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Liver-specific microRNA-122 as prognostic biomarker in patients with chronic systolic heart failure☆

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ABSTRACT

Background: Circulating microRNAs (miRs) have been proposed as potential diagnostic biomarkers in heart failure. Studies investigating the prognostic value of circulating miRs in patients with chronic systolic heart failure (HFrEF) are scarce. The aim of this study was to investigate the prognostic value of circulating miRs in patients with HFrEF.

Methods and results: A pathway-focused microRNA array was performed in derivation case-control cohort of 40 patients with HFrEF who died during the follow-up (cases) and 36 survivors (controls). MicroRNA expression profiling revealed significant differential expression of miR-122, miR-126 and miR-423 between cases and controls. In a validation cohort, circulating levels of these 3 miRs were assessed using qPCR in 234 patients with HFrEF. Primary study endpoints were all-cause and cardiovascular mortality. During a median follow-up time of 3.2 years, 76 patients (32.5%) died. Only miR-122 and miR-423 were independent predictors of the primary endpoint with respective hazard ratios per increase of one standard deviation (HR per 1-SD) of 1.14 (95% CI: 1.02–1.29, p = 0.021) and 1.24 (95% CI: 1.09–1.41, p = 0.001). Adding miR-122 to multivariable model including clinical risk factors and NT-proBNP improved net reclassification index (NRI) by 40.4% (p = 0.004), whereas miR-423 improved NRI by 35.3% (p = 0.012). Adding miR-122, but not miR-423, to the same model improved Harrell's C index from 0.78 (95% CI: 0.73–0.83) to 0.81 (95% CI: 0.76–0.86, p = 0.030).

Conclusion: Circulating miR-122 as a biomarker is predicting all-cause and cardiovascular mortality and improved risk stratification of HFrEF patients. Thus, miR-122 might be a new biomarker for risk assessment in HFrEF.

1. Introduction

Heart failure represents a global health problem [1]. In past decades, mortality rates of acute myocardial infarction, valvular heart disease, and hypertension substantially decreased. At the same time, the prevalence of chronic systolic heart failure (HFrEF) is steadily rising in the aging population [2] and despite significant improvements in management and treatment of HFrEF, the prognosis remains poor in this group of patients, with 5-years survival worse than in most cancers [1]. Consequently, there is a need for improved risk stratification and identification of high-risk patients.

MicroRNAs (miRs) emerged as novel class of biomarkers in cardiovascular disease. Circulating miRs were proposed as biomarkers in coronary artery disease, peripheral artery disease, as well as in acute and chronic heart failure [3–5]. These small, non-coding RNAs regulate crucial pathophysiological processes in HFrEF such as calcium cycling, ventricular remodelling with development of ventricular hypertrophy and fibrosis, contributing ultimately to impaired left ventricular contractility [6]. Circulating miRs are differentially expressed depending on aetiology of HFrEF [7] Several studies demonstrated previously that circulating miRs could distinguish patients with heart failure from other forms of dyspnoea [5,8,9]. Furthermore, p53-responsive miRs predict development of heart failure after acute myocardial infarction [10]. However, studies investigating the prognostic value of circulating miRs in HFrEF are scarce [11,12]. Therefore, we aimed here to analyse the role of circulating miRs as prognostic biomarkers in patients with HFrEF.

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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2. Methods

2.1. Study population

All study participants provided written informed consent. The study protocol complied with the Declaration of Helsinki and was approved by the ethics committee of the Medical University of Vienna. A flow chart of the study design is depicted in Supplemental Fig. 1.

2.1.1. Derivation cohort

Profiling of circulating miRs in patients with HFrEF was performed in 76 patients enrolled at the outpatient department for heart failure at the Medical University of Vienna. Forty cases of non-survivors and 36 survivor controls with similar clinical risk profile were selected for miRs expression profiling. In order to avoid possible confounders on circulating miRs expression profile due to different HFrEF aetiology [7], equal number of cases (non-survivors) and controls (survivors) had ischemic HFrEF.

2.1.2. Validation cohort

Patients in a stable phase of HFrEF were consecutively enrolled at the outpatient department for heart failure at the Medical University of Vienna between January 2008 and July 2013. Inclusion criteria were defined as clinical signs of HFrEF (New York Heart Association (NYHA) functional class ≥2) and either levels of N-terminal pro B-type natriuretic peptide (NT-proBNP) of >500 pg/ml or a left ventricular ejection fraction of <40%. Exclusion criteria were presence of a non-cardiovascular comorbidity reducing life expectancy to <2 years, chronic inflammatory diseases, <18 years of age, and refusal to provide informed consent. Baseline characteristics were assessed by professional health care employees using a standardized patient questionnaire.

2.2. Study endpoints

Primary study endpoints were defined as all-cause mortality and cardiovascular mortality. Data for outcomes were assessed by scanning the national death registry (Statistic Austria) and crosschecking the local electronic clinical database. Death certificates of decedents were obtained to classify as cardiovascular and non-cardiovascular causes of death, using International Classification of Disease-10th Revision criteria.

2.3. Screening phase - microRNA expression profiling

Profiling of circulating miRs in patients with HFrEF was performed in 76 patients, 40 non-survivors and 36 survivors. The expression profile of circulating miRs was determined using pathway-specific miScript miRNA PCR Array Human Cardiovascular disease (Qiagen).

2.4. Validation phase - microRNA preparation and quantification

In the validation phase, differentially expressed miRs from screening phase were prospectively assessed in 234 consecutive patients with HFrEF using quantitative real-time PCR (qPCR) as previously described [4,13]. Blood samples for miRs assessment were taken at single time point, namely at baseline. Detailed description of miRs preparation, expression profiling and quantification can be found in Supplemental material.

2.5. Statistical analysis

Categorical variables are summarized as counts and percentages and are compared by the $\chi 2$-test or by Fisher's exact test as appropriate. Continuous variables are expressed as median and interquartile range (IQR) and compared by the *t*-test or by the Mann-Whitney *U* test in case of non-normal distribution. Univariate and multivariable linear regression models were performed to evaluate the marginal and partial impact of the variables age, sex, NYHA functional class, left ventricular ejection fraction (LVEF), right ventricular dysfunction, NT-proBNP, glomerular filtration estimated by MDRD, C-reactive protein (CRP), myocardial infarction, hypertension, stroke, atrial fibrillation, hypercholesterolemia and diabetes mellitus on the miR-122 and miR-423 levels. Univariate and multivariable Cox proportional hazard regression models were fit to assess whether the miRs expression profile could significantly predict all-cause and cardiovascular mortality. The Benjamini-Hochberg procedure with false discovery rate of 0.1 was applied to adjust for multiple comparisons. Only miRs that remained significant after Benjamini-Hochberg adjustment in univariate Cox regression were entered in multivariable models. Hazard ratios (HR) are given as HR per increase of one standard deviation (HR per 1-SD). Due to their skewed distributions log2-transformed values of miRs concentrations were used within all regression models. Optimal miRs cut-off values were calculated using Cut-off Finder's significance of correlation with survival variable (http://molpath.charite.de/cutoff), as previously described [14]. The optimal cut-off is defined as the point with the most significant log-rank test split. Kaplan-Meier survival plots were constructed in groups according to miRs expression above or below the cut-off value to compare time-dependent discriminative power of circulating miRs. Category-free net reclassification improvement (NRI) was calculated to estimate an improvement in individual risk prediction for the addition of miR-122 and miR-423 to established clinical risk factors and NT-proBNP. In addition, Harrell's C-statistic was applied to further evaluate incremental predictive power of circulating miRs when added to established clinical risk factors and NT-proBNP. Two-sided p-values of ≤0.05 indicated statistical significance. SPSS 22.0 (IBM Corporation, Armonk, NY, USA) and STATA version 12 (StataCorp LLC, College Station, TX, USA) were used for all statistical analyses.

3. Results

3.1. Screening phase

Pathway-focused microRNA array was performed in pooled plasma samples of 40 patients with HFrEF who died during the follow-up (cases) and 36 survivors (controls). Supplemental Table 1 shows clinical characteristics of the screening cohort. Out of 84 analysed miRs, only miR-122, miR-126 and miR-423 were differentially expressed between 2 groups of patients (Supplemental Fig. 2).

3.2. Validation phase

In the second step of the study, quantitative expression of miR-122, miR-126 and miR-423 was prospectively assessed in plasma samples of 234 consecutive patients with HFrEF admitted at outpatient department.

Baseline clinical characteristics of the validation cohort are given in Table 1. Median age was 65 years, 80% of patients were male, and 47.5% of patients had ischemic HFrEF. Almost all patients received beta-blocker therapy (96.6%), over 80% of patients were on angiotensin-converting enzyme inhibitors, and 2/3 of patients had aldosterone antagonists (Table 1). During a median follow-up time of 3.2 years, 76 patients (32.5%) died. Of these, 52 patients (22.2%) died of cardiovascular death and 24 patients (10.2%) died a non-cardiovascular death. Patients who died during the follow-up were older, had higher NYHA functional class and NT-proBNP at study admission, worse renal function, and had more often diabetes and prior stroke.

Baseline concentrations of circulating miRs were determined as described in Methods section. Median concentrations of circulating miR-122, miR-126 and miR-423 are given in Supplemental Table 2.

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Table 1
Baseline clinical characteristics of patient population in the validation phase.

Baseline characteristics	Overall ($n = 234$)	Non-survivor ($n = 76$)	Survivor ($n = 158$)	p-Value
Demographics				
Age (years)	65.1 (56.5–71.5)	67.7 (60.4–75.8)	62.7 (53.2–70.2)	<0.001
Male sex	191 (81.6)	67 (88.2)	124 (78.5)	0.073
BMI	28.4 (25.3–31.6)	27.3 (25.2–31.2)	28.7 (25.3–31.9)	0.249
Active smoking	47 (20.1)	14 (18.4)	33 (20.9)	0.659
Ischemic HFrEF aetiology	107 (45.7)	41 (53.9)	66 (41.8)	0.080
NYHA class				
II	131 (56)	24 (31.6)	107 (67.7)	
III	98 (41.9)	47 (61.8)	51 (32.3)	
IV	5 (2.1)	5 (100)	0 (0)	<0.001
LVEF				
41–50%	79 (33.8)	23 (30.3)	56 (35.4)	
31–40%	79 (33.8)	27 (35.5)	52 (32.9)	
≤30%	76 (32.5)	26 (34.2)	50 (31.6)	0.735
Medical history				
Myocardial infarction	93 (39.7)	34 (44.7)	59 (37.3)	0.279
Hypertension	181 (77.4)	62 (81.6)	119 (75.3)	0.284
Hypercholesterolemia	164 (70.1)	56 (73.7)	108 (68.4)	0.404
Diabetes mellitus	86 (36.8)	36 (47.4)	50 (31.6)	0.019
Stroke	18 (7.7)	12 (15.8)	6 (3.8)	0.001
Atrial fibrillation	102 (43.6)	39 (51.3)	63 (39.9)	0.098
Medication				
Beta blockers	226 (96.6)	74 (97.4)	152 (96.2)	0.646
ACE-inhibitors	189 (80.8)	59 (77.6)	130 (82.3)	0.398
ARB-blockers	108 (46.2)	41 (53.9)	67 (42.4)	0.097
Digoxin	35 (15)	16 (21.1)	19 (12)	0.070
Aldosterone antagonists	153 (65.4)	52 (68.4)	101 (63.9)	0.498
Diuretics	116 (49.6)	55 (72.4)	61 (38.6)	<0.001
Statins	146 (62.4)	52 (68.4)	94 (59.5)	0.187
OAC	108 (46.2)	39 (51.3)	69 (43.7)	0.272
Antiplatelet	100 (42.7)	40 (52.6)	60 (37.9)	0.034
Laboratory parameters				
NT-proBNP	1134 (442–2418)	2315 (1061–4026)	790 (355–1561)	<0.001
CRP	0.3 (0.1–0.6)	0.4 (0.2–0.7)	0.2 (0.1–0.6)	0.046
eGFR (MDRD)	61.2 (45.4–77.8)	49.8 (38.1–63.2)	68.5 (50.3–80.5)	<0.001
Cholinesterase	7.7 (6.6–8.9)	6.8 (5.3–8.4)	7.99 (6.9–9.1)	<0.001
γ-GT	48 (28–91.5)	64.5 (33–161.7)	42 (26–71.7)	<0.001
AST	26 (21–33)	24.5 (20–34)	27 (21−32)	0.274
ALT	25 (18–38)	23 (17–32.7)	27 (18.7–40.2)	0.019

Continuous data are shown as median (interquartile range). Dichotomous data are shown as n (%). BMI, body mass index; HFrEF, heart failure with reduced ejection fraction; NYHA, New York heart association functional classification; LVEF, left ventricular ejection fraction; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blockers; OAC, oral anticoagulation. NT-proBNP, N-terminal pro B-type natriuretic peptide; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine transaminase.

Non-survivors had significantly higher circulating levels of miR-122 and miR-423 as compared to survivors. Concentration of circulating miR-126 was similar in both groups (Supplemental Table 2).

Both miR-122 and miR-423 were positively associated with NT-proBNP in univariable and multivariable linear regression models (Supplemental Table 3). In addition to NT-proBNP, miR-122 was significantly associated with right ventricular dysfunction in multivariable regression model, whereas miR-423 showed significant association with previous myocardial infarction (Supplemental Table 3).

Cox proportional hazard regression models were assessed to investigate the predictive value of circulating miRs for all-cause and cardiovascular mortality. Both miR-122 and miR-423 predicted all-cause mortality with respective HR per 1-SD 1.19 (95% CI: 1.08–1.33; p = 0.001) and HR per 1-SD 1.21 (95% CI: 1.07–1.36; p = 0.002) in univariate Cox regression analysis (Table 2). Furthermore, miR-122 and miR-423 predicted cardiovascular mortality in univariate Cox regression model with respective HR per 1-SD of 1.18 (95% CI, 1.04–1.34; p = 0.011) and HR per 1-SD of 1.18 (95% CI, 1.02–1.36; p = 0.021; Table 2). In contrast, miR-126 showed no association with all-cause and cardiovascular mortality in univariate Cox regression analysis (HR per 1-SD 1.01; 95% CI: 0.84–1.21; p = 0.915; and HR per 1-SD 1.00; 95% CI: 0.99–1.01; p = 0.988; respectively; Table 2).

Table 2
Prognostic value of circulating miRs for all-cause and cardiovascular mortality in patients with HFrEF.

	All-cause mortality			Cardiovascular mortality		
	HR per 1-SD	95% CI	p-Value	HR per 1-SD	95% CI	p-Value
Univariable						
miR-122	1.19	1.08–1.33	0.001	1.18	1.04–1.34	0.011
miR-126	1.01	0.84–1.21	0.915	1.00	0.99–1.01	0.988
miR-423	1.21	1.07–1.36	0.002	1.18	1.02–1.36	0.021
Multivariable model 1						
miR-122	1.21	1.08–1.35	0.001	1.19	1.04–1.36	0.010
miR-423	1.21	1.07–1.38	0.003	1.18	1.01–1.37	0.031
Multivariable model 2						
miR-122	1.16	1.03–1.28	0.012	1.13	0.99–1.29	0.069
miR-423	1.21	1.07–1.42	0.006	1.20	1.02–1.41	0.030
Multivariable model 3						
miR-122	1.15	1.02–1.29	0.021	N/A	N/A	N/A
miR-423	1.27	1.10–1.46	0.001	1.22	1.03–1.45	0.020

Model 1: adjusted for age and sex; Model 2: Model 1 plus NYHA functional classification, LVEF, estimated glomerular filtration rate, type 2 diabetes, BMI, and NT-proBNP; Model 3: Model 2 plus right ventricular dysfunction, cholinesterase, gamma-GT and previous myocardial infarction; miR, microRNA; HR per 1-SD, Hazard ratio per one increase of standard deviation; CI, confidence interval; N/A, not performed since miR-122 was not significantly associated with cardiovascular mortality in multivariable model 2.

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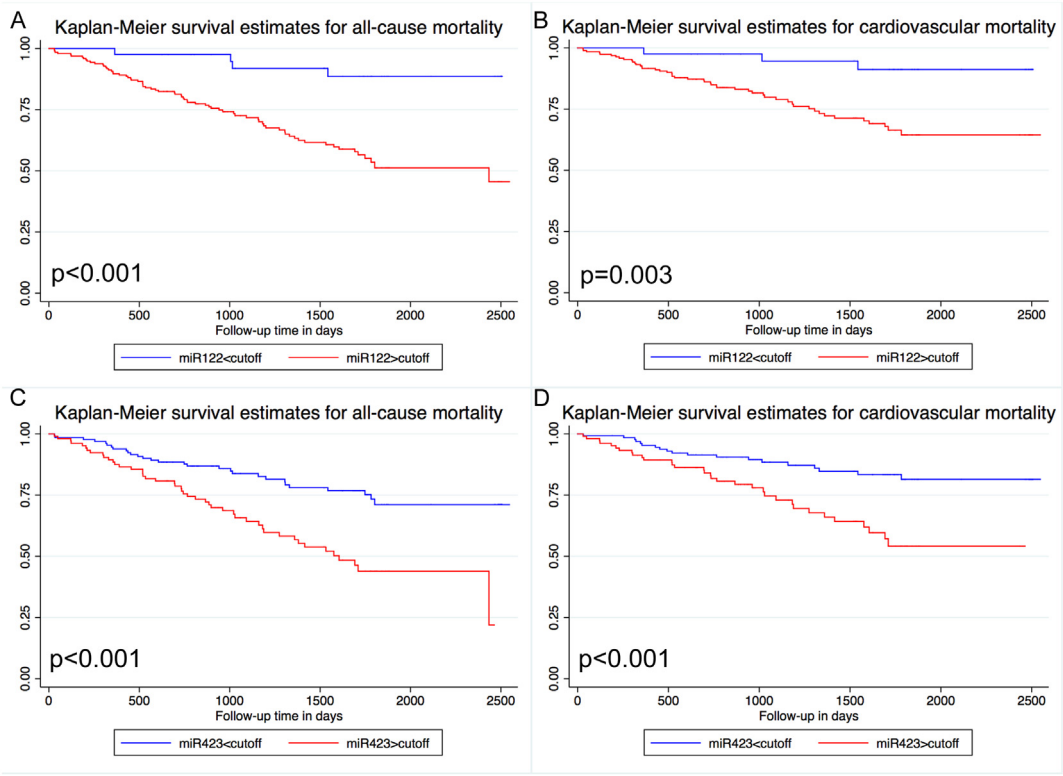

Fig. 1. Kaplan–Meier survival curves for all-cause and cardiovascular mortality. Panels A) and B) depict survival in groups according to baseline miR-122. Panels C) and D) depict survival in groups according to baseline miR-423. The groups with circulating miRs above the cut-off are indicated by the red lines, and blue lines indicate the groups with miRs levels below the cutoff.

Three multivariable models were fit to assess the prognostic value of circulating miR-122 and miR-423 (Table 2). Model 1 was adjusted for age and sex. Both miR-122 and miR-423 independently predicted all-cause and cardiovascular mortality after adjustment for age and sex (Table 2). Model 2 was adjusted for age, sex and the most important clinical risk factors in HFrEF. After adjustment for age, sex, NYHA functional class, LVEF, MDRD, diabetes, BMI, and NT-proBNP, both miR-122 and miR-423 remained significant independent predictors of all-cause mortality with an adjusted HR per 1-SD of 1.16 (95% CI: 1.03–1.28; $p = 0.012$) and HR per 1-SD of 1.21 (95% CI: 1.07–1.42; $p = 0.006$; Table 2), respectively. miR-423 was also an independent predictor of cardiovascular mortality with an adjusted HR per 1-SD of 1.20 (95% CI: 1.02–1.41; $p = 0.030$; Table 2). There was a tendency for miR-122 to independently predict cardiovascular mortality, however it did not reach statistical significance (adjusted HR 1.13; 95% CI: 0.99–1.29; $p = 0.069$; Table 2). Furthermore, miR-122 remained independent predictor for all-cause mortality with HR per 1-SD of 1.15 (95% CI: 1.02–1.29; $p = 0.021$) when right ventricular dysfunction, cholinesterase, gamma-glutamyl transferase (gamma-GT) and previous myocardial infarction were added to multivariable model 2 (multivariable model 3, Table 2). Similarly, miR-423 independently predicted all-cause mortality (HR per 1-SD 1.27; 95% CI: 1.10–1.46; $p = 0.001$) and cardiovascular mortality (HR per 1-SD 1.22; 95% CI: 1.03–1.45; $p = 0.020$) after further adjustment for right ventricular dysfunction, cholinesterase, gamma-GT, and previous myocardial infarction (multivariable model 3, Table 2).

Optimal cut-off values to predict all-cause mortality were calculated for miR-122 and miR-423 as described in methods. Circulating miR-122 was above the threshold of 12 copies/μl plasma in 193 patients (82.5%). Patients with miR-122 above the cut-off had higher all-cause and cardiovascular mortality rates as compared to patients with miR-122 below the cut-off (log-rank: $p < 0.001$ and $p = 0.003$, Fig. 1A and B). Circulating miR-423 was above the cut-off of 3694 copies/μl plasma in 104 patients (44.4%). Patients with circulating miR-423 above the cut-off had higher all-cause and cardiovascular mortality rates as compared to patients with miR-423 below the cut-off (log-rank: $p < 0.001$ and $p < 0.001$, Fig. 1C and D). As depicted in Supplemental Fig. 3A and B, highest all-cause and cardiovascular mortality rates were observed in patients with both miRs above the respective cut-off values (log-rank: $p < 0.001$ and $p = 0.001$).

Risk factors included in a multivariable Cox regression model (age, sex, LVEF, NYHA functional class, MDRD, BMI, and NT-proBNP)

t3.1 **Table 3**
t3.2 miR-122 adds prognostic information on top of clinical risk factors and N-terminal pro-brain natriuretic peptide.

	All-cause mortality			Cardiovascular mortality		
	AUC	95% CI	p-Value	AUC	95% CI	p-Value
Clinical risk factors, and NT-proBNP	0.78	0.73–0.83		0.76	0.69–0.83	
Multivariable model and miR-122	0.80	0.75–0.85	0.030[a]	0.80	0.73–0.86	0.008[a]
Multivariable model and miR-423	0.79	0.74–0.84	0.259[a]	0.77	0.71–0.84	0.413[a]
Multivariable model	0.81	0.76–0.86	0.018[a]	0.80	0.74–0.86	0.017[a]
miR-122 and miR-423			0.319[b]			0.760[b]

t3.10 Multivariable model: age, sex, NYHA functional classification, LVEF, estimated glomerular filtration rate, BMI, and NT-proBNP.
t3.11 [a] As compared to multivariable model.
t3.12 [b] As compared to multivariable model and miR-122; miR, microRNA; CI, confidence interval.

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predicted all-cause mortality with an area under the curve (AUC; Harrell's C-Statistic) of 0.78 (95% CI: 0.73–0.83; Table 3). When miR-122 was added to this model, AUC improved significantly to 0.80 (95% CI: 0.75–0.85; p = 0.030; Table 3). In contrast, there was no improvement of the AUC when miR-423 was added to the same model (AUC = 0.79; 95% CI: 0.74–0.84; p = 0.259). Adding both miR-122 and miR-423 to a multivariable model significantly improved the AUC to 0.81 (95% CI: 076–0.86; p = 0.018). However, adding both miR-122 and miR-423 had no incremental value as compared to miR-122 alone (p = 0.319; Table 3). Similar results were observed for cardiovascular mortality. As shown in Table 3, adding miR-122, but not miR-423, resulted in significant improvement of the AUC from 0.76 (95% CI: 0.69–0.83) to 0.80 (95% CI: 0.73–0.86; p = 0.008). Furthermore, miR-122 and miR-423 showed significant improvements in NRI for all-cause mortality of 40.4% (p = 0.004) and 35.3% (p = 0.012), respectively (Supplemental Table 4). Regarding cardiovascular mortality, miR-423 showed tendency to improve NRI by 29.1%, but it did not reach statistical significance (p = 0.064; Supplemental Table 4).

4. Discussion

The study aimed to identify prognostic relevant circulating miRs in HFrEF and determine their predictive value compared to traditional risk factors and biomarkers in a representative cohort of patients. By performing pathway specific arrays in the screening cohort, we identified 3 candidate miRs, which were further evaluated in the validation cohort. We could show for the first time that miR-122 and miR-423 are independent predictors of all-cause and cardiovascular mortality in patients with HFrEF. Finally, liver-specific miR-122 added prognostic information on top of clinical risk factors and NT-proBNP, and represents a promising complementary biomarker for improved risk stratification in this group of patients.

MiR-122 is a liver-specific microRNA and accounts for >70% of total liver miRs-expression [15]. It regulates cholesterol and fatty acid metabolism [15]. The liver constantly secretes miR-122 into circulation and production of miR-122 is increased upon liver injury [16]. Furthermore, miR-122 has been associated with the risk of developing metabolic syndrome and type 2 diabetes, and statin treatment reduces circulating miR-122 [17]. Interestingly, miR-122 is elevated in acute heart failure as compared to control subjects [18]. In the present study baseline miR-122 circulating levels were higher in patients who died during the follow-up and miR-122 was an independent predictor of all-cause mortality in HFrEF. Increased central venous pressure in right-sided heart failure leads to liver congestion with subsequent atrophy of hepatocytes and liver fibrosis. We could show here that miR-122 is associated with right ventricular dysfunction, and that miR-122 remained an independent predictor of all-cause mortality in HFrEF after adjustment for right ventricular dysfunction, cholinesterase and gamma-GT. We hypothesize that increased levels of miR-122 in HFrEF might reflect beginning liver injury and congestion due to right sided heart failure. Early identification of liver injury is of particular clinical relevance, since liver failure with increased transaminases, prolonged prothrombin time and increased bilirubin in end-stage HFrEF is associated with poor outcome [15,19]. Furthermore, we observed an association of miR-122 with NT-proBNP, the current gold standard for diagnosis and prognosis in HFrEF [20]. In line with this finding, Marques et al. observed an elevated transcardiac gradient suggesting the injured myocardium as an own source of miR-122. However, whether cardiac or hepatic miR-122 production or both contributes the most to the pool of circulating miR-122 in patients with heart failure remains to be answered in further studies [21]. Most importantly, miR-122 turned out as an independent predictor of all-cause mortality in HFrEF after adjustment for NT-proBNP. As each new marker has to add prognostic information on top of currently used parameters, we show here that miR-122 improved both Harrell's C-index and NRI when added to clinical model and provided prognostic information on top of advanced age, reduced LVEF, more severe NYHA functional class, impaired renal function and NT-proBNP. Thus, assessment of miR-122 might potentially reflect involvement of different pathophysiological pathways relevant for clinical outcome in HFrEF.

Several studies suggested a role of miR-432 in acute and chronic heart failure. Expression of miR-423 is increased in failing human myocardium [22]. Tijsen et al. have demonstrated that miR-423 is increased in patients with heart failure and could be used as diagnostic biomarker to differentiate heart failure-induced dyspnoea from non-heart failure-induced dyspnoea [5]. This observation was confirmed in several other studies, demonstrating that miR-423 can discriminate heart failure from other diagnosis with high sensitivity and specificity, either alone or in combination with other biomarkers [9,23]. Furthermore, miR-423 was associated with hypertension-induced heart failure in rats [24]. In contrast, miR-423 failed as biomarker for left ventricular remodelling after myocardial infarction [25]. A recent study showed association of circulating miR-423 with outcome in acute heart failure, but this association was lost after further adjustment for clinical risk factors [26]. In the present study, all patients with HFrEF had high circulating levels of miR-423. We could show that patients who died during the follow-up had higher circulating levels of miR-423, and that miR-423 is an independent predictor of all-cause and cardiovascular mortality in HFrEF. Furthermore, miR423 was associated with NT-proBNP and previous myocardial infarction, but not with LVEF which is in agreement with previous studies [5,24,25]. In addition, it was previously demonstrated that circulating miR-423 is associated with aetiology of HFrEF and to early worsening of renal function in patients with acute heart failure [7,27]. When miR-423 was added to most important clinical risk factors and NT-proBNP it improved NRI for all-cause and cardiovascular mortality, but there was no significant improvement of Harrell's C-index. One could speculate that because of association of miR-423 with multiple prognostic risk factors, there is limited additional benefit of assessing circulating miR-423 for risk stratification in HFrEF.

Since highest mortality rates were observed in a group of patients with high circulating miR-122 and miR-423, we evaluated if assessment of both miRs can further improve risk stratification. We could show here that simultaneous assessment of miR-122 and miR-423 did not further improve the predictive value compared to miR-122 alone.

A previous study reported lower circulating levels of miR-126 in patients with ischemic heart failure and an inverse correlation with BNP [28]. Loss of miR-126 in HFrEF might have a negative impact on neovascularization and cardiac repair capacity [29]. In the present study, there was no association of circulating miR-126 with all-cause and cardiovascular mortality in patients with HFrEF. According to our data, miR-126 may not be suitable as prognostic biomarker in patients with HFrEF.

4.1. Limitations

This study had several limitations. We have screened for 84 circulating miRs previously implicated in cardiovascular disease. We cannot exclude that other miRs may also have prognostic value in this setting. We used cel-miR-39 spike-in control for normalization, as previously described [4,13]. Currently, there are no generally accepted standards for normalization, and using other normalization approaches might result in differently normalized data. The described association of miR-122 with right ventricular dysfunction is hypothesis generating only, and should be confirmed in prospective case-control studies. Furthermore, blood samples for miRs assessment were taken at single time point, namely at baseline. Thus, further insight into disease progression or response to therapy based on possible dynamic changes of miRs expression over time in HFrEF cannot be gained from our study.

5. Conclusion

In conclusion, we could identify miR-122 and miR-423 as independent prognostic biomarkers in HFrEF. Moreover, liver-specific miR-122

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adds prognostic information on top of clinical risk factors and NT-proBNP in HFrEF. Assessment of circulating miR-122 could offer refined risk stratification when added to current gold standard NT-proBNP. Thus, miR-122 might be novel, easily accessible complementary biomarker for improved risk stratification in patients with HFrEF.

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Declaration of competing interest

The authors report no relationships that could be construed as a conflict of interest. Manuel Mayr is named inventor on a patent held jointly by the Medical University of Innsbruck and King's College London for the use of miR-122 as biomarker for metabolic syndrome.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.11.090.

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