ESC HEART FAILURE
ESC Heart Failure 2021; **8**: 3865–3874
Published online 26 June 2021 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.13458

ORIGINAL RESEARCH ARTICLE

Circulating microRNA-29-5p can add to the discrimination between dilated cardiomyopathy and ischaemic heart disease

Martin Brundin[1]*, Dick Wågsäter[2,3], Urban Alehagen[4] and Carl-Johan Carlhäll[1,4,5]

[1]*Department of Clinical Physiology, Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden;* [2]*Division of Drug Research, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden;* [3]*Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden;* [4]*Unit of Cardiovascular Sciences, Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden; and* [5]*Center for Medical Image Science and Visualization, Linköping University, Linköping, Sweden*

Abstract

Aims Heart failure describes a large and heterogeneous spectrum of underlying cardiac diseases. MicroRNAs (miRs) are small non-coding RNAs that in recent years have been shown to play an important role in the pathogenesis of heart failure. Cardiac magnetic resonance imaging is a powerful imaging modality for the evaluation of cardiac characteristics in heart failure. In this study, we sought to compare heart failure patients with a diagnosis of either idiopathic dilated cardiomyopathy (DCM) or ischaemic heart disease (IHD), in the context of serum levels of certain miRs and also magnetic resonance imaging parameters of cardiac structure and function.

Methods and results A total of 135 subjects were studied: 53 patients with DCM (age: 59 ± 12 years, mean ± SD), 34 patients with IHD (66 ± 9 years), and 48 controls (64 ± 5 years). The participants underwent baseline medical examination, blood sampling, and a cardiac magnetic resonance imaging examination at 3 Tesla (Philips Ingenia). The serum levels of seven different miRs were analysed and assessed: 16-5p, 21-5p, 29-5p, 133a-3p, 191-5p, 320a, and 423-5p, all of which have been demonstrated to play potential roles in the pathogenesis of heart failure.

The patients in the DCM and IHD groups had left ventricles that had larger end-diastolic volume ($P < 0.001$), larger mass ($P < 0.001$), and lower ejection fraction ($P < 0.001$) compared with controls. Serum levels of miR-29-5p were increased in DCM compared with IHD ($P < 0.005$) and serum levels of miR-320a were elevated in DCM compared with healthy controls ($P < 0.05$). There was no significant association between miR levels and magnetic resonance imaging parameters of left ventricular structure and function.

Conclusions Circulating miR-320a can add to the discrimination between patients with DCM and healthy controls and circulating miR-29-5p can add to the discrimination between DCM and IHD.

Keywords microRNA; Biomarker; Heart failure; Cardiomyopathy; miRNA-29-5p; miRNA-320a

Received: 2 November 2020; Revised: 30 April 2021; Accepted: 23 May 2021
**Correspondence to: Martin Brundin, Department of Clinical Physiology in Linköping, Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden. Email: brundin75@gmail.com*

Introduction

The prevalence of heart failure (HF) is 2% of the adult population in developed countries and ≥10% among people >75 years of age, with a calculated life-time risk of about 20%.[1,2] In the western world, it is estimated that HF accounts for 1–2% of the annual health care budget.[3,4]

Heart failure includes a wide and heterogeneous spectrum of underlying cardiac abnormalities such as ischaemic heart disease, hypertension, cardiomyopathies, diabetes, exposure to cardiotoxic agents, and valvular disease. With cardiac injury, structural (cardiac remodelling), neurohumoral, cellular, and molecular mechanisms activate to maintain normal physiological function. This, in turn, may lead to

volume overload, sympathetic over-activation, and circulation redistribution.[5] Details of these complex processes remain poorly understood.

In recent years, different novel approaches have revealed underlying mechanisms that seem to play a role in the pathogenesis of HF. For instance, microRNAs (miR)s have been shown to play an important role. miRs are small non-coding RNAs known to be extensively involved in gene regulation, from normal development through to the pathogenesis of disease. Studies have shown that some miRs are expressed in lower or higher concentrations in the myocardium of HF patients, as compared with controls. They are thought to play an important role in progression of HF, by targeting genes that are involved in diverse functions in the cardiac remodelling process, such as in myocyte hypertrophy, increased myocyte loss, and myocardial fibrosis.[3,6,7] For example, it has been shown that miR-29-5p targets extracellular matrix proteins,[8] and that overexpression of miR-320 significantly increased cardiomyocyte apoptosis.[9] In addition to their role in adverse cardiac remodelling, miRs hold promise as biomarkers of disease progression in HF given their presence in circulation and enhanced stability. Furthermore, miRs have become interesting drug targets.[10]

Cardiac magnetic resonance imaging (MRI) has become a powerful and versatile imaging modality for the evaluation of cardiac structure and function. To date, it is a useful and accurate tool, in both research and clinical practice, for the assessment of ventricular size, shape and function in HF patients of different aetiologies.

The purpose of this study was to compare HF patients with a diagnosis of either idiopathic dilated cardiomyopathy (DCM) or ischaemic heart disease (IHD), with healthy controls, in the context of serum levels of certain miRs and also MRI parameters of cardiac structure and function. To our knowledge, this is the first study where different miRs are assessed and compared with cardiac MRI data in well-characterized cohorts of these types of cardiac diseases.

Methods

Study subjects

Study participants were recruited from the Linköping University Hospital outpatient HF clinic. In total 53 patients with DCM and 34 patients with IHD were included.

Inclusion criteria: All included patients, IHD and DCM, had symptoms of HF and previous or current left ventricular (LV) systolic dysfunction [reduced LV ejection fraction (EF) identified with either echocardiography or myocardial scintigraphy]. All included patients were evaluated with coronary angiography. If significant coronary stenoses were found, the diagnosis of IHD was applied. If not, and if the patient exhibited LV dilatation of unknown cause and had no history of IHD, the diagnosis of DCM was applied. Together with reduced LVEF from MRI, our DCM group conforms with the definition of DCM as suggested in previous research.[11–13]

In addition to the two patient groups, 48 healthy controls with no history of prior or current cardiovascular disease or cardiac medication and with a normal electrocardiogram were included in the study.

Exclusion criteria applying to all subjects include the following: contraindications for cardiac MRI, haemodynamically significant valvular disease, and atrial fibrillation or flutter with verified ventricular arrhythmia at the time of inclusion or MRI examination (MRI data quality is hampered by significant ventricular arrhythmia).

To achieve a more homogeneous group of patients, we retrospectively excluded all patients (DCM and IHD) with LVEF (from cardiac MRI) above 57%, in accordance with normal values for cardiac MRI (refer to Kawel-Boehm *et al*.[14] and Pertersen *et al*.[15]).

The study participants underwent physical examination, blood sampling, and cardiac MRI.

The physical examination was performed on the same day as the blood extraction and the imaging was performed within approximately 4 weeks after the blood extraction.

The baseline demographics of the study participants, along with clinical variables, can be found in *Table 1*. The study conforms to the Declaration of Helsinki and was approved by the Research Ethics Review Board of Region Östergötland (number 2010/273-31, 1 November 2010). All study participants gave written informed consent prior to inclusion in the study.

Cardiac MRI

The study participants also underwent cardiac MRI (*Table 2*). This was performed on a clinical 3 T scanner (Philips Ingenia, Philips Medical Systems, Best, the Netherlands) with dedicated cardiac applications. Images for LV structure and function were acquired during end-expiratory breath holds, using balanced steady-state free precession imaging, and reconstructed into 30 timeframes. The number of slices varied according to the size of each patient's heart. The short- and long-axis images had a resolution of 1.0×1.0 mm^2 and a slice thickness of 8.0 mm. Other imaging parameters were: repetition time, 2.8 ms; echo time, 1.4 ms; flip angle, 45 degrees; and parallel imaging with sensitivity encoding with a speed-up factor of 2–3. The LV end-diastolic (EDV) and end-systolic (ESV) volumes as well as LV mass were segmented from the short-axis image stack guided by long-axis images, using research segmentation software (Segment, Medviso AB, Lund, Sweden).

Table 1 Baseline patient characteristics

	DCM	IHD	Control
Baseline demographics			
Number of patients	53	34	48
Age (years)	59 ± 12**	66 ± 9	64 ± 5$^{\#}$
Gender (% male)	68%	88%	38%
BMI (kg/m^2)	28 ± 5	30 ± 5	25 ± 3***, $^{\#\#}$
HF duration (years)	4.7 ± 4.4	5.7 ± 5.6	NA
Systolic BP (mmHg)	130 ± 19	130 ± 20	140 ± 18$^{\#}$
Diastolic BP (mmHg)	79 ± 12	76 ± 16	80 ± 9
Heart rate (1/min)[a]	62 ± 12	66 ± 14	68 ± 12$^{\#}$
Smoking[b]	49%/26%	68%/21%	27%/46%
Comorbidity			
Hypertension	28%	44%	4%***, $^{\#\#}$
Stroke	6%**	18%	0%*
Diabetes	13%*	41%	2%***
Hyperlipidaemia (=on statin treatment)	45%**	82%	2%***, $^{\#\#\#}$
Renal failure	4%	9%	0%
NYHA class			
NYHA I	38%	9%	NA
NYHA II	53%	53%	NA
NYHA IIIa	4%	21%	NA
NYHA IIIb	6%	18%	NA
NYHA IV	0%	0%	NA
Medication			
ACEi or ARB	96%	100%	0%
β-blocker	98%	97%	2%
MRA	36%	32%	0%
Diuretics	57%	50%	4%
Statin	45%	85%	2%
ASA	30%	68%	0%
Clopidogrel	0%	15%	0%
Warfarin	26%	29%	0%

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; DCM, dilated cardiomyopathy; HF, heart failure; IHD, ischaemic heart disease; NYHA, New York Heart Association.
Where relevant, presented values are mean ± standard deviation. In either column, if $P \geq 0.05$, no symbols are used. In the baseline demographics section of the table, we used Bonferroni adjusted Dunn test, and in the comorbidities section, we used Pearson's χ^2 test.
[a]Heart rate data from magnetic resonance imaging.
[b]Presented as Y% /N% where Y stands for yes (now or before) and N stands for never, the remaining patients up to 100% have chosen not to answer the question.
*Statistically significant differences against the IHD group ($P < 0.05$).
**Statistically significant differences against the IHD group ($P < 0.01$).
***Statistically significant differences against the IHD group ($P < 0.001$).
#Statistically significant differences against the DCM group ($P < 0.05$).
##Statistically significant differences against the DCM group ($P < 0.01$).
###Statistically significant differences against the DCM group ($P < 0.001$).

Table 2 MRI parameters

	DCM	IHD	Control
MRI parameters			
EF (%)	40 ± 10	36 ± 10	61 ± 7
EDV (mL)	240 ± 71	240 ± 85	140 ± 33
Indexed EDV (mL/m^2)	120 ± 33	120 ± 43	79 ± 14
ESV (mL)	150 ± 66	160 ± 73	58 ± 19
Indexed ESV (mL/m^2)	75 ± 33	78 ± 38	31 ± 9
LV mass (g)	130 ± 50	150 ± 54	87 ± 23
Indexed LV mass (g/m^2)	66 ± 25	74 ± 25	47 ± 11

DCM, dilated cardiomyopathy; EDV, end-diastolic volume; ESV, end-systolic volume; IHD, ischaemic heart disease; LV, left ventricular; MRI, magnetic resonance imaging.
Values are presented as mean ± standard deviation. There is no significant difference in MRI parameters between the DCM and IHD groups (Bonferroni adjusted Kruskal–Wallis tests). However, every presented parameter differs significantly ($P < 0.001$) between either of the two heart failure groups compared with the control group (Bonferroni adjusted Dunn tests).

Real-time qPCR analysis of miR

In the present study, plasma levels of miR-16-5p, 21-5p, 29-5p, 133a-3p, 191-5p, 320a, and 423-5p were measured, all of which have been found to be of interest in different settings in cardiac remodelling or cardiac injury in animal or human studies. These miRs will be abbreviated as miR-16, 21, 29, 133, 191, 320, and 423.

RNA was extracted from 200 μL plasma of all patients according to the miRNeasy standard protocol (Qiagen, Hilden, Germany) and reverse transcribed using the miRCURY LNA™ Universal RT microRNA PCR, Polyadenylation and cDNA synthesis kit (Exiqon Vedbaek, Denmark) as described previously in more detail.[16] UniSp6 spike-in was used as control for the reverse transcription step. Semi-quantitative real-time PCR amplification of the miRs was performed in a Roche

Lightcycler 480. The primer target sequences used can be seen in *Table 3*.

Plasma concentrations of the different miRs are relative values with arbitrary unit (values from a relative standard curve).

Statistical analysis

Statistical computations were carried out using the freely available software R (GNU general public licence) and SPSS (version 27) was used for all analyses involving adjusting for covariates. Descriptive data are presented as percentages or mean ± standard deviation.

The miR levels were tested separately against group (DCM, IHD, and control), using the Kruskal–Wallis test. $P < 0.05$ was considered significant. The miRs for which significant differences were found (miR-29, miR-320, and miR-423, refer to the Results section) were further investigated for covariance with the baseline variables. The baseline variables found to be of most importance, and for which correction was performed, were gender, age, and body mass index (BMI). Correction was performed using multivariate analysis (ANCOVA), and we found that the miR levels were still significantly different among the three groups. Šidák-adjusted pairwise testing between groups, using the corrected model, was then performed.

Receiver operating characteristics for groups or combinations of groups vs. variables were constructed using 10-fold cross validation. This is carried out by randomly dividing the patients into 10 equally sized groups. One of the groups constitutes the validation set, the remaining nine the training set. An optimal binomial model is fitted to the training set and is then used for predictions on the validation set. A prediction for a patient is, in this context, a number between 0 and 1 that at least intuitively tells 'how much Group A (0) and how much Group B (1) the patient is'. This is repeated 10 times (every group constitutes the validation set exactly once). This yields predictions for all patients, and we can for each threshold value between 0 and 1 (all patients with prediction $<$ threshold are placed in Group A, the rest are placed in Group B) calculate the sensitivity and specificity of the model. The corresponding area under the curve (AUC) was calculated as the mean AUC of 50 such simulations (yielding stable values to at least two decimals).

The relationships between miR levels and MRI parameters of LV structure and function were analysed using linear regression.

Results

Demographic and basic clinical data are complete for all participants (*Table 1*), as are the miR and MRI data. The two HF groups had higher BMI, lower systolic blood pressure, lower heart rate, and a higher proportion of male patients compared with controls. The two patient groups differed mainly in age and comorbidities, with the IHD group being significantly older and sicker. This is also illustrated in the New York Heart Association (NYHA) classification for the two groups.

MRI parameters of LV characteristics differ between HF and controls but not between HF groups, respectively

The MRI parameters included in the study were LV volume and mass (including body surface adjusted versions) and LVEF. As can be seen in *Table 2* and in *Figure 1*, both HF groups had LVs that were dilated, had increased myocardial mass, and had impaired systolic function, as compared with controls. None of the MRI parameters of LV structure and function could discriminate between DCM and IHD.

Circulating miR-29 and miR-320 levels differ between DCM and IHD and between DCM and controls, respectively

Plasma levels of three miRs were found to differ between the three groups (DCM, IHD, and controls). As is clear from *Table 4*, significant differences (not taking covariates into account) were found for miR-29 ($P = 0.0037$), miR-320 ($P = 0.00012$) and miR-423 ($P = 0.0072$). Three baseline variables (age, gender, and BMI) were associated with miR levels in at least one group. Adjusting for these three variables using

Table 3 Primer target sequences for the different miRs

miR	Catalogue number	Mature miR target
miR-16-5p	MIMAT0000069	5′UAGCAGCACGUAAAUAUUGGCG
miR-21-5p	MIMAT0000076	5′UAGCUUAUCAGACUGAUGUUGA
miR-29a-5p	MIMAT0004503	5′ACUGAUUUCUUUUGGUGUUCAG
miR-133a-3p	MIMAT0000427	5′UUUGGUCCCCUUCAACCAGCUG
miR-191-5p	MIMAT0000440	5′CAACGGAAUCCCAAAAGCAGCUG
miR-320a	MIMAT0000510	5′AAAAGCUGGGUUGAGAGGGCGA
miR-423-5p	MIMAT0004748	5′UGAGGGGCAGAGAGCGAGACUUU

Figure 1 Violin plots of the distributions of some of the magnetic resonance imaging parameters measured. The median values and the first and third quartiles are marked. Note that in Table 2, in contrast, values are mean value ± standard deviation. DCM, dilated cardiomyopathy.

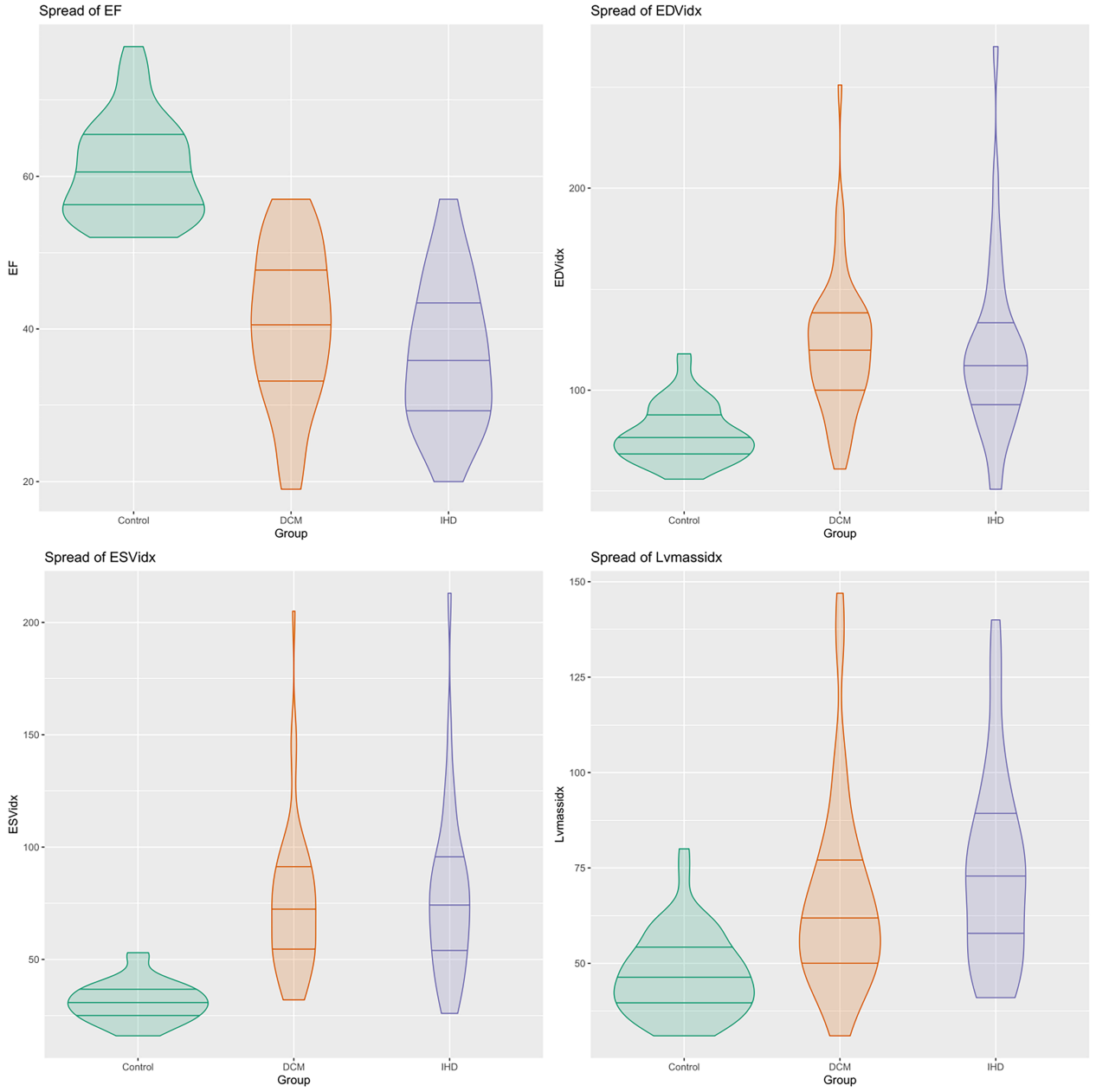


Table 4 miR levels in different groups (without adjustments for covariates)

	DCM	IHD	Control	*P* value
micro-RNA				
miR-16-5p	3100 ± 2000	2900 ± 1500	2600 ± 1200	0.58
miR-21-5p	8000 ± 4800	7100 ± 5300	6500 ± 3000	0.36
miR-29-5p	510 ± 350	330 ± 250	420 ± 230	0.0037
miR-133a-3p	1100 ± 1500	600 ± 640	630 ± 820	0.28
miR-191-5p	3500 ± 2600	2800 ± 1600	2900 ± 1800	0.72
miR-320a	2700 ± 1400	2400 ± 980	1900 ± 1100	0.00012
miR-423-5p	2400 ± 1300	2000 ± 1200	1700 ± 900	0.0072

Values are miR levels (arbitrary unit) presented as mean ± standard deviation. Kruskal–Wallis test was used to test for significant differences between the groups, without adjustments for covariates. The last column shows the corresponding *P* values.

DOI: 10.1002/ehf2.13458

multivariate analysis (ANCOVA), we found that there were significant differences in plasma levels among the three groups, for miR-29 and miR-320 but not for miR-423. Further testing between groups, refer to *Table 5*, showed that miR-29 levels are elevated in DCM compared with IHD ($P < 0.005$) and that miR-320 levels are up-regulated in DCM as compared with controls ($P < 0.05$). No significant difference between any pair of groups was found for miR-423. In *Figure 2*, violin plots show how plasma levels of miR-29 and of miR-320 differ between groups (no adjustment for covariates).

Receiver operating characteristic curves were constructed, *Figure 3*, showing how miR-29 can be used to discriminate between IHD and DCM, AUC ≈ 0.66, and how miR-320 can be used to discriminate between DCM and controls with AUC ≈ 0.70. If combined with BMI, miR-320 discriminates between controls and DCM with AUC ≈ 0.75, which is also shown in *Figure 3*. Combining miR-29 and BMI made no improvement in AUC.

Table 5 miR difference between specific groups, adjusted for covariates

	Control	DCM
(miR-29-5p)		
DCM	0.73	NA
IHD	0.071	0.003
(miR-320a)		
DCM	0.038	NA
IHD	0.71	0.51

Šidák adjusted pairwise comparisons between groups for the different miRs.

Combinations of several miRs and MRI parameters were also assessed, without improving the AUCs significantly.

Relations between miR levels and MRI parameters of LV structure and function were also investigated. Indexed LVEDV, indexed LV mass, and LVEF were fitted to univariate linear models with each of the miRs as variables, respectively. Furthermore, this was performed for IHD only, DCM only, and also for the entire population. All of these regressions came out with R^2 values less than 0.07. Thus, multivariate regression was not pursued.

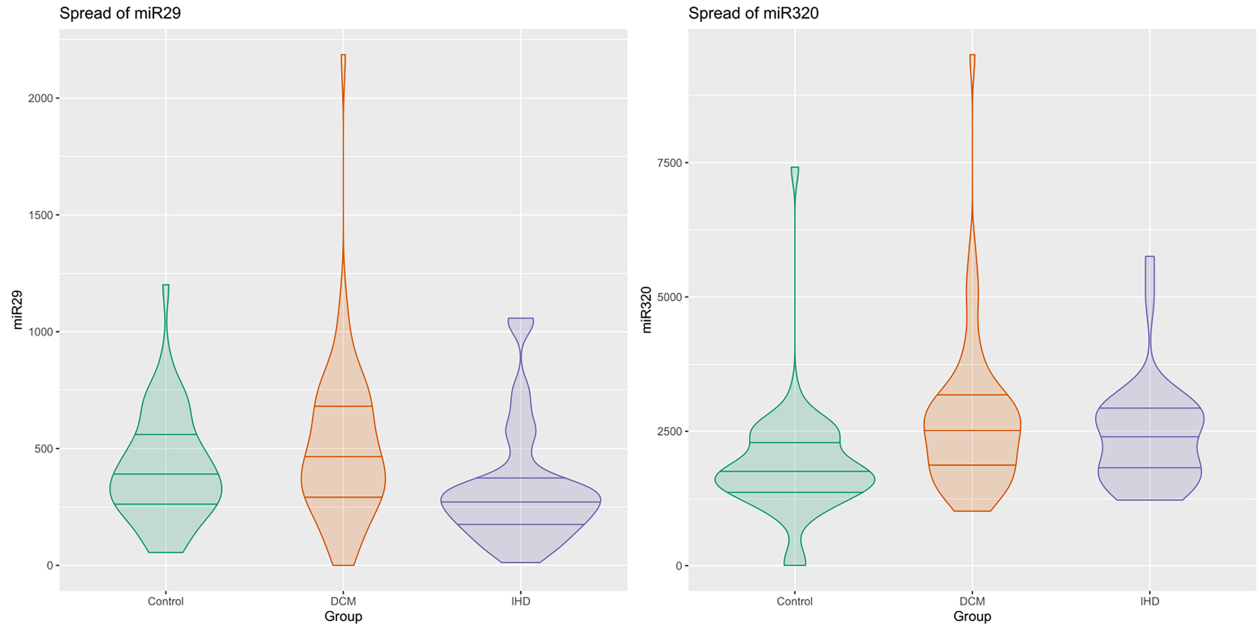
There was no significant relationship between HF duration and any of the miRs.

Discussion

This study investigated differences in plasma levels of certain micro-RNAs, and also MRI parameters, between two well-characterized groups of HF patients (idiopathic DCM and IHD) and a group of healthy controls. The findings propose that plasma levels of miR-29-5p differ between DCM and IHD, and that plasma levels of miR-320a differ between DCM and healthy (controls).

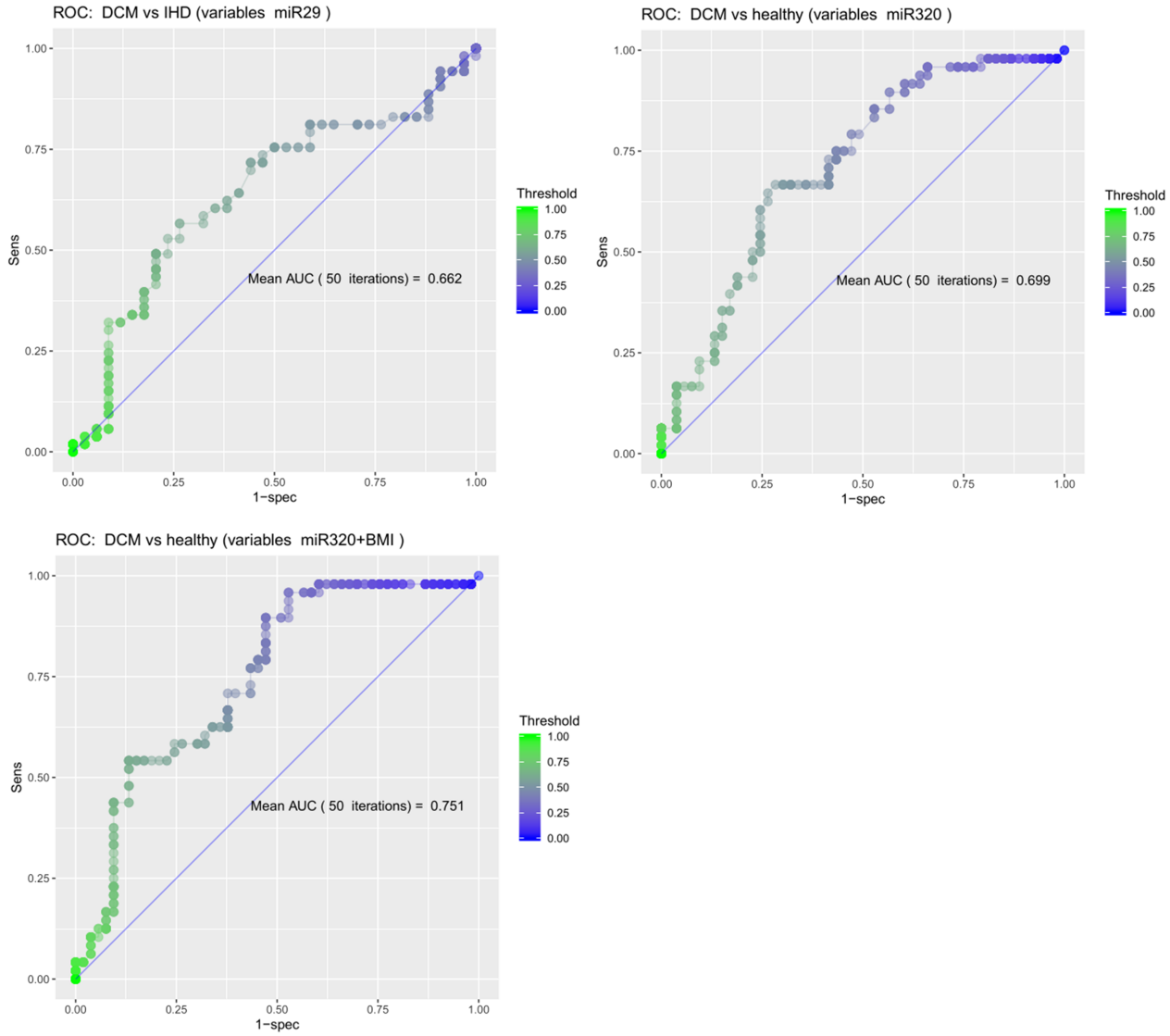
The DCM and IHD groups are quite comparable when it comes to MRI measures of LV structure and function measures (*Table 2*), but quite different in some of the baseline characteristics and comorbidities (*Table 1*). Not surprisingly, diabetes and hypertension are more common in the IHD

Figure 2 Violin plots of the distributions of miR-29-5p and miR-320a in each group, the median values and the first and third quartiles are marked. DCM, dilated cardiomyopathy; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; LV, left ventricular.



DOI: 10.1002/ehf2.13458

Figure 3 Receiver operating characteristics (ROCs) showing the diagnostic accuracy of miR-29-5p levels to discriminate between dilated cardiomyopathy (DCM) and ischaemic heart disease (IHD) and of miR-320a levels to discriminate between DCM and healthy controls. The last ROC curve shows how miR-320a and body mass index together can discriminate better than miR-320a alone between DCM and healthy controls. AUC, area under the curve.



group, as is smoking. Also, the IHD patients have more severe disease according to their NYHA classification.

Several miRs are known to play a role in the pathogenesis of HF, but the exact mechanisms are largely unknown. All of the miRs analysed in this study are known to regulate at least some process in the cardiovascular system. Below, the most interesting miR findings of the current study are discussed.

miRNA-29-5p

We found that miR-29 plasma levels were significantly lower in IHD than DCM. This is, to our knowledge, the first result on miRs and chronic systolic HF that shows a clear difference in serum levels between two types of aetiologically different HF groups. So, for reasons yet to be determined, expression of miR-29 is reduced in IHD as compared with DCM. It is known that miR-29 targets extracellular matrix proteins, and it is shown that miR-29 is associated with atrial fibrotic remodelling.[8] This, however, does not obviously explain its role in the current context. The role of miR-29 in diabetes mellitus has been reviewed recently.[17] Both up-regulation and down-regulation of miR-29 seem to play a role in diabetic cardiovascular complications, including diabetic cardiomyopathy and cardiac fibrosis, suggesting in our case that the down-regulation seen in the IHD group could correspond to

DOI: 10.1002/ehf2.13458

the higher prevalence of diabetes. More directly connected to IHD is the result in the work of van Rooij *et al.*,[18] where it is shown that miR-29 is down-regulated in the cardiac tissue surrounding an infarction. Moreover, miR-29 (among several other miRs) has been described to enhance cardiomyocyte survival and attenuate cardiac fibrosis.[19]

miRNA-320a

The role of miR-320a in cardiovascular pathology is not extensively studied. However, it has been shown that increased expression of miR-320 significantly increased cardiomyocyte apoptosis and that down-regulation had the opposite effect.[9] Furthermore, it has been demonstrated that *in vivo* knockdown of miR-320 in murine hearts led to reduction in the size of myocardial infarction.[20]

The current study confirms that miR-320 levels are significantly higher in HF patients (DCM and IHD) vs. controls and that the diagnostic accuracy is slightly better than for miR-423, with AUC ≈ 0.68.

miRNA-423-5p

miR-423 has been shown to be elevated in HF patients in several other studies[21–25]; in a meta-analysis,[1] it has even been proposed as a possible biomarker for HF. Unfortunately, the HF patient cohorts in the various included studies were not homogeneous, for example, different HF aetiologies were not accounted for and covariates had not been adjusted for. In this study, we did not find any pairwise significant differences in miR-423 levels among the three groups, when adjusted for age, gender, and BMI. In part, this result is not concordant with findings from Rizzacasa *et al.*,[26] which concluded that miR-423 levels in aortic and coronary sinus blood is higher in IHD patients than in non-IHD HF patients and higher than in healthy controls.

Recently, a panel with 30 miRs was tested in small groups of IHD and DCM patients, and the authors suggested that miR-15b-5p and miR-106a-5p could be used to distinguish between the two groups.[27] In this study, there were only 25 patients in each group and the patients were not characterized with advanced cardiac imaging. The only miR analysed in that study and in our study was miR-191-5p.

In a previous study, the same authors analysed circulating miRs and compared these to MRI-based LV mass index in a cohort of 41 patients with hypertrophic cardiomyopathy.[28] The miRs that overlapped with our study were mir-21, miR-29, and miR-133. The authors could not find any significant relationship between these miRs and LV mass index, which is in line with our results in DCM and IHD. However, the authors found a significant relationship between miR-29 and late gadolinium enhancement-based assessment of LV myocardial fibrosis.

Clinical implications

In some cardiovascular diseases, an up-regulation of a certain miR appears to reflect a pathophysiological process, and in some cardiovascular diseases, a down-regulation of the same miR appears to reflect a pathophysiological process. Therefore, it is not obvious that miRs are optimal drug therapy targets, even though some promising results have been proposed.[29] With greater knowledge of in which signalling pathways a given miR is active, and why, it is more than likely that it can be a therapeutic drug target.

miRs have the potential to add to early diagnosis of HF, as well as to help monitor responses to treatment. The value of determining which type of HF a given patient suffers from, if any, via a simple blood sample is significant. This differentiation in HF type can potentially eliminate the need for some expensive, and to some degree harmful, other diagnostic examinations.

Limitations

The different groups were neither gender nor age matched; however, this has been accounted for in all relevant statistical analyses. The majority of the study participants were Caucasian and elderly; therefore, the results are not immediately applicable to other populations.

We did not have access to cardiac tissue as the included patients had no clinical indication to undergo myocardial biopsy procedure. Although we cannot prove that the origin of the miR release is from the heart, the current findings do demonstrate a significant difference in circulating miRs between DCM and IHD.

Although the AUC values of ≈0.7 are not excellent, but near acceptable, the current findings may provide some new perspectives on the discrimination between DCM and IHD.

The presented miR values are relative, arbitrary unit, with values from a relative standard curve. The inter-group differences are relatively small, which correlates well with our general experience of measuring plasma levels of miRs in several other studies. However, a small difference in arbitrary levels could be associated with important clinical differences, as seen in other conditions.

Conclusions

We sought to compare HF patients with a diagnosis of either DCM or IHD, in the context of serum levels of certain miRs

and also MRI parameters of cardiac structure and function. Serum levels of miR-29-5p were increased in DCM compared with IHD and serum levels of miR-320a were elevated in DCM compared with healthy controls. There was no association between miR levels and MRI parameters of LV characteristics. Circulating miR-320a can be used to discriminate between healthy controls and patients with DCM, and circulating miR-29-5p can be used for discrimination between patients with idiopathic DCM and IHD.

Acknowledgements

We thank Emina Vorkapic for her skillful assistance with the microRNA analyses.

We also thank Andreas Bussman, Jennie Kemppi, and Inger Ekman for valuable assistance with the MRI data analyses.

Finally, we thank Mats Fredrikson for carrying out the more sophisticated statistical analyses.

Conflict of interest

None declared.

Funding

This work was supported by the Swedish Medical Research Council (2018-02779 and 2019-01673), the Swedish Heart and Lung Foundation (20170440 and 20190556, and ALF Region Östergötland grant (LIO-797721).

References

1. Metra M, Teerlink JR. Heart failure. *Lancet* 2017; **390**: 1981–1995.
2. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, González-Juanatey JR, Harjola V-P, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P, ESC Scientific Document Group. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 2016; **37**: 2129–2200.
3. Gomes CPC, Schroen B, Kuster GM, Robinson EL, Ford K, Squire IB, Heymans S, Martelli F, Emanueli C, Devaux Y. Regulatory RNAs in heart failure. *Circulation* 2020; **141**: 313–328.
4. Liao L, Allen LA, Whellan DJ. Economic burden of heart failure in the elderly. *Pharmacoeconomics* 2008; **26**: 447–462.
5. Tanai E, Frantz S. Pathophysiology of Heart Failure. *Compr Physiol* 2015; **6**: 187–214.
6. Calderon-Dominguez M, Belmonte T, Quezada-Feijoo M, Ramos-Sánchez M, Fernández-Armenta J, Pérez-Navarro A, Cesar S, Peña-Peña L, Vea À, Llorente-Cortés V, Mangas A, de Gonzalo-Calvo D, Toro R. Emerging role of microRNAs in dilated cardiomyopathy: evidence regarding etiology. *Transl Res* 2020; **215**: 86–101.
7. Topkara VK, Mann DL. Role of microRNAs in cardiac remodeling and heart failure. *Cardiovasc Drugs Ther* 2011; **25**: 171–182.
8. Dawson K, Wakili R, Ördög B, Clauss S, Chen Y, Iwasaki Y, Voigt N, Qi XY, Sinner MF, Dobrev D, Kääb S, Nattel S. MicroRNA29: a mechanistic contributor and potential biomarker in atrial fibrillation. *Circulation* 2013; **127**: 1466–1475 1475e1-28.
9. Tian ZQ, Jiang H, Lu ZB. MiR-320 regulates cardiomyocyte apoptosis induced by ischemia-reperfusion injury by targeting AKIP1. *Cell Mol Biol Lett* 2018; **23**: 41.
10. Vegter EL, van der Meer P, de Windt LJ, Pinto YM, Voors AA. MicroRNAs in heart failure: from biomarker to target for therapy. *Eur J Heart Fail* 2016; **18**: 457–468.
11. Merlo M, Cannatà A, Gobbo M, Stolfo D, Elliott PM, Sinagra G. Evolving concepts in dilated cardiomyopathy. *Eur J Heart Fail* 2018; **20**: 228–239.
12. Merlo M, Cannatà A, Pio Loco C, Stolfo D, Barbati G, Artico J, Gentile P, de Paris V, Ramani F, Zecchin M, Gigli M, Pinamonti B, Korcova R, di Lenarda A, Giacca M, Mestroni L, Camici PG, Sinagra G. Contemporary survival trends and aetiological characterization in non-ischaemic dilated cardiomyopathy. *Eur J Heart Fail* 2020; **22**: 1111–1121.
13. Sinagra G, Elliott PM, Merlo M. Dilated cardiomyopathy: so many cardiomyopathies! *Eur Heart J* 2020; **41**: 3784–3786.
14. Kawel-Boehm N, Hetzel SJ, Ambale-Venkatesh B, Captur G, Francois CJ, Jerosch-Herold M, Salerno M, Teague SD, Valsangiacomo-Buechel E, van der Geest RJ, Bluemke DA. Reference ranges ("normal values") for cardiovascular magnetic resonance (CMR) in adults and children: 2020 update. *J Cardiovasc Magn Reson* 2020; **22**: 87.
15. Petersen SE, Khanji MY, Plein S, Lancellotti P, Bucciarelli-Ducci C. European Association of Cardiovascular Imaging expert consensus paper: a comprehensive review of cardiovascular magnetic resonance normal values of cardiac chamber size and aortic root in adults and recommendations for grading severity. *Eur Heart J Cardiovasc Imaging* 2019; **20**: 1321–1331.
16. Wanhainen A, Mani K, Vorkapic E, de Basso R, Björck M, Länne T, Wågsäter D. Screening of circulating microRNA biomarkers for prevalence of abdominal aortic aneurysm and aneurysm growth. *Atherosclerosis* 2017; **256**: 82–88.
17. Dasare AP, Gondaliya P, Srivastava A, Kalia K. A therapeutic approach towards microRNA29 family in vascular diabetic complications: a boon or curse? *J Diabetes Metab Disord* 2019; **18**: 243–254.
18. van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, Hill JA, Olson EN. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. *Proc Natl Acad Sci U S A* 2008; **105**: 13027–13032.
19. Moghaddam AS, Afshari JT, Esmaeili SA, Saburi E, Joneidi Z, Momtazi-Borojeni AA. Cardioprotective microRNAs: lessons from stem cell-derived exosomal microRNAs to treat cardiovascular disease. *Atherosclerosis* 2019; **285**: 1–9.
20. Ren XP, Wu J, Wang X, Sartor MA, Qian J, Jones K, Nicolaou P, Pritchard TJ, Fan GC. MicroRNA-320 is involved in the regulation of cardiac ischemia/reperfusion

injury by targeting heat-shock protein 20. *Circulation* 2009; **119**: 2357–2366.
21. Ellis KL, Cameron VA, Troughton RW, Frampton CM, Ellmers LJ, Richards AM. Circulating microRNAs as candidate markers to distinguish heart failure in breathless patients. *Eur J Heart Fail* 2013; **15**: 1138–1147.
22. Fan KL, Zhang HF, Shen J, Zhang Q, Li XL. Circulating microRNAs levels in Chinese heart failure patients caused by dilated cardiomyopathy. *Indian Heart J* 2013; **65**: 12–16.
23. Goren Y, Kushnir M, Zafrir B, Tabak S, Lewis BS, Amir O. Serum levels of microRNAs in patients with heart failure. *Eur J Heart Fail* 2012; **14**: 147–154.
24. Seronde MF, Vausort M, Gayat E, Goretti E, Ng LL, Squire IB, Vodovar N, Sadoune M, Samuel JL, Thum T, Solal AC, Laribi S, Plaisance P, Wagner DR, Mebazaa A, Devaux Y, GREAT network. Circulating microRNAs and outcome in patients with acute heart failure. *PLoS ONE* 2015; **10**: e0142237.
25. Tijsen AJ, Creemers EE, Moerland PD, de Windt LJ, van der Wal AC, Kok WE, Pinto YM. MiR423-5p as a circulating biomarker for heart failure. *Circ Res* 2010; **106**: 1035–1039.
26. Rizzacasa B, Morini E, Mango R, Vancheri C, Budassi S, Massaro G, Maletta S, Macrini M, D'Annibale S, Romeo F, Novelli G, Amati F. MiR-423 is differentially expressed in patients with stable and unstable coronary artery disease: a pilot study. *PLoS ONE* 2019; **14**: e0216363.
27. Onrat ST, Onrat E, Ercan Onay E, Yalım Z, Avşar A. The genetic determination of the differentiation between ischemic dilated cardiomyopathy and idiopathic dilated cardiomyopathy. *Genet Test Mol Biomarkers* 2018; **22**: 644–651.
28. Roncarati R, Viviani Anselmi C, Losi MA, Papa L, Cavarretta E, da Costa Martins P, Contaldi C, Saccani Jotti G, Franzone A, Galastri L, Latronico MVG, Imbriaco M, Esposito G, de Windt L, Betocchi S, Condorelli G. Circulating miR-29a, among other up-regulated microRNAs, is the only biomarker for both hypertrophy and fibrosis in patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2014; **63**: 920–927.
29. Hinkel R, Ramanujam D, Kaczmarek V, Howe A, Klett K, Beck C, Dueck A, Thum T, Laugwitz KL, Maegdefessel L, Weber C, Kupatt C, Engelhardt S. AntimiR-21 prevents myocardial dysfunction in a pig model of ischemia/reperfusion injury. *J Am Coll Cardiol* 2020; **75**: 1788–1800.