.

Results: MiR-133a expression increased by 4.6-fold (P<0.001) and miR-221 expression increased by 2.0-fold (P<0.001) in the elderly HF patients relative to the healthy controls. ROC curves were generated and AUC values of 0.863 for miR-133a (CI95%: 0.800–0.927), 0.718 for miR-221 (CI95%: 0.622–0.813), and 0.895 for NT-proBNP (CI95%: 0.841–0.948) were obtained. Unlike NT-proBNP, miR-133a and miR-221 were found to be unaffected by age, BMI, renal function, albumin, or Hb levels. More importantly, the diagnostic value of NT-proBNP was found to be improved when combined with any of the examined miRNA biomarkers alone or in a panel. When combining miR-133a with NT-proBNP, an AUC value of 0.975 (CI95%: 0.950–0.999) was obtained, which was significantly higher than for NT-proBNP alone (z=2.395, P=0.016).

Conclusions: miR-133a and miR-221 can serve as potential HF diagnostic biomarkers in elderly patients. Moreover, the diagnostic accuracy of NT-proBNP can be improved by the addition of miR-133a.

MeSH Keywords: Biological Markers • Heart Failure • MicroRNAs

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Background

Heart failure (HF) is the terminal stage for most types of cardiovascular diseases and continues to be a health concern in most countries. Regardless of the diagnosis and treatment, the mortality rate of HF is still very high, with mortality occurring in half of patients within 5 years of diagnosis. In patients with advanced HF, mortality occurs in half of the patients within the first year of diagnosis [1]. HF prevalence in the general population has been estimated to be 1–2%, and further increased to 20% in people age > 80 years [2]. In elderly patients, it is difficult to diagnose HF early due to an increased number of chronic diseases and atypical HF symptoms.

MicroRNAs (miRNAs) are a class of endogenous, single-stranded, noncoding RNAs that regulate gene expression by targeting mRNAs for cleavage or translational repression [3]. miRNAs play important roles in cardiac differentiation, proliferation, and maturation, as well as pathological remodeling in response to stress or injury [4–7]. In addition, miRNAs are actively released for circulation and are protected from subsequent degradation, indicating that these may be potentially utilized as biomarkers for various diseases. Several studies have investigated the potential of circulating miRNAs for the diagnosis of cardiovascular diseases, including HF [8,9].

In heart and skeletal muscle, hsa-miR-133a-3p (miR-133a) is specifically expressed. Significantly higher plasma levels of miR-133a have been observed in patients diagnosed with acute myocardial infarction (AMI) compared to healthy controls [10,11]. Furthermore, in a chronic HF canine model, miR-133 was significantly increased in HF cardiomyocytes when compared to the control [12]. Additionally, in a congestive HF rabbit model, circulating miR-133 was upregulated by 2.6–3.3 fold [13]. However, peripheral blood mononuclear cell-derived miR-133a is not a diagnostic biomarker for hypertensive patients with HFpEF and hypertensive patients without HFpEF [14].

Another miRNA of interest is hsa-miR-221-3p (miR-221), which is highly expressed in endothelial cells and is involved in tumor development, obesity, and cardiovascular disease [15–17]. Furthermore, miR-221 has been found to be differentially expressed in the plasma of HF patients [18]. miR-221 can be used to distinguish HF with preserved ejection fraction (HFpEF) and HF with reduced ejection fraction (HFrEF), with miR-221 upregulated in HFpEF and downregulated in HFrEF [18].

In the present study, circulating miR-133a and miR-221 levels were examined in elderly patients with HF and in healthy controls using quantitative real-time polymerase chain reaction (qRT-PCR). The diagnostic efficacies of these miRNAs were also evaluated relative to the well-established biomarker N-terminal prohormone of brain natriuretic peptide (NT-proBNP).

Material and Methods

Participants

From October 2015 to April 2016, 94 elderly patients with HF and 31 healthy volunteers were recruited through the Department of Cardiology, Zhoupu Hospital, Shanghai University of Medicine and Health Sciences. All patients and healthy volunteers were 65 years old or older. The inclusion criteria for the heart failure group were chronic heart failure patients, diagnosed by heart failure specialized cardiologist according to the 2009 Focused Update: ACCF/AHA Guidelines for the Diagnosis and Management of HF in Adults. Comorbidities for heart failure, such as hypertension, diabetes mellitus, hyperlipidemia, coronary artery disease, chronic permanent atrial fibrillation, and chronic obstructive pulmonary disease (COPD), did not preclude recruitment. Patients with severe hepatic and renal dysfunction, a simultaneous infection, a malignant tumor, or connective tissue diseases were excluded. The remaining patients were assigned to 1 of 3 groups according to the functional classification system of the New York Heart Association (NYHA), which included the NYHA II (N=32), NYHA III (N=32 patients), and NYHA IV (N=30 patients) groups. The control group, which consisted of 31 healthy individuals, were matched in terms of the age, sex, and ethnicity with the HF group and showed no clinical history of cardiac disease. The Zhoupu Hospital, Shanghai University of Medicine and Health Sciences Medical Ethics Committee approved this study (ZPYYLL-2015-23).

Clinical assessment

The patients were assessed by trained research physicians who gathered information on HF-related symptoms such as NYHA class and who conducted a physical examination that consisted of body mass index (BMI) determination. Serum levels of albumin (A), creatinine (Cr), hemoglobin (Hb), and urea nitrogen (BUN) were measured using a biochemistry automated analyzer (Beckman Coulter Inc, Fullerton, CA, USA).

NT-proBNP and CRP measurements

Plasma NT-proBNP levels were measured with an electrochemiluminescence immunoassay (Elecsys proBNP II assay; Roche Diagnostics, Indianapolis, IN, USA) using an automated Cobas e601 analyzer (Roche Diagnostics, Mannheim, Germany) following the manufacturer's instructions. Quality control measurements were performed within 2 standard deviation (SD) values. NT-proBNP detection was within the range 5 pg/mL to 35 000 pg/mL. C-reactive protein (CRP) levels were measured using an immunoturbidimetric assay (Hitachi 912 chemistry analyzer; Roche Diagnostics, Basel, Switzerland), which measures concentrations ranging from 0.3 to 350 mg/l.

Echocardiography

Doppler echocardiography was conducted by 1 of 2 investigators who were blinded to the ongoing results of this study, as recommended by the European Society of Echocardiography. The data are expressed as the mean of 3 sequential measurements of the cardiac cycle. Left ventricular ejection fraction (LVEF) was calculated as described by Simpson [19].

Sample collection and preparation

Blood samples were collected from each participant using sodium citrate tubes. The samples were left to stand for about 3 h at 4°C and then centrifuged at 1500 g for 10 min at room temperature. The plasma was isolated from each sample and then stored at –80°C.

RNA extraction and analysis

RNA was isolated from the plasma samples with a Qiagen miRNeasy Serum/Plasma kit (Qiagen, Hilden, Germany) following the manufacturer's specifications. The synthetic *C. elegans* miR-39 (5'-UCA CCG GGU GUA AAU CAG CUU G-3') was added to the plasma samples prior to the RNA isolation step. Quality assessment and quantification of RNA was then performed with a Nanodrop spectrophotometer (ND-1000; Nanodrop Technologies, Wilmington, DE, USA). The integrity of the extracted RNA was assessed by gel electrophoresis. RNA re-isolation was performed for samples that did not meet the quality and quantity criteria.

Reverse transcription

Reverse transcription reactions were performed using a miScript Reverse Transcription Kit (Qiagen) in a 20-µl RT reaction: 0.1 µg total RNA or 1 µl synthesized miRNA mixture of hsa-miR-133a, hsa-miR-221 and cel-miR-39 (10^{-4} fM of each), 1 µl miScript Reverse Transcriptase Mix, 4 µl 5× miScript RT buffer, and RNase-free water. RT reactions were carried out on an ABI 2720 Thermal Cycler (Applied Biosystems, Foster, CA, USA) under the following conditions: 37°C for 60 min, 95°C for 5 min, and then hold at 4°C. RT products were stored at –20°C prior to use.

qRT-PCR

The qRT-PCR reactions were performed using miScript SYBER Green PCR master mix (Qiagen). PCR reactions (20 µl) contained 1 µl cDNA template, 1 µl specific upstream microRNA primer (10 µ*M*), 2 µl 10× miScript Universal Primer, 10 µl 2× QuantiTect SYBR Green PCR Master Mix, and RNase-free water. qRT-PCR was carried out on an ABI 7500 Sequence Detection system (Applied Biosystems) under the following conditions: 95°C for 15 min, 40 cycles of 94°C for 15 s, 55°C for 30 s, and 70°C for 30 s. The specific upstream microRNA primers of has-miR-133a, has-miR-221, and cel-miR-39 are 5'-AGC TGG TAA AAT GGA ACC AAA T-3', 5'-AGC TAC ATT GTC TGC TGG GTT TC-3' and 5'-TCA CCG GGT GTA AAT CAG CTT G-3', respectively. qRT-PCR assays were tested initially using a serial dilution of cDNA of synthesized miRNAs. qRT-PCR efficiencies of target miRNAs (has-miR-133a and has-miR-221) and reference miRNA cel-miR-39 were similar and were greater than 87%. The circulating miR-133a and miR-221 levels were analyzed using the ΔΔCt method, normalized using exogenous spike-in control cel-miR-39.

Data analysis and statistics

Distributions of continuous variables were tested for normality using the Kolmogorov-Smirnov test. If a normal distribution was detected, the variables are displayed as a mean ±SD, unless otherwise indicated. Differences between groups (control and HF) were analyzed using an independent-samples t test, while differences among multiple groups (control, NYHA II, NYHA III, NYHA IV) were assessed using a one-way ANOVA. If a skewed distribution was observed, variables are displayed as a median and interquartile range (IQR). Differences between 2 independent groups were analyzed using the Mann-Whitney *U* test, while differences among multiple independent groups were analyzed using the Kruskal-Wallis test. Categorical clinical variables are displayed as a count and percentage and were compared using the chi-square test. The relationships among numerical variables were assessed with Pearson's or Spearman's correlation tests. All statistical assessments were performed using SPSS v. 19.0 (SPSS Inc., Chicago, IL, USA), using *P*<0.05 an indicator for statistical significance.

Results

Patient characteristics

The study cohort consisted of 94 elderly HF patients and 31 age-, sex-, and BMI-matched healthy controls (Table 1). Patients with HF were classified as NYHA II (n=32), NYHA III (n=32) or NYHA IV (n=30) based on their functional clinical status. Age, sex, and BMI differences among the NYHA II, NYHA III, NYHA IV, and control groups were not significantly different (Table 2). In terms of cardiac dysfunction, the HF groups exhibited significantly lower LVEFs and elevated NT-proBNP, CRP, Cr, and BUN levels relative to the control.

Elevated plasma miR-133a and miR-221 levels in HF patients

RNA was isolated from HF and control plasma samples and circulation levels were analyzed using qRT-PCR. The HF patients

Table 1. Characteristics of the heart failure (HF) and control groups.

Variables	Control		HF		P
Male: Female	12: 19		45: 49		0.539
Age (years)	74.5±8.8		77.4±8.4		0.125
BMI (kg/m^2)	22.4±3.3		22.9±3.7		0.402
A (g/L)	39.6±3.6		37.8±3.3		0.010
Hb (g/L)	127.8±11.7		122.6±18.1		0.179
LVEF (%)	66.9±5.5		55.2±11.7		0.000
BUN (mmol/L)	5.5±1.4		8.5±4.8		0.000
Cr (μmol/L)	59	(50–75)	76.5	(64.8–113.0)	0.000
NT-proBNP (ng/L)	183.0	(133.0–262.0)	2425	(613.8–8165.8)	0.000
CRP (mg/L)	3.6	(2.0–7.4)	9.5	(4.0–20.6)	0.004
miR-133a ($2^{-\Delta\Delta Ct}$)	2.5	(1.0–4.1)	11.6	(5.2–28.0)	0.000
miR-221 ($2^{-\Delta\Delta Ct}$)	0.8	(0.6–1.3)	1.6	(0.9–3.0)	0.002

NYHA – New York Heart Association; BMI – body mass index; A – albumin; Hb – hemoglobin; LVEF – left ventricular ejection fraction; BUN – blood urea nitrogen; Cr – creatinine; NT-proBNP – N-terminal pro-brain type natriuretic peptide; CRP – C-reactive protein. Values are means ±SD or a median with an interquartile range.

Table 2. Characteristics of the heart failure (HF) sub groups (NYHA II, NYHA III, and NYHA IV) and control groups.

Variables	Control	NYHA II	NYHA III	NYHA IV	P
Male: Female	12: 19	14: 18	18: 14	13: 17	0.539
Age (years)	74.5±8.8	75.3±8.3	78.3±7.2	78.8±9.2	0.125
BMI (kg/m^2)	22.4±3.3	23.6±3.7	23.0±3.3	22.1±3.8	0.402
A (g/L)	39.6±3.6	38.9±2.8	37.4±3.9	37.0±2.6	0.010
Hb (g/L)	127.8±11.7	124.5±13.9	126.6±16.6	116.3±21.7	0.179
LVEF (%)	66.9±5.5	60.3±7.0	56.9±8.7	47.8±14.5	0.000
BUN (mmol/L)	5.5±1.4	5.9±2.0	8.7±3.8	11.2±6.1	0.000
Cr (μmol/L)	59.0 (50.0–75.0)	69.5 (59.3–80.8)	77.5 (65.5–102.5)	105.5 (74.5–143.8)	0.000
NT-proBNP (ng/L)	183.0 (133.0–262.0)	399.5 (184.5–1567.0)	2415.0 (1287.5–5655.0)	9220.0 (5690.3–16900.0)	0.000
CRP (mg/L)	3.6 (2.0–7.4)	7.1 (3.4–13.8)	10.6 (6.1–32.8)	13.3 (2.8–42.4)	0.004
miR-133a ($2^{-\Delta\Delta Ct}$)	2.5 (1.0–4.1)	16.6 (6.3–44.8)	8.9 (5.5–20.2)	12.4 (4.7–27.5)	0.000
miR-221 ($2^{-\Delta\Delta Ct}$)	0.8 (0.6–1.3)	1.8 (0.9–5.4)	1.3 (0.9–2.1)	1.6 (0.8–2.6)	0.002

NYHA – New York Heart Association; BMI – body mass index; A – albumin; Hb – hemoglobin; LVEF – left ventricular ejection fraction; BUN – blood urea nitrogen; Cr – creatinine; NT-proBNP – N-terminal pro-brain type natriuretic peptide; CRP – C-reactive protein. Values are means ±SD or a median with an interquartile range.

showed 4.6-fold higher miR-133a (*P*<0.001; Figure 1A) and 2.0-fold higher miR-221 (*P*<0.001; Figure 1C) expression levels compared to the controls. After adjusting for age, sex, diabetes, hypertension, BMI, BUN, Cr, CRP, and LVEF, both miR-133a and miR-221 were shown to have a significant association with HF (*P*<0.001). Additionally, miR-133a and miR-221 expression levels were examined in the 3 HF groups relative to the control group (Figure 1B, 1D). miR-133a expression was increased 6.6-fold in NYHA II (*P*<0.001), 3.6-fold in NYHA III (*P*<0.001), and 5.0-fold in NYHA IV (*P*<0.001) patients relative to the controls,
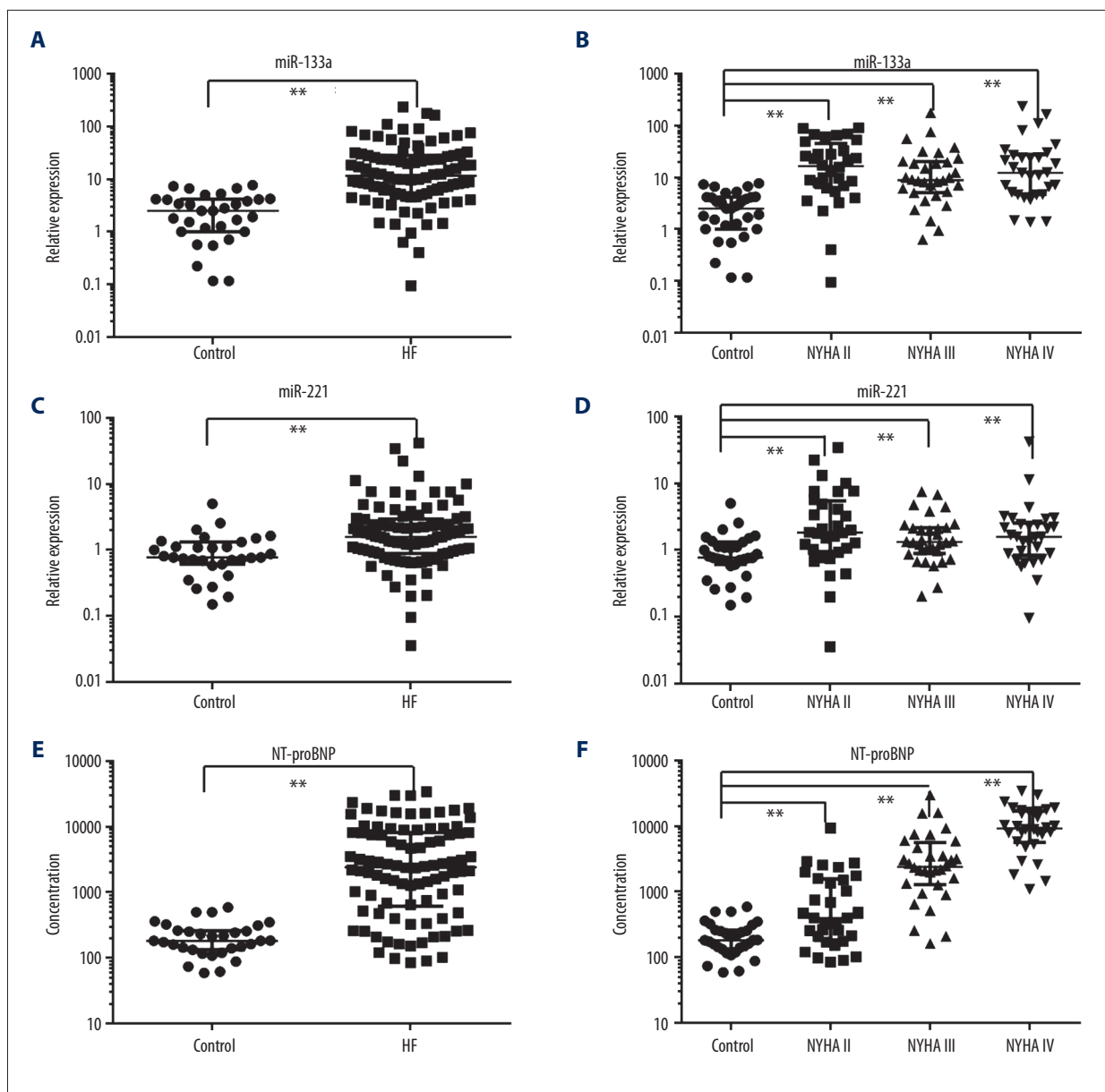
Figure 1. Comparison of miRNAs and NT-proBNP levels in HF patients and healthy controls. Differences between 2 independent groups were analyzed using a Mann-Whitney U test, while differences among multiple independent groups were analyzed using a Kruskal-Wallis test. (**A**) miR-133a levels in HF patients and healthy controls. (**B**) miR-133a levels in the NYHA II, NYHA III, NYHA IV, and healthy control groups. (**C**) miR-221a levels in HF patients and healthy controls. (**D**) miR-221 levels in the NYHA II, NYHA III, NYHA IV, and healthy control groups. (**E**) NT-proBNP levels in HF patients and healthy controls. (**F**) NT-proBNP levels in the NYHA II, NYHA III, NYHA IV, and healthy control groups. ** P<0.01.

while miR-221 expression was increased by 2.3-fold (*P*<0.001), 1.6-fold (*P*=0.008), and 2.0-fold (*P*=0.004), respectively. As expected, the concentrations of NT-proBNP in all HF groups were significantly higher than in the control group (Figure 1E, 1F).

Correlation of miR-133a and miR-221 with other clinical parameters

To identify possible correlations between miR-133a or miR-221 expression and other clinical parameters, Spearman rank correlation analysis was performed. The results showed that miR-133a is significantly correlated with NT-proBNP, CRP, LVEF and heart functional status (*P*<0.05), while miR-221 is only

Table 3. P values for the correlation coefficients between miRNAs or NT-proBNP levels and clinical parameters.

	miR-133a	miR-221	NT-proBNP
Gender	0.360	0.525	0.648
Age	0.507	0.465	0.004
BMI	0.288	0.803	0.026
NYHA	0.000	0.022	0.000
A	0.300	0.556	0.000
Hb	0.330	0.050	0.009
LVEF	0.000	0.061	0.000
BUN	0.473	0.581	0.000
Cr	0.125	0.304	0.000
CRP	0.001	0.149	0.000
NT-proBNP	0.002	0.147	N/A

NYHA – New York Heart Association; BMI – body mass index; A – albumin; Hb – hemoglobin; LVEF – left ventricular ejection fraction; BUN – blood urea nitrogen; Cr – creatinine; NT-proBNP – N-terminal pro-brain type natriuretic peptide; CRP – C-reactive protein.

significantly correlated with cardiac functional status ($P<0.05$; Table 3). Additionally, the biomarker NT-proBNP was found to be significantly correlated with age, BMI, BUN, albumin level, Hb level, CRP level, Cr level, LVEF, and heart functional status, but not sex.

Assessment of miR-133a and miR-221 diagnostic potential

To investigate whether miR-133a or miR-221 can discriminate between the HF and the control groups, receiver operating characteristic (ROC) curves were constructed for each miRNA based on the qRT-PCR data. The results indicated that both miRNAs depicted significant values for the area under the curve (AUC) (Figure 2A, 2B). The AUC value for miR-133a was 0.863 (CI95%: 0.800–0.927; $P<0.001$) and for miR-221 it was 0.718 (CI95%: 0.622–0.813; $P<0.001$). Furthermore, the diagnostic ability of miR-133a (81.9% sensitivity and 80.7% specificity) was higher than that of miR-211 (64.9% sensitivity and 74.2% specificity). The combined use of miR-133a and miR-221 slightly improved the diagnostic efficiency (AUC=0.871, Figure 2C). These findings were then compared to an ROC curve generated for NT-proBNP (Figure 2D). NT-proBNP levels showed an AUC value=0.895 (CI95%: 0.841–0.948; $P<0.001$), 73.4% sensitivity, and 100% specificity.

### Comparison of the diagnostic efficacy of NT-proBNP with and without the miRNAs

While neither miR-133a nor miR-221 are as effective as NT-proBNP as an HF diagnostic biomarker, the addition of either miRNA to NT-proBNP improved its diagnostic ability (Figure 2E, 2F). However, ROC curve analysis showed that miR-133a provided a greater enhancement than did miR-221. When combining miR-133a and NT-proBNP, the AUC value was 0.975 (CI95%: 0.950–0.999), which was significantly higher than NT-proBNP alone (z=2.395, P=0.016; Table 4). Furthermore, the combination of both miRNAs with NT-proBNP did not offer any substantial additional enhancement relative to the addition of miR-133a alone (Figure 2G, Table 4).

Discussion

This study investigated the levels of circulating miR-133a and miR-221 among elderly patients diagnosed with HF. The plasma miR-133a and miR-221 levels of the elderly HF patients were significantly upregulated relative to the healthy controls, and ROC curve analysis indicated significant AUC values for both miRNAs. Therefore, miR-133a and miR-221 can serve as potential HF diagnostic markers. However, the well-established biomarker NT-proBNP was shown to be more effective as an HF diagnostic biomarker than the examined miRNAs. However, combining the miRNAs with NT-proBNP significantly improved the diagnostic accuracy when compared to NT-proBNP alone.

The use of circulating miRNA-based biomarkers will enable a non-invasive method for comprehensive disease analysis. MiRNAs are highly stable in plasma and are readily detectable quantitatively using qRT-PCR and microarray assays [20]. Thus, circulating miRNAs may be potentially utilized as biomarkers in the diagnosis or prognosis of cardiovascular diseases, which include HF, hypertension, and myocardial infarction [21–23]. Previous studies have shown that specific plasma miRNAs are highly specific for cardiovascular pathologies and thus may be used for diagnostics and monitoring.
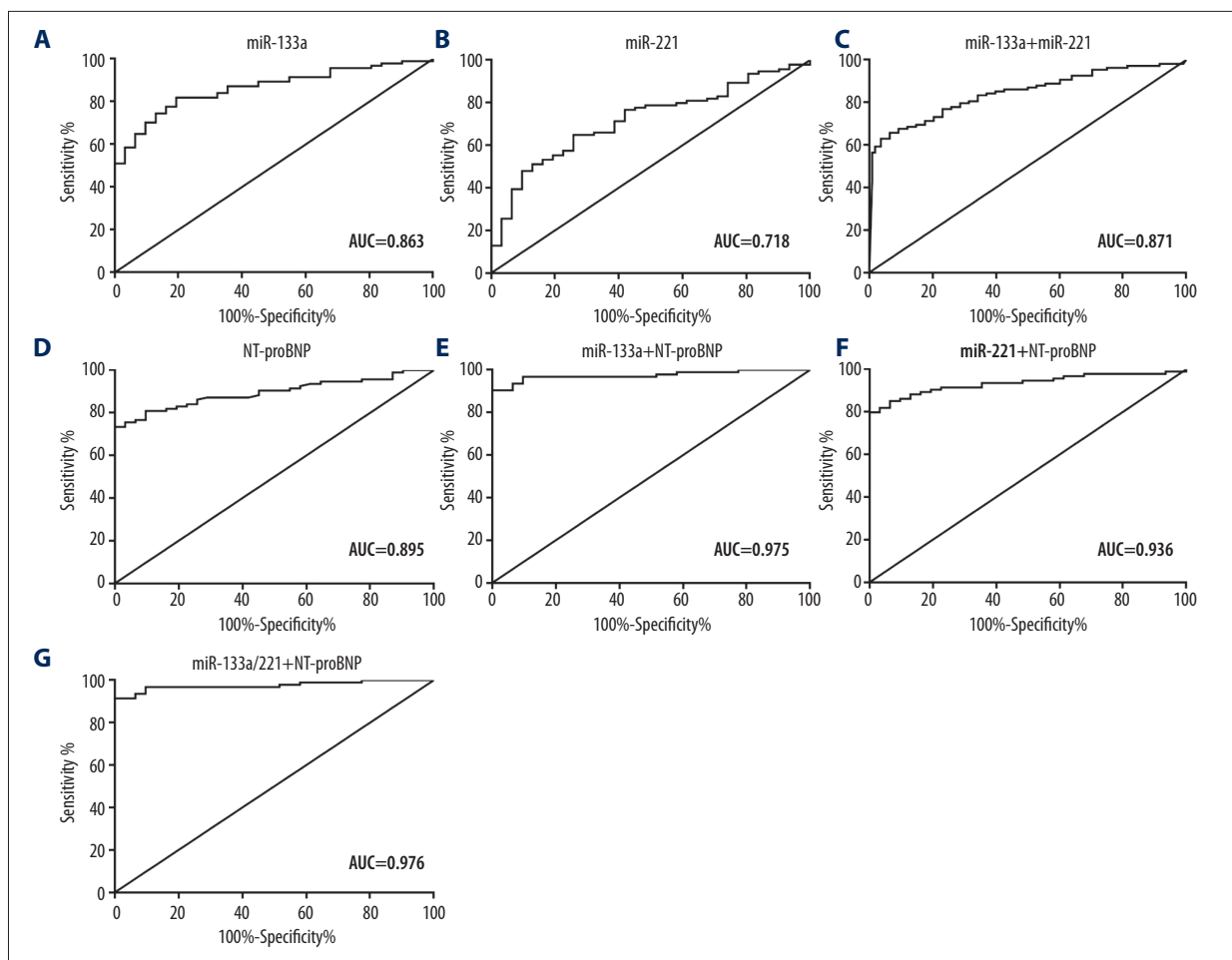
Figure 2. Receiver operating characteristic (ROC) analysis. ROC curves were generated for miR-133a (**A**), miR-221 (**B**), miR-133a combined with miR-221 (**C**), NT-proBNP (**D**), miR-133a combined with NT-proBNP (**E**), miR-221 combined with NT-proBNP (**F**), and miR-133a/221 combined with NT-proBNP (**G**).

Table 4. Comparison of the diagnostic efficacy of NT-proBNP with and without miRNAs.

Biomarkers	AUC ±SE (95%CI)	P	Sensitivity% (95%CI)	Specificity% (95%CI)	NT-proBNP Comparison
miR-133a	0.863±0.033 (0.800–0.927)	<0.001	81.9 (72.6–89.1)	80.7 (62.5–92.6)	P=0.201
miR-221	0.718±0.049 (0.622–0.813)	<0.001	64.9 (54.4–74.5)	74.2 (55.4–88.1)	P=0.007
NT-proBNP	0.895±0.027 (0.841–0.948)	<0.001	73.4 (63.3–82.0)	100 (88.8–100.0)	N/A
miR-133a + miR-221	0.871±0.032 (0.799–0.824)	<0.001	65.7 (56.0–74.6)	93.5 (87.1–97.4)	P=0.263
miR-133a + NT-proBNP	0.975±0.013 (0.950–0.999)	<0.001	90.4 (82.6–95.5)	100 (88.8–100.0)	P=0.016
miR-221 + NT-proBNP	0.934±0.021 (0.892–0.976)	<0.001	79.8 (70.3–87.4)	100 (88.8–100.0)	P=0.311
miR-133a/221 + NT-proBNP	0.976±0.012 (0.951–1.000)	<0.001	91.5 (83.9–96.3)	100 (88.8–100.0)	P=0.016

An earlier investigation involving HF patients indicated the upregulation of miR-423-5p in plasma samples and its association with NT-proBNP levels and NYHA classification [24]. Another study screened for changes in the levels of plasma miRNA of ischemic HF patients relative to healthy controls, which was inversely correlated to NT-proBNP and miR-126 levels [25]. Furthermore, plasma miR-126 levels were shown to increase when a patient's symptoms are ameliorated during a regression from class IV to class III [25].

Here, a relatively large sample size was utilized and the qRT-PCR results consistently showed that miR-133a and miR-221 were upregulated in the plasma of elderly HF patients. However, miR-133a had a better discriminatory ability when identifying HF than did miR-221, due to a higher specificity. In this study, HF patients were not further divided into HFpEF or HFrEF groups. One study found that miR-221 is upregulated in HFpEF, while it is downregulated in HFrEF [18]. This may explain the lower diagnostic efficiency of miR-221. While the confirmed diagnostic biomarker NT-proBNP performed better than miR-133a or miR-221, NT-proBNP was significantly affected by age, BMI, renal function, albumin, and Hb levels. Unlike NT-proBNP, miR-133a and miR-221 did not show any correlations with these factors. Furthermore, when combining these miRNAs with NT-proBNP, an improved HF diagnostic efficacy was obtained. These findings suggest that circulating miRNAs can further enhance the diagnostic abilities of NT-proBNP, and may facilitate an early and accurate diagnosis of HF and serve as an improved prognostic tool.

This study has several limitations. It had a relatively small study population. In addition, the population in this study was limited to the elderly. Although a previous study indicated that plasma miR-133a and miR-221 were not significantly associated with age in a healthy population [26], it is still necessary to evaluate the diagnostic performance of these 2 miRNAs in young and middle-aged heart failure patients.

Conclusions

Circulating miR-133a and miR-221 levels were significantly increased in elderly HF patients. Furthermore, unlike NT-proBNP, miR-133a and miR-221 were not affected by age, BMI, renal function, albumin, or Hb levels. More importantly, the combined use of miR-133a and NT-proBNP significantly improved the diagnostic accuracy when compared to NT-proBNP alone.

Acknowledgments

Conflicts of interest

None.

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