International Journal of Cardiology 203 (2016) 564–569

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

MicroRNAs relate to early worsening of renal function in patients with acute heart failure☆

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ARTICLE INFO

Article history:
Received 12 May 2015
Received in revised form 9 October 2015
Accepted 27 October 2015
Available online 11 November 2015

Keywords:
Circulating microRNA
Acute heart failure
Worsening renal function

ABSTRACT

Background: Deregulation of microRNAs (miRNAs) may be involved in the pathogenesis of heart failure (HF) and renal disease. Our aim is to describe miRNA levels related to early worsening renal function in acute HF patients.
Method and results: We studied the association between 12 circulating miRNAs and Worsening Renal Function (WRF; defined as an increase in the serum creatinine level of 0.3 mg per deciliter or more from admission to day 3), absolute change in creatinine and Neutrophil Gelatinase Associated Lipocalin (NGAL) from admission to day 3 in 98 patients hospitalized for acute HF.
At baseline, circulating levels of all miRNAs were lower in patients with WRF, with statistically significant decreased levels of miR-199a-3p, miR-423-3p, and miR-let-7i-5p (p-value < 0.05). The increase in creatinine during the first 3 days of hospitalization was significantly associated with lower levels of miR-199a-3p, miR-27a-3p, miR-652-3p, miR-423-5p, and miR-let-7i-5p, while the increase in NGAL was significantly associated with lower levels of miR-18a-5p, miR-106a-5p, miR-223-3p, miR-199a-3p and miR-423-3p. MiR-199a-3p was the strongest predictor of WRF, with an Odds Ratio of 1.48 (1.061–2.065; p-value = 0.021) and a C-index of 0.701.
Conclusions: Our results show that the levels of circulating miRNAs at hospital admission for acute HF were consistently lower in patients who developed worsening of renal function. MiR-199a-3p was the best predictor of WRF in these patients.

1. Introduction

MicroRNAs (miRNAs) are involved in the regulation of several cell processes, including proliferation, stress response, inflammation and apoptosis [1]. Their deregulation might therefore play a role in the pathogenesis of heart failure (HF) [2,3]. This is supported by studies showing that the expression levels of miRNAs change significantly with disease severity [4]. In addition, specific miRNA patterns were related to the diagnosis and prognosis in HF [5–8]. We recently showed that certain

☆ 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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http://dx.doi.org/10.1016/j.ijcard.2015.10.217
0167-5273/

circulating miRNAs were significantly reduced in acute HF (rapid onset of signs and symptoms associated with volume overload requiring immediate treatment) in comparison with chronic HF (signs and symptoms caused by abnormalities of the heart) patients and healthy controls and that they were associated with a higher risk of mortality at 180 days [9].

Renal impairment is common in patients with acute HF [10]. Worsening of renal function (WRF) in HF patients is the consequence of multiple mechanisms including renal hypoperfusion, increased renal venous and intra-abdominal pressure, neurohormonal and inflammatory activation, and drug therapy for HF [11]. Several definitions of WRF have been used in literature, some of the most common being an increase in creatinine ≥0.3 mg/dL, or an increase in GFR >20% [10,12].

MiRNAs play a role in renal development, maintenance of renal function, and progression of kidney injury [13]. MiRNAs are thought to be key regulators of kidney responses to acute damage, such as acute kidney injury following renal Ischemia/Reperfusion (I/R) due to hypoperfusion as in acute HF [14]. However, to date, the association between miRNAs and changes in renal function in patients with acute HF has not been studied. In the present study, we describe circulating miRNA levels that are associated to early changes in renal function in patients with acute decompensated HF.

2. Methods

2.1. Population and study procedure

This study is a post hoc case control analysis of the Placebo-controlled Randomized Study of the Selective A1 Adenosine Receptor Antagonist Rolofylline for Patients Hospitalized with acute HF and Volume Overload to Assess Treatment Effect on Congestion and Renal Function (PROTECT) trial, a multicenter, randomized, double-blind, placebo-controlled trial with neutral results. Study design and main results have been published previously [15,16]. The trial was approved by the local Ethics Committee and performed in accordance with the Declaration of Helsinki. Written informed consent was provided by all patients. One hundred patients hospitalized for acute HF with mild to moderate renal impairment were randomly selected. Two patients were excluded from the analyses since renal function variables were not assessed. Medical history and physical examination were recorded at admission. Serum creatinine and Neutrophil Gelatinase Associated Lipocalin (NGAL) were assessed daily from baseline until discharge or Day 6. For NGAL measurements, plasma samples were frozen at −80 °C. NGAL levels were detected by Alere Inc. (San Diego, USA), through sandwich enzyme-linked immunosorbent assays (ELISA) on a microtiter plate. From a panel of 375 circulating miRNAs, twelve indicative miRNAs (miR-let-7i-5p, miR-16-5p, miR-18a-5p, miR-26b-5p, miR-27a-3p, miR-30e-5p, miR-106a-5p, miR-199a-3p, miR-223-3p, miR-423-3p, miR-423-5p, miR-652-3p) were selected on the basis of their distinctive profile in patients affected by acute HF, as studied previously by our group [9]. These miRNAs were identified in independent discovery and validation cohorts, ranging from patients with acutely decompensated HF to patients with stable chronic HF and healthy controls. From fifteen most significantly different miRNAs characterized in the discovery cohort, these 12 miRNAs were confirmed to have reduced levels in the acute HF patients of the validation cohort. In particular, miR-18a-5p, miR-26b-5p, miR-27a-3p, miR-30e-5p, miR-106a-5p, miR-199a-3p and miR-652-3p passed the Bonferroni correction threshold and were significantly lower in acute HF patients compared with healthy controls. In our study, miRNA levels were measured in plasma on the first day of hospitalization. Early worsening renal function (WRF) was defined as an increase in the serum creatinine level of 0.3 mg per deciliter (26.5 μmol per liter) or more on day 3 [17]. Absolute change of serum creatinine and NGAL was calculated as difference between their value on day 3 and baseline.

2.2. RNA isolation and quantitative real-time PCR of CIRCULATING microRNAs

RNA isolation, reverse transcription reactions and real-time Polymerase Chain Reaction (PCR) were performed according to the manufacturer's protocol (Exiqon, Vedbaek, Denmark), using respectively the miRCURY RNA isolation kit-Biofluids for liquid samples, the Universal cDNA Synthesis Kit and a LC480 instrument.

Initial data was analyzed with the Exiqon GenEx qPCR analysis software to obtain raw Threshold Cycles (CT) values. CT values indicate the fractional cycle number at which the amount of an amplified target reaches a fixed threshold [18]. The Delta-CT method was used to calculate relative changes in miRNA expression determined from real-time quantitative PCR experiments.

The data are presented as the fold change in miRNA expression normalized against endogenous reference genes. Normalization was performed against geometrical mean of miR-30a-5p and miR-194-5p.

In short, the CT value of the reference gene was subtracted from the CT value of the target miRNA to obtain the Delta-CT value. Therefore, when a specific miRNA is more expressed, the number of cycles needed to be normalized to the endogenous reference gene is lower.

2.3. Statistical analysis

Continuous variables are presented as mean ± standard deviation when normally distributed or median with corresponding 25th and 75th percentiles (interquartile range) when non-normally distributed. Categorical variables are presented as frequencies and percentages. Student's t-test or ANOVA (normal distribution),Wilcox or Kruskall–Wallis (skewed distribution) and Chi-square (categorical variables) tests were used for group comparisons. Trends of miRNAs were analyzed across tertiles of renal variables delta over the 3 days. The exact binomial test was performed to test the likelihood of the occurrence of multiple miRNAs being significant predictors of WRF. The predictive value of miRNAs for WRF was assessed using logistic regression models and C-statistics. The most significant miRNAs were included into a model and its ability to discriminate between WRF and non-WRF was tested with the receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC). A p-value < 0.05 was considered statistically significant for all statistical procedures used.

All analyses were performed using R: A Language and Environment for Statistical Computing, version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Baseline characteristics of patients

Twelve indicative miRNAs were measured at baseline in a total of 98 patients hospitalized with acute decompensated HF from the PROTECT trial. According to the definition of WRF described in the methods section, we identified 17 patients with WRF and 81 patients without WRF. Patients with WRF were more often female (55.6% vs 23.5%, $p = 0.016$), had a higher systolic blood pressure (126.9 ± 15.9 mmHg vs 117.8 ± 17.1 mmHg, $p = 0.047$) and a greater use of calcium-antagonists (23.5% vs 7.4%, $p = 0.046$) compared to patients without WRF. Other clinical and laboratory baseline characteristics, renal function included, were similar between patients with and without WRF (Table 1).

3.2. microRNAs and renal function

Circulating levels of all miRNAs had a similar trend toward lower expression in patients with WRF, with statistically significant reduced levels of miR-199a-3p, miR-423-3p, and miR-let-7i-5p ($p < 0.05$; Table 2 and Fig. 1). The occurrence of three miRNAs out of twelve

Table 1
Baseline characteristics of patients with worsening and non-worsening renal function (2 NA).

Variable	no WRF	WRF	*p*-Value
N=	81	17	
Demographics			
Sex % male(n)	55.6 (45)	23.5 (4)	0.016
Age (years)	68.8 ± 11.3	69.5 ± 12.7	0.801
BMI (kg/m^2)	28.8 ± 7.2	30.8 ± 5.8	0.297
LVEF (%)	33.4 ± 12.1	34.1 ± 15.4	0.893
HFPEF (%)	21.1 (8)	28.6 (2)	0.66
Systolic blood pressure (mm Hg)	117.8 ± 17.1	126.9 ± 15.9	0.047
Diastolic blood pressure (mm Hg)	70.4 ± 11.8	74.8 ± 10.8	0.166
Heart Rate (beats/min)	77.4 ± 15.6	85.5 ± 15	0.053
Rolofylline administration %(n)	64.2 (52)	58.8 (10)	0.676
Clinical profile			
Atrial fibrillation on presentation	60 (18)	50 (5)	0.58
Orthopnea %(n)	95.1 (77)	94.1 (16)	0.872
Rales %(n)	58 (47)	47.1 (8)	0.407
Edema %(n)	66.7 (54)	70.6 (12)	0.754
Jugular venous pressure %(n)	43.8 (32)	35.7 (5)	0.573
Medical history			
Hypertension %(n)	81.5 (66)	88.2 (15)	0.504
Diabetes mellitus %(n)	40.7 (33)	58.8 (10)	0.172
Hypercholesterolemia %(n)	48.1 (39)	47.1 (8)	0.935
Smoking %(n)	17.3 (14)	11.8 (2)	0.576
Ischemic heart disease %(n)	77.5 (62)	64.7 (11)	0.267
Myocardial infarction %(n)	55 (44)	29.4 (5)	0.055
PCI %(n)	24.1 (19)	17.6 (3)	0.569
CABG %(n)	24.1 (19)	29.4 (5)	0.643
Peripheral vascular disease %(n)	8.6 (7)	5.9 (1)	0.706
Atrial fibrillation %(n)	56.8 (46)	58.8 (10)	0.878
NYHA class			0.418
1	16 (13)	11.8 (2)	
2	49.4 (40)	76.5 (13)	
3	29.6 (24)	11.8 (2)	
ICD therapy %(n)	25.9 (21)	11.8 (2)	0.21
CRT therapy %(n)	11.1 (9)	0 (0)	0.149
Stroke %(n)	8.6 (7)	11.8 (2)	0.685
COPD %(n)	16 (13)	11.8 (2)	0.656
Prior medication use			
ACE inhibitors or ARB %(n)	69.1 (56)	58.8 (10)	0.41
Beta blockers %(n)	74.1 (60)	88.2 (15)	0.21
Mineralocorticoid receptor antagonists %(n)	48.1 (39)	58.8 (10)	0.424
Calcium antagonists %(n)	7.4 (6)	23.5 (4)	0.046
Nitrates %(n)	27.2 (22)	41.2 (7)	0.25
Digoxin %(n)	35.8 (29)	41.2 (7)	0.676
Laboratory values			
Creatinine (mg/dL)	1.4 [1.2–1.9]	1.5 [1.3–1.9]	0.731
Creatinine clearance (ml/min)	46.1 [35.1–62.7]	41.7 [34.8–52]	0.248
Blood urea nitrogen (mg/dL)	32 [25–45]	30 [25–48]	0.717
Sodium (mmol/L)	140 [136–142]	139 [137–144]	0.693
Potassium (mmol/L)	4.3 [3.9–4.7]	4.4 [4.2–4.7]	0.274
Hemoglobin (g/dL)	12.1 ± 1.7	11.4 ± 1.6	0.141
Anemia %(n)	50 (38)	56.2 (9)	0.649
Total cholesterol (mmol/L)	135.2 ± 45.3	157.5 ± 71.3	0.102
Triglycerides (mmol/L)	96.2 ± 46.2	114.5 ± 93.9	0.232
BNP (mg/dL)	1315.5 [867.3–2966]	1157.2 [664–3113]	0.887
NGAL (ng/mL)	82.1 [50.8–161.7]	90.1 [77.5–159.2]	0.306

WRF, worsening renal function; BMI, body mass index; LVEF, left ventricular ejection fraction; BP, blood pressure; HFPEF, heart failure with preserved ejection fraction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; NYHA, New York Heart Association; ICD, internal cardiac defibrillator; CRT, cardiac resynchronization therapy; COPD, chronic obstructive pulmonary disease; ACE, angiotensin-converting enzyme; ARB, aldosterone receptor blocker; BNP, brain natriuretic peptide; NGAL, Neutrophil Gelatinase Associated Lipocalin; NA, not assessed.

Table 2
Baseline microRNAs levels in patients with and without WRF (creatinine increase ≥0.3 mg/dl on day 3). MicroRNA levels are presented as median with corresponding interquartile ranges (in the square brackets); the values reported are calculated as Delta Ct. Normalization was performed against geometrical mean of miR-30a-5p and miR-194-5p.

Variable	non-WRF	WRF	*p*-Value
N=	81	17	
miR-18a-5p	−0.7 [−1.6–0.6]	0.3 [−0.3–0.6]	0.107
miR-106a-5p	−3.4 [−4.5–2.4]	−2.9 [−3.3–2.2]	0.088
miR-26b-5p	−0.5 [−1.4–0.4]	−0.3 [−0.7–1.1]	0.304
miR-223-3p	−6.2 [−7.6–5]	−5.8 [−6.9–4.9]	0.367
miR-199a-3p	−1.4 [−3.2–0]	0 [−1–1.3]	0.012
miR-27a-3p	−3 [−4.4–1.7]	−2.3 [−3–0.9]	0.084
miR-652-3p	−1.1 [−2.3–0.2]	0 [−1.2–0.5]	0.064
miR-423-5p	−3.4 [−4.7–2.6]	−2.8 [−3.4–2]	0.096
miR-423-3p	−1.8 [−2.9–0.4]	−1 [−1.4–0.1]	0.032
miR-16-5p	−8.7 [−9.7–7.5]	−8.4 [−9–7]	0.325
miR-30e-5p	−3.3 [−4.4–2.1]	−2.6 [−2.9–1.7]	0.053
miR-let-7i-5p	−3.2 [−4–2.2]	−2.3 [−2.8–1.7]	0.034

being associated with WRF was validated through the exact binomial test, that produced a $p = 0.020$. Hereby we statistically demonstrate that the association of these miRNAs to WRF was not a chance finding due to multiple testing. Table 3 shows the levels of all miRNAs across tertiles of absolute change in creatinine from day 1 to day 3, where the 3rd tertile represents the highest change in creatinine from baseline (Supplementary Fig. A.1).

Statistically significant low levels were found in patients with the highest increase in serum creatinine for miR-199a-3p, miR-27a-3p, miR-652-3p, miR-423-5p, miR-let-7i-5p. MiR-199a-3p, miR-18a-5p, miR-106a-5p, miR-223-3p and miR-423-3p were also significantly reduced in patients with the greatest change in NGAL levels (3rd tertile) from day 1 to day 3 (Table 4 and Supplementary Fig. A.2).

The remaining miRNAs showed a similar consistent trend characterized by lower levels in patients with greater increases in NGAL, but these associations were not statistically significant.

Lower levels of all miRNAs were related to an increased risk of WRF, although not all associations reached statistical significance. MiR-199a-3p was a significant predictor of WRF, with an Odds Ratio (OR) of 1.48 (1.061–2.065; $p = 0.021$) and a C-index of 0.701 (Supplementary Table A.1). Combining miR-199a-3p with four other statistically significant miRNAs, miR-423-3p, miR-423-5p, miR-let-7i-5p, miR-223-3p, we established a more sensitive model (AUC:0.777) to distinguish between WRF and non-WRF (Fig. 2).

4. Discussion

Lower levels of miRNAs were consistently associated with early worsening of renal function during hospitalization for acute HF. In particular, miR-199a-3p was associated with change in several renal parameters and was a significant predictor of WRF.

Recent studies showed that miRNAs play an important role in homeostasis of renal function and progression of kidney diseases [19]. Inhibition of miRNA function through inactivation of Dicer, an enzyme required for the production of mature miRNAs, resulted in multiple abnormalities such as irregular areas of the glomerular basement membrane, podocyte apoptosis and depletion, capillary dilation, glomerulosclerosis and altered expression of renin in the juxtaglomerular cells [20].

The progress in discovering miRNAs and characterizing their functions in kidney diseases has been rapid but understanding the specific role, in cardio–renal pathophysiology, of the miRNAs examined in our study is still limited. Data available about their role are summarized in the Supplementary Table A.2. There are relatively few studies available regarding changes in circulating miRNA levels in patients with renal insufficiency. Neal et al. investigated the levels of five circulating miRNAs (miR-16, miR-21, miR-155, miR-210, miR-638) in patients with

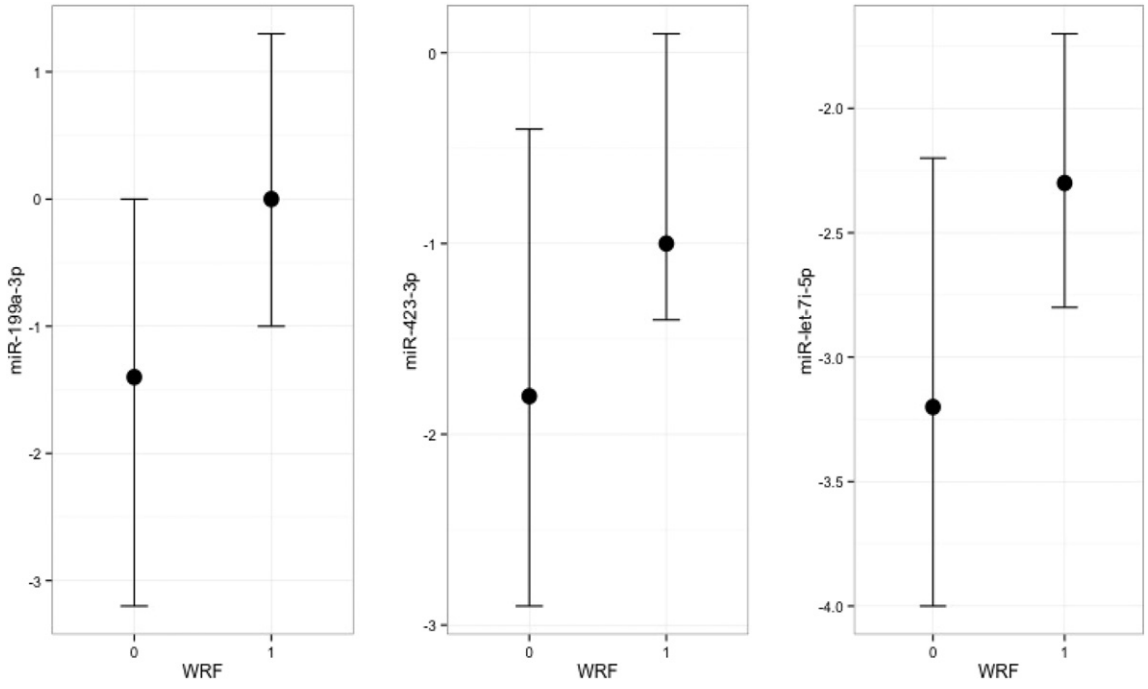

Fig. 1. Significant baseline microRNA levels in patients with (1) and without (0) WRF; the graphs represent median (dot) and interquartile ranges.

different stages of chronic kidney failure; they were reduced in patients with severe chronic kidney disease (CKD) in comparison to patients with mild CKD or normal renal function; a significant correlation was displayed between miRNAs circulating levels and estimated glomerular filtration rate [21]. Low circulating levels of specific miRNAs (miR-16 and miR-320) were also detected in plasma of critically ill patients with acute kidney injury (AKI) requiring renal replacement therapy [22].

There is currently no study that related specific miRNAs to renal function in patients with acute HF.

Our results showed that levels of miR-199a-3p, miR-423-3p, miR-let-7i-5p were significantly lower in patients with early WRF, being in accordance with the previous studies that revealed reduced circulating miRNAs in the setting of AKI and severe CKD. The associations we found were not a chance finding, as demonstrated by the significant result obtained with the exact binomial test. We found a consistent trend toward decreased levels of all miRNAs. In addition to the miRNAs mentioned above, the absolute increase in creatinine was directly associated with low levels of miR-27a-3p, miR-423-5p and miR-652-3p.

Previous studies suggested that miR-199a-3p might prevent kidney injury, resulting from hypoxia, and may control inflammation and apoptosis signaling, which mediate tubular damage after I/R injury [23]. Data from a murine model showed that downregulation of miR-199a is required in hypoxia-induced apoptosis through the hypoxia-inducible factor1α-p53 pathway [24]. The transcription factor TWIST1/miR-199/214 axis is down-regulated in dilated cardiomyopathy [25] and it is a critical regulator of energy metabolism expression, facilitating a metabolic shift from predominant reliance on fatty acid utilization in the healthy myocardium toward increased reliance on glucose metabolism at the onset of HF [26].

Many studies have identified NGAL as an early marker of ischemic or toxic tubular injury and, in kidney I/R injury murine models, the tissue expression of certain miRNAs correlated with its renal expression and plasma levels [27,28]. We also demonstrated a potential role of miRNAs in tubular damage, since reduced levels of miR-199a-3p, miR-18a-5p, miR-106a-5p, miR-423-3p and miR-223-3p were correlated with an increase of NGAL.

The mechanism behind the reduced levels of circulating miRNAs in kidney dysfunction is still unclear. Potential explanations are that in

Table 3
Baseline microRNAs for absolute change of creatinine, expressed in tertiles, from baseline through day 3. MicroRNA levels are presented as median with corresponding interquartile ranges (in the square brackets); the values reported are calculated as Delta Ct. Normalization was performed against geometrical mean of miR-30a-5p and miR-194-5p.

Variable	1st tertile	2nd tertile	3rd tertile	*p*-Value
N=	34	34	30	
miR-18a-5p	−0.7 [−2.1–1]	−1.1 [−1.6–0]	0 [−1.2–0.8]	0.275
miR-106a-5p	−3.6 [−4.8–2.7]	−3.7 [−4.5–2.5]	−3 [−3.4–2.1]	0.064
miR-26b-5p	−0.8 [−1.6–0]	−0.3 [−1.5–0.5]	0 [−0.7–1]	0.106
miR-223-3p	−6.1 [−7.6–5]	−6.7 [−8.2–5.1]	−5.7 [−6.7–4.8]	0.214
miR-199a-3p	−2 [−3.1–0.3]	−1 [−3.3–0.1]	−0.4 [−1.3–1.2]	0.006
miR-27a-3p	−3.3 [−4.6–2.5]	−3.3 [−4.4–1.8]	−2.5 [−3.3–0.9]	0.026
miR-652-3p	−1.6 [−2.2–0.2]	−1.1 [−2.3–0]	0 [−1.2–0.6]	0.034
miR-423-5p	−4.2 [−4.8–2.8]	−3.1 [−4.7–2.5]	−2.8 [−4–2.1]	0.046
miR-423-3p	−1.6 [−2.8–0.6]	−1.8 [−2.9–0.5]	−1.2 [−2–0]	0.139
miR-16-5p	−8.7 [−9.5–7.5]	−8.7 [−10–7.8]	−8.1 [−9.1–6.9]	0.263
miR-30e-5p	−3.4 [−4.1–2.3]	−3.3 [−4.9–2]	−2.6 [−3.2–1.4]	0.134
miR-let-7i-5p	−3.5 [−4.3–2.4]	−3.2 [−3.7–2.3]	−2.6 [−3.2–1.7]	0.01

Table 4
Baseline microRNAs for absolute NGAL change, expressed in tertiles, from baseline through day 3. MicroRNA levels are presented as median with corresponding interquartile ranges (in the square brackets); the values reported are calculated as Delta Ct. Normalization was performed against geometrical mean of miR-30a-5p and miR-194-5p.

Variable	1st tertile	2nd tertile	3rd tertile	*p*-Value
N=	33	32	33	
miR-18a-5p	−0.9 [−2.3–0.5]	−0.4 [−1.3–0.2]	0 [−1.2–0.9]	0.042
miR-106a-5p	−3.7 [−5–2.4]	−3 [−4.1–2.7]	−2.8 [−3.8–2.2]	0.046
miR-26b-5p	−1 [−2.2–0]	−0.3 [−1.4–0.7]	−0.3 [−1–0.4]	0.089
miR-223-3p	−6.9 [−8.5–5.3]	−5.7 [−7.2–5]	−6 [−7.1–4.9]	0.045
miR-199a-3p	−2 [−3.9–0]	−1.2 [−2.9–0.1]	−0.8 [−1.6–0.3]	0.011
miR-27a-3p	−2.8 [−5.1–1.9]	−3 [−4.1–2]	−2.8 [−3.9–1.3]	0.146
miR-652-3p	−1.6 [−2.6–0.1]	−1 [−1.8–0.4]	−0.1 [−1.3–0.4]	0.067
miR-423-5p	−4.2 [−4.9–2.6]	−3.2 [−4.7–2.6]	−2.9 [−4–2.3]	0.073
miR-423-3p	−2 [−3.5–0.9]	−1.3 [−2.8–0.4]	−1.3 [−2.1–0.1]	0.039
miR-16-5p	−8.8 [−9.6–7.6]	−8.6 [−10–7.4]	−8.7 [−9–7.4]	0.414
miR-30e-5p	−3.1 [−4.7–2.2]	−3.3 [−4.3–1.9]	−2.9 [−3.3–1.9]	0.19
miR-let-7i-5p	−3.5 [−4.5–1.9]	−2.9 [−3.7–2.1]	−2.8 [−3.3–2.3]	0.113

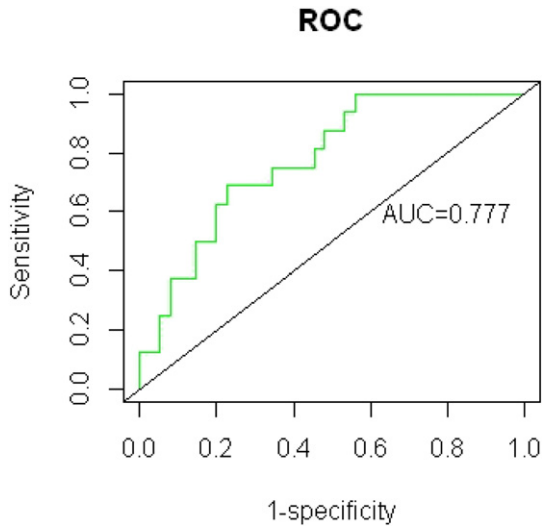

Fig. 2. ROC curve (green line) of microRNA model (including miR-199a-3p, miR-423-3p, miR-423-5p, miR-let-7i-5p, and miR-223-3p) predicting early worsening renal function.

patients with renal dysfunction, circulating miRNAs are secreted to a lower extent by cells, are increasingly taken up by other circulating or resident cells or tissues, or are degraded [29]. The metabolic alterations of renal failure (i.e. hyperkalemia, metabolic acidosis) and the increased amounts of RNA degrading enzymes in circulation following injury might lead to the degradation of certain circulating miRNAs [22].

We showed that miR-199a-3p, miR-let-7i-5p and miR-423-3p had the strongest association with (worsening of) renal function in patients with acute HF. Identifying HF patients at risk of developing WRF is important in preventing progression of disease and adverse events, through early adjustments of medication management (e.g. diuretics) and improved treatment with Renin-Angiotensin-Aldosterone-System-blockers before discharge. However, WRF is difficult to predict, since there is no consensus about its definition [30]. While previous studies have merely shown associations between miRNAs and disease activity, our results suggest a potential role as predictors for WRF, supported by the consistency of lower levels of miRNAs in WRF patients. Circulating miR-199a-3p was already found to be associated with HF diagnosis in regression and receiver operating characteristic analyses in a population of breathless patients [31].

The present data do not allow concluding whether these circulating miRNAs are just markers or mediators of WRF, although previous data [23] have suggested a role for miR-199a-3p in limiting kidney injury. While studies in the cardiovascular and cancer field have displayed the functional mechanism of miRNAs, evidences in patients with renal failure are mostly descriptive.

Although further analysis is needed to prove a causative pathophysiological role of circulating miRNAs in WRF, the possibility to use them as biomarkers in this setting emerges as an exciting field. They have many of the essential characteristics of good biomarkers: they are highly stable and resistant to pH variations and longterm storage at room temperature; they can be reliably analyzed and quantified by real-time PCR. The analysis is extremely sensitive and, since it is sequence-based, it is also very specific [32].

Thus, miRNAs may represent candidate biomarkers of disease severity, since lower levels of circulating miRNAs were associated with increasing severity of HF [9]; in association with additional well-known biomarkers and clinical parameters, they may facilitate early diagnosis, functional assessment and evaluation of disease progression and therapeutic response in HF patients.

A better understanding about their role might give more detailed insights into the pathophysiology of HF and WRF and result in specific biomarker-targeted therapeutic approaches and ideally novel targets for therapy, leading us toward personalized therapeutic treatment of patients with acute HF [33].

4.1. Study limitations

The major limitations are the small sample size of the studied population, its etiologic heterogeneity and the retrospective analysis of data. Our conclusion cannot be generalized as we selected a specific panel of miRNAs relying on their characteristic profile in patients with acute HF. Our predictive miRNA model was not optimized for association with clinical parameters. Finally, we mainly showed associations between miRNA levels and renal function and we cannot provide molecular insights into the underlying mechanisms that cause dysregulation of circulating miRNAs in the setting of WRF in acute HF, since dedicated experimental studies are necessary to further elucidate pathophysiological pathways in which miRNAs are involved.

5. Conclusion

WRF during a hospital admission for acute HF was consistently associated with lower baseline levels of several circulating miRNAs. MiR-199a-3p, that is related to the prevention of kidney injury resulting from hypoxia and may control inflammation and apoptosis signaling, was the strongest predictor of WRF. A better understanding of the role of circulating miRNAs will be necessary to develop new diagnostic, prognostic and/or therapeutic tools in this setting.

Funding

This study was supported by a Grant from the Dutch Heart Foundation: Approaching Heart Failure By Translational Research Of RNA Mechanisms (ARENA). The PROTECT trial was supported by NovaCardia, a subsidiary of Merck.

Conflict of interest

N.B., J.M.T.M., E.S.O., E.L.V., M.A.E.V., P.V.D.M., R.A.D.B., P.V.D.H., D.S., and H.L.H. have nothing to disclose. A.A.V. has received speaker and consultancy fees from Merck and NovaCardia. C.M.O.C. is a consultant to Merck. D.J.V.V. has received Board Membership fees from Amgen, BG Medicine, Biocontrol, Johnson & Johnson, Novartis, Sorbent and Vifor. D.M.B. is an employee of Merck. E.B. is co-founder and member of scientific advisory board of InteRNA Technologies B.V., which develops microRNA therapeutics for cancer. No other conflicts were reported. G.C. and B.D. are employees of Momentum Research Inc, which was contracted to perform work on the project by Merck & Co, Inc. H.C.D was an employee of NovaCardia and a consultant to Merck. J.R.T. has received research funds and consulting fees from Merck, the producer of rolofylline for the conduct of this study and has also received research funds and consulting fees from Abbott, Amgen, Biogen Idec, Corthera, Cytokinetics, Johnson and Johnson/Scios, Novartis, Relypsa and Solvay for research in related areas. J.G.C. was on the Steering Committee for the study, served on the Advisory Board for MSD, and received payments for both. M.M. has received honoraria and reimbursements from NovaCardia, sponsors of the study, and from Merck, that purchased the rights to rolofylline after completion of the PROTECT pilot study. M.M.G. has received institutional research support and served on a scientific Advisory Board for Merck. P.P. has received honoraria from Merck. Y.M.P. has a minor stake (<5%) in University spin-off which commercializes IP in the field of biomarkers and microRNAs.

Acknowledgements

We acknowledge the support from the Netherlands CardioVascular Research Initiative: the Dutch Heart Foundation, Dutch Federation of University Medical Centres, the Netherlands Organisation for Health Research and Development and the Royal Netherlands Academy of Sciences. Alere and Singulex kindly provided assays and performed biomarker measurements.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2015.10.217.

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