Associations between volume status and circulating microRNAs in acute heart failure

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In recent years, several differentially expressed circulating microRNAs (miRNAs) have been identified in heart failure (HF).[1] Few studies have described circulating miRNA profiles in patients with acute HF (AHF) and the majority have specifically shown a downregulation of miRNA expression compared with that in control subjects.[2,3] Acute HF is often characterized by signs of volume overload, such as pulmonary congestion, peripheral oedema, jugular venous dilatation and ascites. Fluid retention in HF can also lead to haemodilution, and a change in haemoglobin concentration may be used as a marker for change in volume status in patients hospitalized with AHF. In addition, it has been shown that an increase in haemoglobin is related to better decongestion and an improved outcome.[4,5] Circulating miRNAs may behave similarly in relation to changes in intravascular volume, in which fluid overload may contribute to low miRNA levels in AHF patients. Indeed, we previously found that circulating miRNA levels were lowest in patients admitted for AHF, increased gradually in patients with more stable forms of HF and were highest in healthy control subjects.[3] This leads to the hypothesis that fluid overload may contribute to lower miRNA concentrations in plasma. Therefore, we aimed to investigate the effect of change in volume status (reflected by change in haemoglobin concentration) on change in circulating miRNA levels in patients hospitalized with AHF.

A total of 100 patients from the Placebo-Controlled Randomized Study of the Selective A1 Adenosine Receptor Antagonist Rolofylline for Patients Hospitalized with Acute Decompensated Heart Failure and Volume Overload to Assess Treatment Effect on Congestion and Renal Function (PROTECT) were studied. The main results of the PROTECT trial and the characteristics of the current study population have been previously described.[3,6] Patients with haemoconcentration were defined as having an increase in haemoglobin levels at day 7 compared with baseline (hospital admission) levels, whereas patients without haemoconcentration were identified according to stable or decreased haemoglobin levels at day 7.

Table 1 Change in normalized – ΔCt circulating microRNA levels on day 7 compared with baseline in patients with and without haemoconcentration

MicroRNA	Mean ± standard deviation change		*P*-value
	No haemo-concentration	Haemo-concentration	
Δ let-7i-5p	−0.6 ± 1.2	0.1 ± 1.5	0.014
Δ miR-16-5p	−0.4 ± 2.1	0.7 ± 1.7	0.012
Δ miR-18a-5p	−0.2 ± 1.3	−0.2 ± 2.0	0.860
Δ miR-26b-5p	−0.1 ± 1.9	0.3 ± 2.1	0.319
Δ miR-27a-3p	−0.5 ± 1.9	0.6 ± 2.5	0.027
Δ miR-30e-5p	−0.4 ± 1.2	0.6 ± 1.8	0.004
Δ miR-106a-5p	−0.3 ± 1.2	0.1 ± 1.7	0.247
Δ miR-199a-3p	−0.3 ± 1.9	0.2 ± 2.4	0.368
Δ miR-223-3p	−0.2 ± 1.5	0.1 ± 2.0	0.476
Δ miR-423-3p	−0.5 ± 1.7	0.3 ± 2.2	0.079
Δ miR-423-5p	−0.2 ± 1.6	0.7 ± 1.5	0.010
Δ miR-652-3p	−0.2 ± 1.4	0.5 ± 2.0	0.059

Blood samples at baseline and day 7 were collected as previously reported.[6] The expression levels of 12 circulating miRNAs previously related to HF (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 and miR-652-3p) were determined using quantitative reverse transcription polymerase chain reaction; the methods have been described in detail elsewhere.[3] GenEx Professional software (Multid Analyses AB, Gothenburg, Sweden) was used to analyse raw threshold cycle (Ct) values. The miRNAs of interest were normalized against the endogenous reference miRNAs miR-30a-5p and miR-194-5p and presented as −ΔCt values. Group differences were assessed with *t*-tests for normally distributed variables, Mann–Whitney U-tests for non-normally distributed continuous variables, and χ^2 tests for binomial and categorical variables. Simple and multiple linear regression analyses were performed to investigate the associations between haemoconcentration and change in miRNA levels. *P*-values of <0.05 were considered to indicate statistical significance. Analyses were performed in R version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

Complete data for haemoglobin levels at baseline and day 7 were available for 90 of the 100 patients. Patients with a haemoglobin increase on day 7 [mean ± standard deviation (SD): 0.7 ± 0.6 g/dL] also showed increases in both haematocrit (mean ± SD: 2.4 ± 2.8%) and albumin (mean ± SD: 0.1 ± 0.3 g/dL); these findings were significantly different from those in patients without haemoconcentration, in whom haemoglobin decreased (mean ± SD: −0.8 ± 0.6 g/dL), in parallel with decreases in haematocrit (mean ± SD: −2.5 ± 2.9%) and albumin (mean ± SD: −0.2 ± 0.3) ($P < 0.001$ for all comparisons). Patients with haemoconcentration had baseline characteristics similar to those of patients with no haemoconcentration. However, patients with haemoconcentration lost significantly more weight during hospitalization than patients without haemoconcentration (mean ± SD: −2.8 ± 2.6 kg vs. −1.1 ± 3.5 kg; $P = 0.02$) and showed a trend towards a better diuretic response. Although patients with haemoconcentration experienced fewer clinical adverse events in absolute numbers (including mortality and rehospitalization attributable to HF within 180 days, and cardiovascular or renal disease within 60 days), no statistically significant differences in outcome parameters were found in this subset of patients.

The majority of miRNAs (except for miR-18a-5p) showed a clear increase in expression levels on day 7 compared with those at baseline in patients with haemoconcentration, whereas miRNAs were decreased in patients without haemoconcentration (*Table 1*). Changes in expression levels of the miRNAs let-7i-5p, miR-16-5p, miR-27a-3p, miR-30e-5p and miR-423-5p differed significantly between patients with and without haemoconcentration. We also investigated

haemoconcentration as a predictor of change in miRNA levels despite important factors reflecting disease status. Linear regression models showed that the presence of haemoconcentration was significantly related to increases of let-7i-5p [B = 0.78, 95% confidence interval (CI) 0.16–1.39; $P = 0.01$], miR-16-5p (B = 1.07, 95% CI 0.24–1.90; $P = 0.01$), miR-27a-3p (B = 1.10, 95% CI 0.13–2.06; $P = 0.03$), miR-30e-5p (B = 1.01; 95% CI 0.33–1.68; $P = 0.004$) and miR-423-5p (B = 0.90, 95% CI 0.23–1.58; $P = 0.01$). These associations remained significant for let-7i-5p (B = 0.74, 95% CI 0.07–1.42; $P = 0.03$), miR-16-5p (B = 0.98, 95% CI 0.06–1.89; $P = 0.04$), miR-30e-5p (B = 0.92, 95% CI 0.18–1.67; $P = 0.02$) and miR-423-5p (B = 0.81, 95% CI 0.08–1.54; $P = 0.03$) after correction for parameters previously reported as prognostically important in AHF patients in the PROTECT study, including age, previous HF hospitalization, peripheral oedema, systolic blood pressure, serum sodium, blood urea nitrogen, creatinine and albumin.[7]

In HF, circulating miRNAs have been mainly described in relation to their (modest) roles as potential biomarkers. For example, it has been shown that miRNAs can distinguish between HF and other causes of dyspnoea, as well as between HF with reduced ejection fraction and HF with preserved ejection fraction.[1] However, information on the function and regulation of circulating miRNAs in HF is limited. It has been postulated that circulating miRNAs reflect pathophysiological processes underlying HF and can exert a paracrine function.[8] Furthermore, there are several hypotheses which may explain the downregulation of miRNA levels in HF, such as an increased uptake by organs or reduced production. In this study, we show that several miRNAs which were previously found to be downregulated in (acute) HF change in parallel with haemoglobin in patients admitted with AHF. More specifically, in patients with haemoconcentration, circulating miRNAs levels increased, whereas in patients without haemoconcentration miRNA levels decreased. Interestingly, most of these associations remained significant after correction for clinically relevant parameters reflecting disease status. This suggests that change in volume status may partly explain change in circulating miRNA levels, although the absolute change is modest and therefore other contributing pathophysiological mechanisms should also be considered.

The limitations of this study should be acknowledged. As this is a post hoc study in a relatively small patient population, the results should be regarded as hypothesis-generating and as requiring further investigation in larger, prospective studies. However, this is the first study to address the potential influence of volume status on circulating miRNA levels in AHF patients and it may provide more knowledge about the complex nature of circulating miRNAs in HF.

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