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"Transcoronary concentration gradients of circulating miRNAs in Heart Failure"

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SUMMARY AND PURPOSES

Circulating levels of microRNA (miRs) are emergent promising biomarkers for cardiovascular disease. Altered expression of miRs has been related to heart failure (HF) and cardiac remodeling. To identify the potential source for miRs released into the circulation, we measured the concentration gradients across the coronary circulation to assess their usefulness to diagnose HF of different etiologies. Circulating miRs were measured by TaqMan polymerase chain reaction in EDTA-plasma simultaneously obtained from the aorta (Ao) and the coronary venous sinus (CVS) in patients with non-ischemic HF (NICM-HF, n=23), or ischemic HF (ICM-HF, n=23), as well as from control subjects with no HF (n=11). We found differential modulation of circulating levels of the miRs 423, 34a, 21-3p and 126 across the etiology HF groups. Interestingly, we found a positive transcoronary gradient for the miR-423 ($p<0.001$) and the miR-34a ($p<0.001$), only in the ICM-HF group. On the contrary, a positive transcoronary gradient was found for the miR-21-3p ($p<0.001$) only in the NICM-HF group. Finally, despite the dramatic downregulation observed for the miR-126 in HF patients ($p<0.001$) compared to controls, no significant variations were observed in its transcoronary gradient.

The present findings suggest that circulating levels of miRs are differentially expressed in patients with HF of different etiologies. The presence of a transcoronary concentration gradient suggests a selective release of miR by the failing heart into the coronary circulation. The presence of etiology-specific transcoronary concentration gradient in HF patients might provide important information to better understand their role in HF, and suggests they could be useful biomarkers to distinguish HF of different etiologies.

1.INTRODUCTION

The prevalence of Heart Failure (HF) has esponentially increased over the last decades and it has been estimated that it will further grow, reaching up to 10% of the general population in 2030 (1). HF is associated with high mortality rates, up to 50% at 5 years and over 70% at 10 years from its first diagnosis (2). For this reason, several biomarkers have been developed over the last decades, in an attempt to warrant a timely diagnosis and guide the optimal clinical management. However, even currently established biomarkers present some limitations (3). In particular, they do not allow an etiology-based diagnosis.

Micro RNAs (miRs) are short, non-coding RNAs that modulate gene expression at a post-transcriptional level, through sequence specific binding of target messenger-RNAs. They are deeply involved in development of the cardiovascular system and in the pathophysiology of cardiovascular diseases (4-6). Moreover, they can be detected in the circulating blood (7). Since circulating miRs are seemingly quite stable, they may be exploited as blood-borne biomarkers (8). In fact, several studies reported an association between circulating levels of specific miRs and cardiovascular diseases, including acute myocardial infarction (9-11), myocarditis (12), tako-tsubo (13), acute and chronic heart failure (14), stable coronary artery disease (15), in-stent restenosis (5,16-17) type 2 diabetes (18), or in association to different levels of platelet activity (19-20).

Importantly, since specific miRs are differentially expressed in different cell types, circulating levels of specific miRs might reflect different biological alterations, which could provide useful hints to the underlying aetiopathogenesis (21).

In this context, we evaluated the transcoronary expression gradients of selected circulating microRNAs in heart failure patients, to investigate whether a specific release from the heart could be found for different aetiologies underlying heart failure.

2. METHODS

2.1 Study sample

A total of 75 subjects undergoing coronary angiography in the catheterization laboratories of the Federico II University of Naples and the Magna Graecia University of Catanzaro were enrolled. Patients were classified into groups according to their clinical status as follows:

1) patients with no evidence of cardiovascular disease undergoing percutaneous closure of a patent foramen ovale (control group);

2) patients with HF with an underlying ischemic cardiomyopathy (ICM), undergoing cardiac catheterization for implantation of an implantable cardioverter defibrillator (ICD);

3) patients with HF but no underlying ischemic cardiomyopathy (NICM) undergoing cardiac catheterization for ICD implantation.

Exclusion criteria were a known history of leukopenia, thrombocytopenia, or severe hepatic or renal dysfunction, as well as ongoing inflammatory or malignant disease. In addition, diagnosis of myocarditis or the presence of cardiogenic shock were also exclusion criteria.

On the basis of the expected expression values of miR-423 and its standard deviation we calculated that 60 patients would have been required to have an 80% chance of detecting a 2-fold increase in miR expression levels between the ICM-HF and the NICM-HF groups (primary study outcome), at a significance level of 0.05. However, the total number of HF patients included was raised to 64 to account for asymmetrical distribution (consecutive inclusion independently of the etiological group) and eventual technical issues with detection of miRs in some samples. Patients undergoing cardiac

catheterization for percutaneous occlusion of a PFO with no evidence of cardiac impairment during the enrollment period were also included to serve as a control group.

The ethics review board of the Magna Graecia University of Catanzaro, Italy, approved the protocols, and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each individual.

2.2 Blood collection and storage

Blood samples were simultaneously obtained from the coronary venous sinus and the aortic bulb during the cardiac catheterization procedure, before administration of heparin or any contrast agent and before starting any interventional procedure. After centrifugation, samples were transferred to RNase/DNase-free tubes and stored at −80°C.

2.3 RNA preparation

Total RNA in plasma was isolated by using TRI Reagent BD following the instructions from the manufacturer with modifications. To date, no housekeeping miRNA has been established and validated to normalize for the miRNA content. Therefore, we supplemented the samples (after addition of TRIzol LS) with 5 nmol/L *Caenorhabditis elegans* miR-39 (cel-miR-39), as described previously (8).

2.4 Quantitative reverse transcriptase-polymerase chain reaction (real time - PCR) analysis

RNA was obtained as outlined above and diluted 1:10. Diluted RNA (5 μL) was reverse transcribed using the TaqMan microRNA Reverse Transcription kit (ABI) according to the instructions of the manufacturer. Subsequently, 3 μL of the product was used for detecting miRNA expression by quantitative (q)PCR using TaqMan microRNA Assay kits (ABI) for the corresponding microRNA. cel-miR-39 was used for normalizing the data. MicroRNA levels are expressed as $2^{-\Delta CT}$[microRNA - cel miR-39].

2.5 Statistical analysis

The quantitative data were analyzed by means of the Kruskal Wallis, the Mann-Whitney U test or the Wilcoxon test (paired sample), as specified in the figure legends. For categorical variables, Fischer's exact test or the $\chi 2$ test were used. Pearson correlation was used to compare levels of microRNAs with other factors. All *p*-values are two-sided and less than 0.05 was considered a statistically significant difference. All statistical calculations were performed using IBM SPSS Statistics 23.0 for Windows.

3. RESULTS

3.1 Study sample

A total of 75 subjects were included. Eleven control subjects undergoing percutaneous closure of patent foramen ovale with no further structural heart anomalies and normal cardiac function, 41 patients with HF and ICM and 23 patients with HF and NICM undergoing consecutively cardiac catheterization were enrolled. The clinical characteristics of the study groups are summarized in table 1. Overall, HF patients presented a marked reduction of left ventricular function (left ventricular ejection fraction = 29.7% ± 6.8%) and substantially increased plasma NT-ptoBNP levels (2944 ± 5671). Accordingly, 32% of these patients were in NYHA class III or IV. As reported in table I, HF patients were on optimal medical treatment, with 68% of them on betablockers, 45% on ACE inhibitors/ARBs and 45% on potassium sparing diuretics.

3.2 MiR selection process

Specific miRs to be measured in the present study were selected among those already known to be associated with heart failure. Briefly, PubMed and the Cochrane Library electronic databases were searched for using the following keywords: “circulating microRNAs” and “heart failure”. After removal of duplicates, a total of 101 articles were available. Full texts of these articles were evaluated by two operators (JS and SDR), in order to select the most promising circulating miRs to be evaluated in association to Heart Failure. Among the most represented, the miR-423 was first selected since several reports had described its potential usefulness as a circulating biomarkers in HF, despite conflicting results (14,22-24). We also found several reports of association between the miR-34a, heart failure and alterations in left ventricular function in response to different risk factors and in various LV damage models, such as post-ischemic HF (25-26), LV hypertrophy (27), hyperglycemic damage (28), or doxorubicin-induced cardiac toxicity (29-30). In more recent times, several reports have demonstrated a deep involvement of the passenger strand of miR-21, the so called miR-21* (miR-21-3p) in the pathophysiology of cardiac hypertrophy and heart failure (31-34). Most interestingly, it was found that intercellular transfer of miR-21-3p through exosomes plays a key role in these processes (35-36). Not surprisingly, a modulation of circulating levels of miR-21-3p were reported in LV dysfunction due to LV overload (37).

In addition, the miR-126 was selected because of multiple original reports demonstrating its association with HF. In fact, it is involved in various molecular mechanisms underlying HF (38-40). Moreover, several reports

described an association between circulating levels of miR-126 and the clinical prognosis of HF patients (41-43).

On the other hand, some miRs were not selected, despite several reports were available on their association, due to case-specific reasons. For example, although an association of the miR-208 was reported with cardiac damage, remodelling, left ventricular function and survival in HF patients (12, 26, 44) it was excluded from our analyses for the technical difficulty in measuring the extremely low expression levels of this specific miR and the high degree of variability observed in our population (data not shown).

3.3 MiRs levels in aortic and coronary sinus blood

As illustrated in figure 1A, circulating plasma levels of the miR-423 were significantly elevated in both the aorta (10-fold, $p=0.043$) as well as the coronary sinus (15-fold, $p<0.001$) only in HF patients with ischemic aetiology whereas no substantial differences were observed in the NICM HF group (figure 1A).

Similarly, concentrations of the miR-34a presented a similar, increase in both the aorta (6.6-fold, $p=0.011$) and the CVS (16.5-fold, $p<0.001$) of ICM HF patients (figure 1A).

On the other hand, levels of the miR-21-3p were significantly increased in both the aorta (4.5-fold, $p=0.003$) and the coronary sinus (11.9-fold, $p<0.001$) of HF patients with non-ischemic aetiology (NICM), as compared to the control group. Vice-versa, no significant increase was observed in the group of patients with HF of ischemic aetiology (ICM) (figure 1B).

On the contrary, plasma levels of the miR-126 were substantially reduced both in aorta (23.3-fold, $p<0.001$) and in the CVS (7.8-fold, $p<0.001$) of patients with HF of ischemic origin. A similar modulation was observed in the aorta (31.2-fold, $p<0.001$) and in the CVS (29.3-fold, $p<0.001$) of patients from the NICM HF group (figure 1B).

3.4 Transcoronary concentration gradients

In order to test whether the modulation in the levels of circulating miRs observed in HF patients were heart-specific, miR levels were measured in blood samples obtained from the aorta and CVS (45). Transcoronary concentration gradients were calculated as the ratio between miRs levels measured in the coronary venous sinus and those measured in the aorta (Figure 2).

As shown in figure 3A, there was a positive transcoronary concentration gradient for the miR-423 only in the group of patients with HF of ischemic aetiology ($p=0.006$), but not in HF patients with NICM ($p=0.951$), suggesting its selective release into the coronary circulation in this etiological subgroup of HF (figure 3A). Accordingly, a significant transcoronary gradient was confirmed at the Wilcoxon rank test in the ICM HF group ($p<0.001$), but not in controls ($p=0.155$) or NICM HF patients ($p=0.296$).

Similarly, we found a more modest, although significant, positive transcoronary concentration gradient for the circulating levels of the miR-34a (figure 3B) in the ICM HF group ($p<0.001$). Accordingly, a significant transcoronary gradient was confirmed at the Wilcoxon rank test in the ICM HF group ($p<0.001$), but not in controls ($p=0.646$) or NICM HF patients ($p=0.177$).

In contrast, a positive transcoronary concentration gradient for the miR-21-3p was found only in the group of patients with HF of non-ischemic aetiology (NICM) ($p=0.036$), suggesting its selective release into the coronary circulation in this etiological subgroup of HF (figure 3C). In fact, no gradient was documented in the ICM HF group ($p=0.798$). Accordingly, a significant transcoronary gradient was confirmed at the Wilcoxon rank test in NICM HF group ($p<0.001$), but not in controls ($p=0.600$) or ICM HF patients ($p=0.198$).

Finally, the transcoronary concentration gradient of the circulating miR-126 presented no significant variation in the group of patients with HF of ischemic aetiology (p=0.611) or non-ischemic aetiology (p=0.749), compared to the control (figure 3D).

Importantly, treatment with calcium channel blockers had a significant impact on the transcoronary concentration gradient of the miR-423 (r=0.423; p=0.002) and miR-126 (r=0.546; p<0.001), while ongoing treatment with ARBs was significantly associated with the aortic concentration of the miR-423 (r=0.294; p=0.032).

3.5 Correlation between circulating MiRs concentrations and prognostic parameters

In order to further document an association between circulating concentrations of miRs and clinical parameters known to be associated to long-term prognosis in heart failure patients, we correlated individual miRs levels with known prognostic predictors (46-52) and found that the CVS concentration of miR-34a was significantly correlated with hemodynamic parameters, such as systolic blood pressure ($r=0.289$; $p=0.025$) diastolic blood pressure ($r=0.258$; $p=0.047$), and heart rate ($r=0.349$; $p=0.006$), as well as with triglyceridemia ($r=0.364$; $p=0.027$). Finally, the transcoronary gradient of miR-34a was correlated with heart rate ($r=0.597$; $p<0.001$) and age ($r=0.253$; $p=0.041$).

Moreover, the coronary venous plasma concentration of miR-126 was also significantly associated with both the systolic ($r=0.462$; $p<0.001$) and the diastolic ($r=0.462$; $p=0.035$) left ventricular function. Similar correlations were found with aortic levels of miR-126 ($r=0.417$ with LV-EF; $p=0.001$ and $r=0.410$ with E/A; $p=0.058$). In addition, CVS levels of miR-126 were also correlated to patients' age ($r=-0.255$; $p=0.035$) and gender ($r=-0.277$; $p=0.020$), as well as with HDL cholesterol ($r=0.389$; $p=0.023$). Similar correlations were found with aortic levels of miR-126: $r=0.423$ with age ($p<0.001$); $r=0.510$ with HDL cholesterol ($p=0.002$). Finally, CVS levels of miR-21-3p were significantly correlated with both tele-diastolic diameter (TDD) ($r=0.358$; $p=0.011$), tele-systolic diameter (TSD) ($r=0.359$; $p=0.013$), and LV-EF ($r=-0.283$; $p=0.026$). Similarly, aortic levels of miR-21-3p were significantly correlated with LV-EF ($r=-0.250$; $p=0.046$). Transcoronary concentration gradients of miR-21-3p were

significantly correlated with TDD ($r=0.447$; $p=0.001$) and TSD ($r=0.472$; $p<0.001$).

4. DISCUSSION

The results of the present study document for the first time aetiology-specific transcoronary concentration gradients of circulating miRs in HF patients. In particular, while circulating levels of miR-423 and miR-34a were higher in HF patients, particularly in the ischemic aetiology subgroup, compared to the control group, expression levels of miR-21-3p were dramatically more elevated in the non-ischemic HF subgroup. On the contrary, circulating levels of miR-126 were reduced in both HF subgroups. These results suggest that the circulating levels of specific miRs reflect at least in part specific pathophysiological processes underlying HF in different aetiological subgroups. Moreover, the differential modulation of circulating miRs during the transcoronary passage further supports this hypothesis and most probably reflects cell type-specific expression levels and release mechanisms associated with myocardial remodelling in patients with heart failure.

Several studies reported the involvement of miRs in various pathogenic mechanisms underlying heart failure, such as remodelling, hypertrophy, apoptosis, and hypoxia (53-54). Importantly, a dynamic modulation of miRs was reported across different disease stages (55), or in association with specific aetiologies of HF (56), with obvious implications for their prognostic potential.

Among the most investigated circulating miRs in HF, the miR-423 was first proposed as a disease biomarker by Tijsen et al., reporting a promising discriminatory ability to identify HF-associated dyspnoea from dyspnoea of different origins (14). In line with these results, Goren et al. evaluated the expression levels of 186 circulating miRs in blood samples from 30 HF patients and reported a 1.5-fold increase of miR-423 levels compared to healthy

volunteers (57). In this context, Goldraich et al. (58) reported a modest but statistically significant transcoronary concentration gradient of the miR-423 in 16 patients with HF of different aetiologies, despite no difference was observed in the levels of miR-423 in venous, arterial or coronary sinus samples between HF patients and the control group. Although these conflicting results raised come concern and several debates around this research topic (23,59-60), they kept the spotlights on miR-423. Among the more recent reports, Seronde et al. found that lower levels of the circulating miR-423 are associated to a poorer prognosis in patients with acute HF (41), while Kuosmanen et al. described differential expression levels of miR-423 in pericardial fluid samples obtained from HF patients of different aetiologies, although the differences reported were not statistically significant, most probably due to the small sample size (61).

Several reasons were suggested to explain the large discrepancies reported about the circulating levels of miR-423 and their clinical significance in Heart Failure patients. In fact, most studies were small in sample size, they often used heterogeneous diagnostic criteria, and grouped different pharmacological treatments across different disease stages (23). Most importantly, HF patients were evaluated independently of their etiology and/or treatment received before and/or during the study. In fact, around half of all HF patients included in the studies by Goren et al. and Tijsen et al had an ischemic/atherosclerotic etiology but the limited sample size did not allow to check for inter-group differences. Hence, the modulation in the expression levels of miR-423 could have been driven by the higher levels to be found in the ICM subgroup. On the other hand, no specific etiology subgroups were reported in the study by Goren and colleagues.

On the contrary, in the present study we looked into different etiology subgroups and indeed found that multiple circulating miRs are differently regulated between ischemic and non-ischemic HF patients. Our results are in line with previous findings by Kousmanen et al., reporting different levels of the miR-423 in the pericardial fluid, depending on the specific aetiology underlying HF (61). Moreover, our finding of a transcoronary concentration gradient of miR-423 in one specific aetiology subgroup further suggests that the modulation of its expression levels reflects the pathophysiological background of HF.

In addition, the different regulation of the circulating miRs selected for the present analysis across different HF subgroups represents a potential added value for their clinical use as biomarkers. In fact, if positive transcoronary gradients were found for the miR-423 and the miR-34a in ischemic HF, patients with non-ischemic HF presented a positive concentration gradient for miR-21-3p. On the other hand, the circulating levels of miR-126 were decreased in both HF subgroups, compared to the control group. Thus, if confirmed in large prospective validation studies, the combined measurement of these miRs could be very helpful for the etiological diagnosis of HF.

The finding of a modulation in the levels of these specific miRs is particularly interesting, since they are all involved in the pathophysiology of left ventricular dysfunction. In particular, the miR-34a had been previously associated to left ventricular remodelling and clinical prognosis (26), and a modest increase in its levels was reported in HF patients (43). In line with those preliminary observations, we found in the present study that miR-34a levels are indeed increased with Heart Failure, with a positive transcoronary concentration gradient, particularly in the ICM-HF subgroup, which was

significantly correlated with hemodynamic parameters, such as heart rate and blood pressure. A modulation in the expression levels of both the miR-21-5p and the miR-21-3p had been previously reported. However, the latter was more interesting as it is involved in the pathophysiology of heart failure and left ventricular hypertrophy, and it mediates cell-to-cell communications between fibroblasts and cardiomyocytes (35-36). To this regard, in the present study CVS levels of the miR-21-3p were significantly correlated with multiple parameters of left ventricular function, such as LV ejection fraction, tele-diastolic- and tele-systolic-diameters. Also in line with our findings, lower circulating levels of miR-126 had been reported in HF patients, where its levels were correlated with age, left ventricular function, NT-proBNP levels and left atrium volume (62). Interestingly, circulating levels of miR-126 were shown to correlate with the cardiac repair potential (43). Similarly, miR-126 levels were significantly correlated with age, gender, cholesterol levels and left ventricular function.

Measured concentrations of circulating miRs may be affected by multiple parameters, including tissue expression, sample processing and storage, the release by specific cells into the circulation and their stability. In addition, the binding with plasmatic proteins or complex macromolecular complexes, as well as their packaging in microvesicles or circulating exosomes also potentially affects measured levels of circulating miRs. All these factors may have contributed to the inter-individual variance in the measured miRs levels. However, the limited sample volume did not allow to test for those factors in the present study.

In summary, circulating concentrations of selected miRs are significantly modified upon passage through the coronary circulation. Most interesting,

individual miRs present a specific transcoronary concentration gradient in different etiological patients' subgroups, suggesting their potential utility to discriminate between different aetiologies underlying HF. Future studies will have to address whether the aetiology-specific transcoronary circulation gradient of miRs are reflected in different peripheral blood concentrations to verify their actual clinical utility in heart failure.

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6. FIGURES AND TABLES

	ICM-HF	NICM-HF	ICM-HF + NICM-HF
	(n=41)	(n=23)	(n=64)
Age (years)	68.4± 10.5	65.1 ±13.2	67.2 ± 11.5
Male gender	83%	78%	81%
Family Historyof CAD	28%	35%	30%
NYHA grade Class III/IV	31%	32%	32%
Diabetes	44%	35%	43%
Hypertension	73%	65%	72%
Smoking	68%	50%	62%
Dyslipidemia	71%	50%	66%
AF	8%	15%	10%
BMI (kg/m²)	27.3 ± 6.1	29.3 ± 5.4	28.0 ± 5.9
Systolic BP (mmHg)	119.8 ± 15.1	121.1 ± 17.3	120.2 ± 15.7
DiastolicBP (mmHg)	74.0 ± 8.1	73.4 ± 12.1	73.8 ± 9.4
Heart Rate	72.3 ± 14.0	71.5 ± 9.0	72.0 ± 12.5
HDL Chol(mg/dl)	44.5 ± 12.7	52.1 ± 20.7	46.1 ± 14.6
LDL Chol(mg/dl)	79.6 ± 34.2	73.3 ± 38.8	78.4 ± 34.6
Triglycerides (mg/dl)	125.4 ± 49.4	132.0 ±76.4	127.1 ± 56.4
Creatine (mg/dl)	1.3 ± 0.5	1.4 ± 1.3	1.3 ± 0.8
NT-proBNP (pg/ml)	2507 ± 3731	3742 ± 8205	2944 ± 5671
DTD (mm)	61.9 ± 6.1	68.2 ± 10.9	63.8 ± 8.3
DTS (mm)	47.4 ± 7.5	54.5 ± 13.4	49.5 ± 10.0
LVEF (%)	31.7 ± 6.7	25.3 ± 4.9	29.7 ± 6.8
β-Blockers	68%	69%	68%
ACEI/ARB	50%	31%	45%
CCB	8%	8%	8%
K sparingduretics	43%	54%	45%
ASA	75%	39%	66%
Clopidogrel	38%	15%	32%
Ticlopidine	5%	8%	6%
Anticoagulants	20%	15%	19%
Statins	80%	31%	68%

HF, Heart Failure; CAD, coronaryarterydisease, NYHA,New York Heart Association; LVEF, leftventricularejectionfraction; AF, atrialfibrillation; BMI, Body Mass Index; HDL, high-densitylipoprotein; LDL, low-densitylipoprotein; NT-proBNP, N-terminal pro-B-type natriureticpeptide; DTD, telediastolicdiameter; DTS, telesystolicdiameter; ACEI, angiotensin-convertingenzymeinhibitor; ARB, angiotensinreceptorblockers; CCB, Calcium channelblockers.

Table 1: baseline characteristics of heart failure patients.

	No HF (n=11)
Age (years)	49.0 ± 10.8
Male gender	18%
Family Historyof CAD	18%
NYHA grade Class III/IV	none
Diabetes	none
Hypertension	27%
Smoking	46%
Dyslipidemia	18%
AF	none
BMI (kg/m2)	27.7 ± 12.2
Systolic BP (mmHg)	125.0 ± 8.7
DiastolicBP (mmHg)	77.5 ± 6.3
Heart Rate	65.0 ± 7.0
HDL Chol(mg/dl)	142.3 ± 19.2
LDL Chol(mg/dl)	61.0 ± 23.6
Triglycerides (mg/dl)	94.8 ± 60.7
Creatine (mg/dl)	0.9 ± 0.2

HF, Heart Failure; CAD, coronaryarterydisease, NYHA,New York Heart Association; LVEF, leftventricularejectionfraction; AF, atrialfibrillation; BMI, Body Mass Index; HDL, high-densitylipoprotein; LDL, low-densitylipoprotein; NT-proBNP, N-terminal pro-B-type natriureticpeptide; DTD, telediastolicdiameter; DTS, telesystolicdiameter.

Supplementary Table 1. Baseline characteristics, control group.

Figure 1

A.

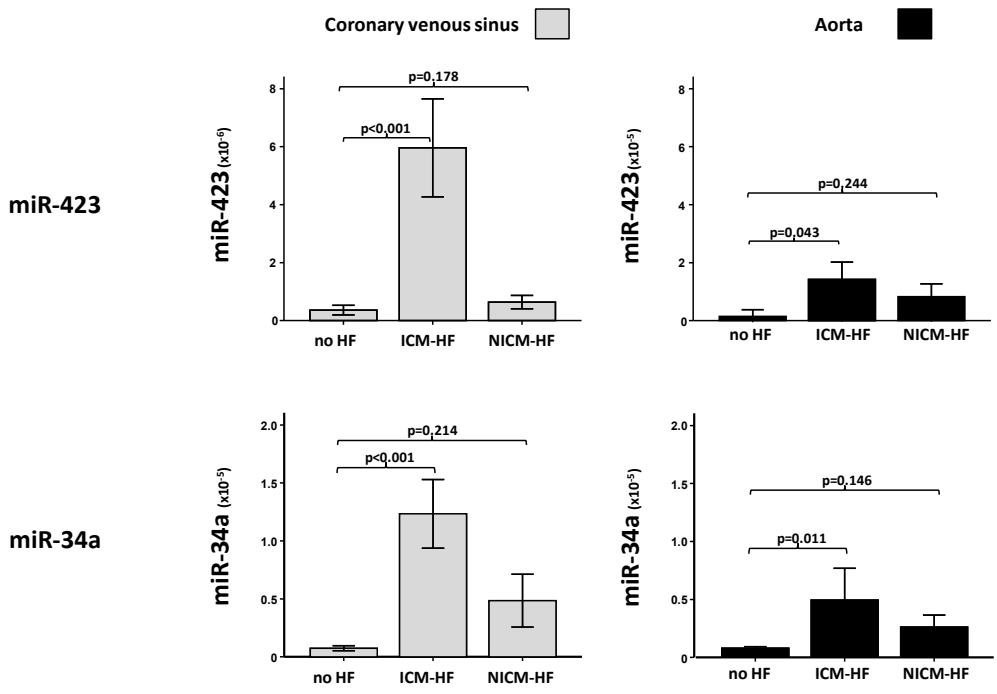

Figure 1 A. Circulating miRs levels in coronary sinus and aortic blood

A, levels of the miR-423 and the miR-34a, measured in coronary sinus (gray, left) and aortic blood (black, right), in the 3 different groups of patients.

Figure 1

B.

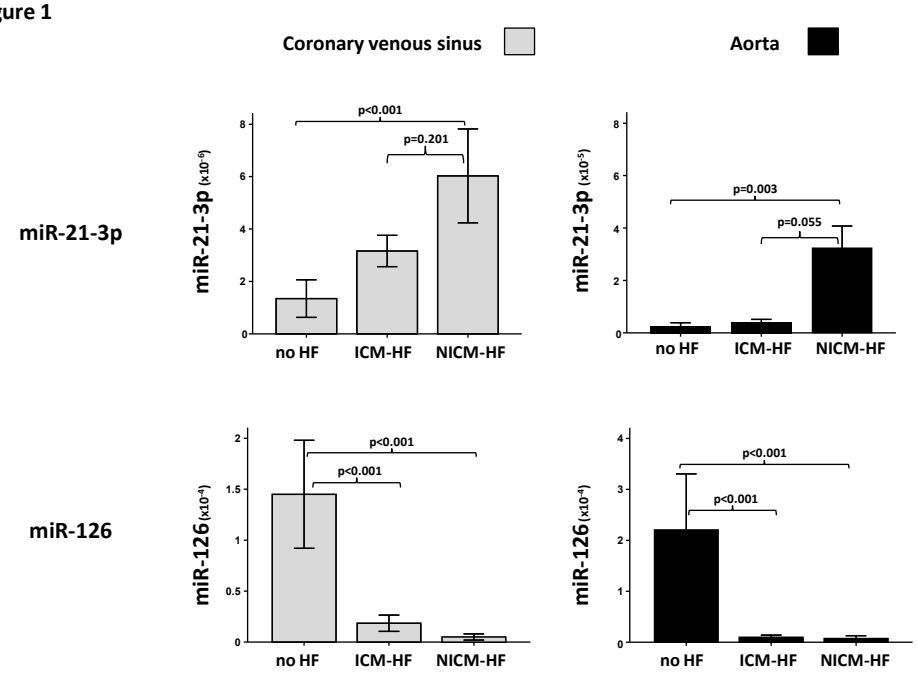

Figure 1 B. Circulating miRs levels in coronary sinus and aortic blood.

Levels of the miR-21-3p and the miR-126, measured in coronary sinus (gray, left) and aortic blood (black, right), in the 3 different groups of patients.Between groups comparisons were performed using the Mann-Whitney U test.

Figure 2

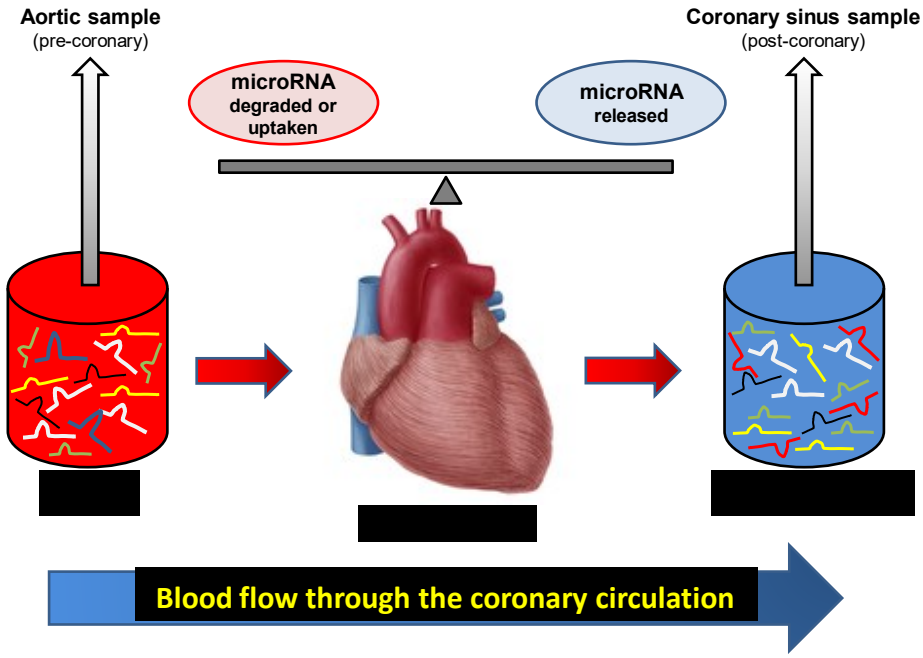

Figure 2. Transcoronary concentration gradients: scheme.

Experimental scheme: transcoronary concentration gradients of circulating microRNA are calculated as the ration between the expression levels detected in coronary sinus samples and those detected in aortic samples.

Figure 3

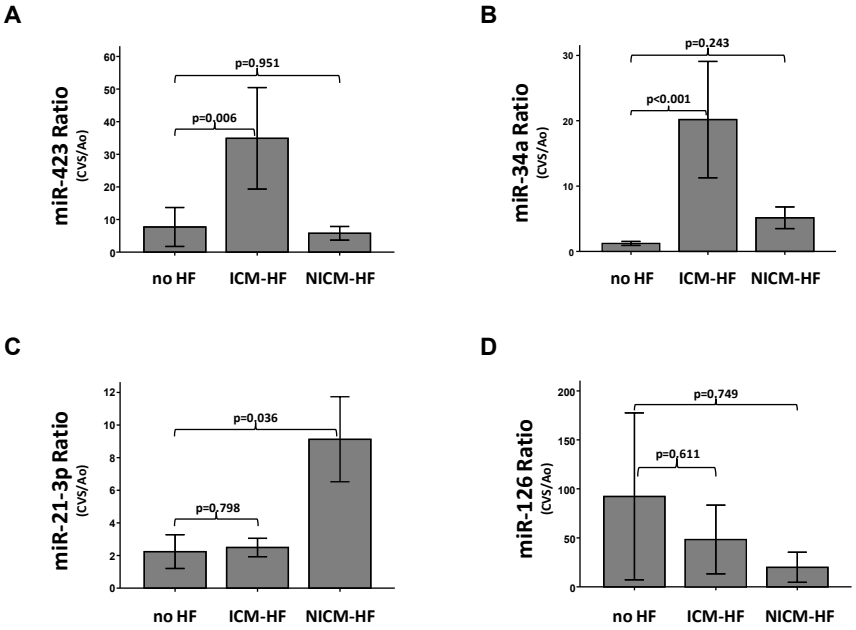

Figure 3. Transcoronary concentration gradient of circulating miRs.

Transcoronary concentration gradients, expressed as the ratio between their expression levels in the coronary venous sinus and the aorta ([coronary venous sinus]/[aorta]), for the miR-423 (A), miR-34a (B), miR-21-3p (C) and the miR-126 (D). Between groups comparisons were performed using the Mann-Whitney U test.